Journal of Applied Polymer Science

Incorporation of Theophylline in a Chitosan/Chondroitin Sulfate Hydrogel Matrix: *In Vitro* Release Studies and Mechanical Properties According to pH Changes

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

Group of Polymeric Materials and Composites, Chemistry Department, Maringá State University, Avenue Colombo, 5790 - CEP 87020-900, Maringá, Paraná, Brazil

Correspondence to: A. R. Fajardo (E-mail: drefajardo@hotmail.com)

ABSTRACT: Hydrogels based on natural polymers have been widely applied as vehicles for controlled drug release because of their advantages and interesting properties. In this study, a physical hydrogel based on chitosan and chondroitin sulfate (CS) was formed under mild conditions to act as a potential device for the controlled release of theophylline (TH). *In vitro* CS and TH release studies at pH 2 and 8 were performed. Under acid conditions (pH 2), the fraction of TH released (ca. 0.87) was higher than that of CS (ca. 0.13). On the other hand, under basic conditions, the fractions released of both substances were similar (ca. 0.57). In addition, the system presented in this work was able to sustain the TH release in a controlled way for 30 h. The variation of the pH affected the mechanical properties and contributed to form ordered regions within the hydrogel network, as observed through compression tests and wide-angle X-ray scattering analysis. The experimental data and discussion presented in this article will contribute to the development of a new vehicle for controlled TH release; this ensures the efficacy of the drug and reduced the number of daily doses administered. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: drug delivery systems; gels; porous materials

Received 8 March 2012; accepted 16 July 2012; published online
DOI: 10.1002/app.38357

INTRODUCTION

The development of hydrogels based on natural polymers to act as devices for controlled drug release are a promising research field.[1–3] Hydrogels are formed by crosslinked macromolecular networks that can easily swell in water or in biological fluids.[4] Two possible routes describe the crosslinking among the macromolecules: chemical crosslinking, which is based on irreversible covalent bonds,[5] and physical crosslinking, which comprises reversible ionic interactions.[5] In the biomedical field, hydrogels that are physically crosslinked present great advantages over those that are chemically crosslinked.[6] Physically crosslinked hydrogels do not require the use of crosslinking agents, auxiliary molecules, or organic solvents to be formed. Thus, the level of toxicity of the final material is very low or even nonexistent. Hydrogels formed by the polyion complexation of chitosan (CHI) and chondroitin sulfate (CS) are good examples of physically crosslinked hydrogels.[7,8] CHI is a linear polymer obtained from the partial or total deacetylation of the biopolymer chitin.[9,10] Its cationic nature and unique properties, such as low toxicity,[11] biodegradability,[11] and biocompatibility;[12] makes CHI to be a good candidate for application in hydrogel formulation. Several researchers have reported the formation of polyion complexes between CHI and CS, another natural polymer.[13–15] CS, a glycosaminoglycan, is an important component of connectives tissues and the extracellular matrix.[15] CS has been used to combat diseases related to ligaments, such as osteoarthritis.[16,17] Moreover, CS is a water-soluble biopolymer with an anionic nature that complexes easily with CHI. Hydrogels formed from a polyion complexation of CHI/CS have an interesting pH-responsive behavior. Several drug-delivery systems based on CHI/CS polyion complexes have been investigated. Sui et al.[18] and Ganza-Gonzáles et al.[19] discussed the formation and application of CHI/CS-based microcapsules for controlled heparin and metoclopramide release. One worthy aspect of the CHI/CS polyion complex is that even when the system type (microsphere, microcapsule, scaffold, membrane, etc.) is changed, the pH dependency for drug release is retained.

Theophylline (TH), a bronchus–dilator drug, is widely used to treat asthma and presents anti-inflammatory effects.[20,21] Its use in the treatment of asthma is restricted because of its short half-life, which is approximately 6 h.[22] For this reason, three to four daily doses have to be administrated to prevent large

variations of concentration in the blood plasma.[23] The oscillation of TH concentration in blood plasma is undesirable because it may cause adverse effects in the gastrointestinal and cardiac systems.[24] One way to overcome this limitation is to encapsulate TH within a controlled-release device. Miyazaki et al.[25] reported the successful encapsulation and the controlled TH release from dextran derivatives and cellulose acetate butyrate based microspheres. In this study, we investigated the viability of a hydrogel formed by a polyion complex of CHI and CS to act as a suitable device for controlled TH release.

EXPERIMENTAL

Materials

CHI was purchased from Golden-Shell Biochemical [Yuhuan, China, 85% deacetyled, and viscometric molar mass (M_V) of 87 $\times$ 10^3 g/mol, calculated according to methodology described by Mao et al.[26]]. Briefly, the intrinsic viscosity of the CHI solutions (acetic acid = 2 wt %/0.2 mol/L sodium acetate) with different concentrations was measured with an Ubbelohde-type capillary viscometer (model Cannon 100/E534, Cole-Palmer, Chicago, USA) at room temperature. The Mark–Houwink constants used for CHI with a deacetylation degree of 85% were $K = 1.38 \times 10^{-5}$ and $a = 0.85$. CS, kindly supplied by Solabia (Maringá, Brazil), presented an M_V equal to 22 $\times$ 10^3 g/mol. M_V was determined according to the method proposed by Wasteson.[27] The viscometry of different solutions composed of 1, 2, 3, 4, and 5 wt % CS and 0.2 mol/L sodium chloride was also measured. For these experimental conditions, the value of the constant K was 5.0 $\times$ 10^{-5} and that of a was 1.14. Crystalline TH, with a minimum purity of 99%, was purchased from Sigma-Aldrich (St. Louis, USA). All of the reactants were analytical grade and were used without further purification.

Hydrogel Formation

CHI solution (concentration = 1.75 wt %) was obtained by a solubilization of 3.5 g of CHI in 200 mL of aqueous acid solution (0.57 mol/L of hydrochloric acid) at 50°C. CS solution (concentration = 25.0 wt %) was obtained by the solubilization of 12.5 g of CS in 50 mL of distilled water. TH (1 g) was added to the CS solution. The forming solutions (CHI and CS/TH solutions) were mixed under vigorous stirring at room temperature. The resulting suspension was stored for 24 h. The hydrogel formed was collected and purified in distilled water overnight. The pH of the media was adjusted to 7 with the addition of 0.2 mol/L sodium hydroxide. After this step, the CHI/CS/TH hydrogel was cut into small pieces with a cubic format. These cubes were dried at room temperature for 24 h.

FTIR Analysis

From the dried hydrogel, we prepared a KBr pellet, which was characterized by infrared spectroscopy with a transform infrared spectroscope (Shimadzu Scientific Instruments, model 8300, Kyoto, Japan), operating in the region from 4000 to 500 cm^{-1} and a resolution of 4 cm^{-1}. Also characterized by the FTIR technique were the pure polysaccharides (CHI and CS) and TH.

In Vitro CS- and TH-Release Studies

The CHI/CS/TH hydrogels samples were previously weighed (ca. 1 g) and then deposited in cylindrical containers to swell in 25-mL buffer solutions (pH 2 and 8) at 37°C. Aliquots were collected from the stock solutions at different time intervals to quantify the fraction of CS and TH released. The quantification was performed in a Surveyor Plus chromatographic workstation (Thermo Fisher Scientific, Waltham, USA). The spectrophotometer detector was based on a model Surveyor Plus PDA detector with a diode array and operated by ChromChest software and a PolySep-GFC-P 6000 chromatographic column (300 $\times$ 7.8 mm, Phenomenex). Buffer solutions (pH 2 and 8) with a concentration of 50 mmol/L and a constant ionic strength (0.1 mol/L) were used as a mobile phase (flowing at 0.5 mL/min). The eluted solution was monitored at $\lambda = 210$ nm. Also, the standard curves of the absorbance peak area the against the CS and TH concentrations (wt %) at pH 2 and 8 ($R^2 \approx 0.999$) were plotted. For this, solutions of known concentrations of CS (0.2, 0.4, and 0.8 wt %) and TH (0.05, 0.1, and 0.2 wt %) were prepared. In addition, the hydrogel composition was estimated by HPLC analysis of the residual solution collected after hydrogel precipitation.

Mechanical Properties

The compression elastic modulus (E) was determined for the swollen hydrogel samples. The samples were previously swollen for 50, 100, 200, and 450 h in media with different pHs (2–10). The compression tests were performed in a Texturometer (Stable Micro System, model TA.TXT2, Godalming, UK). The device was equipped with a cell loading of 5 N and a bodyproof with a section area of 126 mm^2. The experimental parameters were adjusted at a deformation equal to 10% and at a test speed of 1 mm/s. The compression tests were performed at room temperature and quickly to prevent the loss of the absorbed liquid. The data generated by the equipment were force per displacement, which were subsequently converted to stress and deformation. The stress was obtained from the compression force needed to compress the gel with the follow eq. (1):[28]

$$\sigma = \frac{F_{max}}{A} = E(\lambda - \lambda^{-2}) \quad (1)$$

where σ is the compression stress, F_{max} is the force exerted to deform 10% of the samples, A is the sectional area of the bodyproof that compresses hydrogel, and λ is the relative deformation. We used eq. (1) to determine E for each sample. The value of E was determined from the slope of the line obtained according to eq. (1): σ versus $\lambda - \lambda^{-2}$, where $\lambda - \lambda^{-2}$ is the strain calculated from the eq. (2):

$$\lambda = \frac{\Delta L}{L_0} \quad (2)$$

where ΔL is the deformation of the sample and L_0 is the initial sample length.

Solid-State ^{13}C-NMR and Wide-Angle X-Ray Scattering (WAXS) Analysis

The samples previously swollen for 450 h (after the determination of E) were crushed into smaller pieces and then lyophilized (Christ Gefriertrocknungsanlagen, Harz, Germany) at −55°C

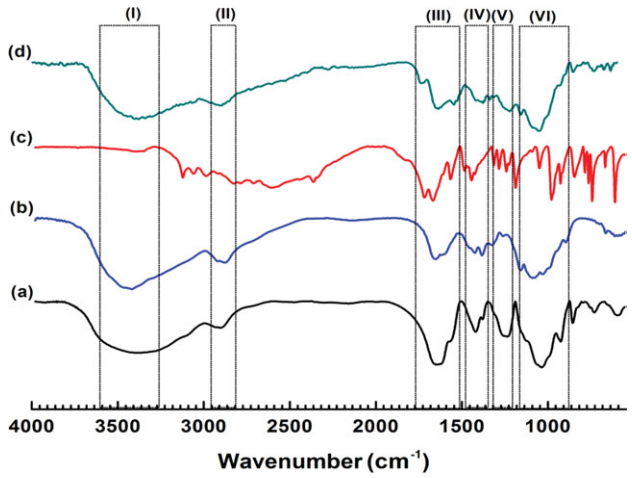

Figure 1. FTIR spectra of the (a) pure CS, (b) pure CHI, (c) pure TH, and (d) CHI/CS/TH hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for 24 h. The lyophilized samples were characterized by WAXS and solid-state ^{13}C-NMR techniques. The WAXS profiles were recorded on a diffractometer (Shimadzu model XRD-600, Kyoto, Japan) equipped with Ni-filtered Cu Kα radiation. The WAXS profiles were collected in a scattering range of $2\theta = 5$–70° with a resolution of 0.02° at a scanning speed of 2°/min. Solid-state ^{13}C-NMR spectra were obtained in a Varian spectrometer (Oxford model 300, Palo Alto, USA) at a frequency of 75.5 MHz. The relaxation delay and the angle pulse used were 3 s and 45°, respectively. A line broadening of 3 Hz was used.

RESULTS AND DISCUSSION

FTIR Analysis

Hydrogel formation by the complexation of oppositely charged polyions was supported mainly by intense electrostatic interactions among charged groups.[29] For polyion complexes based on CHI and CS, electrostatic interactions were formed among the protonated amino groups ($-NH_3^+$) from CHI with the sulfate ($-OSO_3^-$) and carboxylate ($-COO^-$) groups from CS. The protonation of amino groups from CHI occurred in acid medium ($-NH_2 + H^+ \rightarrow -NH_3^+$), whereas the appearance of sulfate and carboxylic ions groups on the CS backbone was observed in media where the pH was close to the pK_a of these groups ($pK_{aOSO3H} \approx 2.6$ and $pK_{aCOOH} \approx 4.57$).[30]The CHI/CS/TH hydrogel was characterized by the FTIR spectroscopy technique, the spectra obtained from the hydrogel, and the pure polyelectrolytes are show in Figure 1.

The FTIR spectra in region I (3300–3560 cm^{-1}) showed the vibrational stretching of the hydroxyl group and the symmetric and asymmetric NH stretching on CS [Figure 1(a)] and CHI [Figure 1(b)].[31,32] Close to this range, in the TH [Figure 1(c)] spectrum, a band assigned to the NH vibrational stretching was observed at 3130 cm^{-1}. Region II showed the bands relative to C—H bond stretching. Region III (1750–1500 cm^{-1}) showed a band assigned to the amide band in the CS spectrum, the band assigned to the C=O stretching from amide group, the NH_2 deformation in the CHI spectrum,[31,32] and the bands of asymmetric and symmetric C=O bond stretching and C=N bond stretching in the TH spectrum.[33] Region IV (1350–1500 cm^{-1}) showed a band assigned to the C—O vibrational stretching, the coupling of the OH angular vibration, the C—O stretching of the primary alcoholic group in the CS spectra, and a band referent to CH_3 deformation in the CHI spectra. Region V (1200–1300 cm^{-1}) showed the characteristic band of CS and the S=O vibrational stretching of the sulfate group.[34] Finally, region VI (900–1200 cm^{-1}) showed bands assigned to the C—O vibration stretching on the CS spectrum, the vibration stretching of C—N on the CHI spectra,[34,35] and a band referent to N–CH_3 bonding on the TH spectra.

The hydrogel spectra [Figure 1(d)] exhibited great part of the bands characteristics of CHI and CS. Figure 1(d) shows the displaced bands assigned to S=O and C=O bonds and NH_2 deformation due to the electrostatic interaction among the functional groups from CHI and CS. Also, in the region III, the hydrogel spectrum showed the bands assigned to the asymmetric and symmetric C=O bond stretching and C=N stretching due to the presence of TH. Thus, the hydrogel FTIR analysis allowed us to infer that the material obtained was formed by the polyionic complexation between CHI and CS and that the drug was successfully loaded into the hydrogel network.

CS and TH Release Studies

After hydrogel formation, its composition was estimated by HPLC analysis of the residual materials. Each gram of hydrogel was composed by 84 wt % CHI, 15 wt % CS, and 1 wt % TH, respectively. Thus, each gram of the CHT/CS/TH hydrogel has 10 mg of TH encapsulated. With the final dry weight of the CHI/CS/TH hydrogel (c.a. 13.31 g), the initial amount of TH (1 g), and the hydrogel composition, the TH encapsulation efficiency was estimated to be 13.3%. For future pharmaceutical applications, this low encapsulation efficiency could be overcome through changes in the loading methodology. In this study, *in situ* loading was applied; however, in the future, the drug loading could be performed after hydrogel formation.

In vitro TH-release studies with the loaded hydrogel were performed to determinate the cumulative fraction of TH released. The fraction of TH released was determined for the CHI/CS/TH hydrogel samples immersed at pH 2 and 8 by the HPLC technique. Some researchers have discussed CS release from CHI/CS polyionic complexes as a function of the pH of the medium.[36–38] In fact, those studies have demonstrated that the CHI/CS hydrogels showed a maximum liquid uptake capacity when they were immersed in a medium with pHs between 6 and 8. After it reached a maximum value, the liquid uptake capacity of the CHI/CS hydrogel decreased because of the release of part of the CS chains. This phenomenon of releasing occurred when the CHI/CS hydrogel was swollen in media with a high pH value.[36–38] For this reason, in this study, the amount of CS released from the hydrogel matrix was also determined.

Figure 2 shows the fractions of CS [Figure 2(a)] and TH released [Figure 2(b)] from the CHI/CS/TH hydrogels immersed at pH 2 and 8. According to the data presented in Figure 2(a), it was implicit that the CS release was directly affected by the pH of the medium. Sui et al.[18] and Ganza-Gonzales et al.[19] also

Applied Polymer SCIENCE

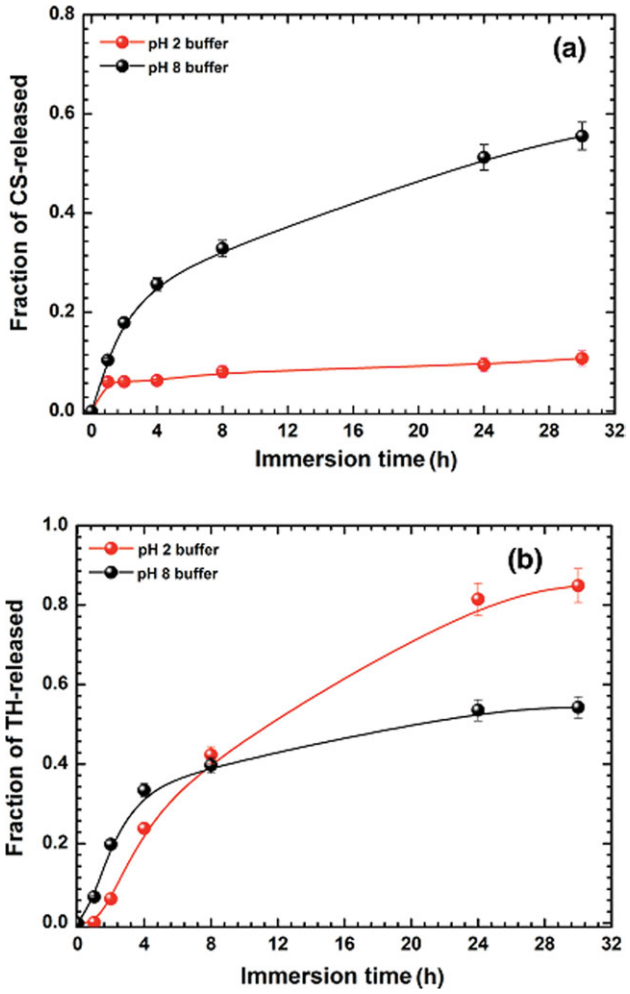

Figure 2. Time-dependent cumulative fractions of (a) CS and (b) TH released from the hydrogel samples swelled at pH 2 and 8. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed that the release of a solute from CHI/CS microspheres were directly affected by the pH of the medium. At pH 2, the maximum fraction (Fr_{max}) of CS released was reached quickly after 1 h. The Fr_{max} of CS released was close to 0.13. After this, the CS release became slow and reached equilibrium. The fraction of CS released did not change up to the end of the study. On the other hand, at pH 8, even after 30 h of immersion, the fraction of CS released did not reach equilibrium. Moreover, the Fr_{max} of CS evaluated in this study was 0.58, four times higher than the Fr_{max} evaluated at pH 2.

This great difference between the fractions of CS released when the hydrogel samples were immersed under these pH conditions could be explained by the following: when the polyionic complexes of CHI and CS are formed, usually in acidic media, the amino groups from CHI are protonated ($—NH_3^+$). The sulfate and carboxylic groups from CS, which have low pK_a values, are deprotonated ($—OSO_3^-$ and $—COO^-$). Thus, the polycationic groups from CHI and the polyanionic groups from CS interact strongly through electrostatic interactions. The electrostatic interactions are responsible for the formation of complexes that form the hydrogel polymer network. Also, this strong interaction among the CS and CHI chains promotes the formation of a highly entangled and stable network. When the hydrogel is swollen in a medium with low pH, the stability of the polymer network is maintained because of the preservation of strong electrostatic interactions. Thus, the CS chains remain entrapped within the hydrogel network and are not released into the external environment. Under high-pH conditions the hydrogel network is destabilized because the $—NH_3^+$ groups from CHI react with the OH^- anions; this prevents the stabilization of the anionic groups from CS. The nonstabilization of these groups increases the negative charge density within the hydrogel network and promotes the anion–anion repulsion among the CS chains. Thus, CS chains that were previously attached to the CHI chains get mobility. In addition, the amount of liquid that diffuses through the hydrogel network increases dramatically because of macromolecular expansion. This allows a great number of CS chains to be conducted outward in the hydrogel network, as was observed for the samples immersed at pH 8.

Figure 2(b) shows the TH-release curves at pH 2 and 8. Again, in this case, the pH of the medium directly affected the release profile. Comparing the release curves for both media tested, we observed that at pH 2, the TH-release rate was lower than that at pH 8 after the first hour of immersion. At pH 2, we observed that after 8 h of immersion, the fraction of TH released was no higher than 0.4, and even after 30 h, the release did not achieve equilibrium. At pH 2, the hydrogel network remained stable and prevented CS release; this promoted a higher release of encapsulated TH because of the physicochemical affinity between the drug and the solvent under acid conditions. For this reason, we observed that the fraction of TH released at pH 2 was greater than the fraction of CS released when the hydrogel was immersed at pH 8.

The trend of the curve for the hydrogel samples immersed at pH 8 shifted to a horizontal straight-line behavior, which characterized the equilibrium of TH release. For these samples, the Fr_{max} of TH released was equal to 0.56, which was the lowest value compared to those of the samples immersed at pH 2 for 30 h. At pH 8, the hydrogel network was destabilized; this caused the hydrolytic cleavage of CS.[37,39] Because of the increase in liquid that diffused through the hydrogel network, small fragments of the CS chains were easily transported to the swelling media and prevented TH release. Therefore, there was a decrease in the fraction of TH released at pH 8 [see Figure 2(b)]. Thus, the CHI/CS/TH hydrogel could be applied efficiently as a device for the controlled release of TH under acidic conditions (e.g., in the stomach region). Figure 3 illustrates the CS- and TH-release behavior under different pH conditions.

The results show that the CHI/CS/TH hydrogel had some interesting advantages over previously reported systems for controlled drug release (e.g., TH). Sui et al.[18] and Ganza-Gonzales et al.[19] described systems based on CHI/CS microcapsules for the controlled release of TH and metoclopramine. In both studies, the authors used crosslinking agents to form

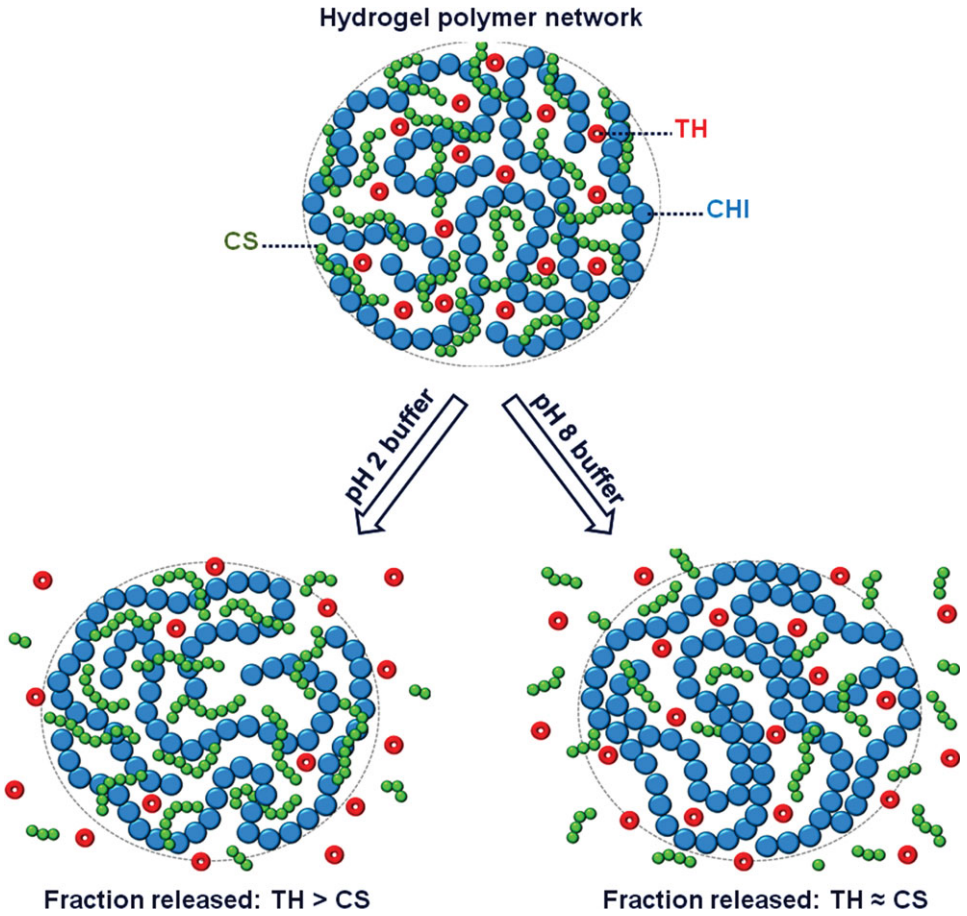

Figure 3. Schematic illustration of CS and TH release from the hydrogel network under different pH conditions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

their system; we avoided this in our work to reduce the level of toxicity in the system. In addition, their release studies showed a fast drug release during the first hours (ca. 70–100% of the drug encapsulated), which indicated that a great amount of drug was encapsulated on the surface of the microspheres, and the drug release occurred no later than 8 h. During the first hours, our system released about 40% of the TH encapsulated and was able to release TH in a controlled way for up to 30 h; this increased its efficiency to act as a device for a controlled-release system. Miyazaki et al.[25] observed that for a system based on dextran derivatives and cellulose acetate microspheres, the controlled release of TH occurred for no longer than 8 h and was not affected by the pH of the media, which decreased the specificity of the device. Hydrogels that respond to diverse stimuli (e.g., pH, temperature, light, pressure, and eletric field) have the ability to respond to minute changes in ambient stimuli and to exhibit dramatic property changes. These changes could modulate the drug-release rate to improve the drug efficiency in specific parts of the body (e.g., gastric or colon regions).

In this study, the release of CS and TH was treated as a partition phenomenon with a mathematical model published in our laboratory.[40] The cited model allowed us to predict the whole profile of solute release from the hydrogel networks. The model was applied to the data presented in Figure 2(a, b). According to that model, the parameter that determined the partition activity (α) could calculated from eq. (3):

$$\alpha = \frac{Fr_{\max}}{1 - Fr_{\max}} \tag{3}$$

where α expresses the physicochemical affinity of the drug with hydrogel and with solvent phases. The chemical nature of the solvent that is in contact with the hydrogel can affect both the release rate and the value of α as well.[38] With $\alpha > 0$, we observed the diffusion of solute in the solvent phase. From the values of $Fr_{\max}$ extracted from the curves shown in Figure 2(a, b), the respective values of α were obtained and are presented in Table I. The values of $Fr_{\max}$ and α were treated by the use of eq. (3) (details for obtaining this equation can be found in ref. 39). We obtained the rate constant for CS and TH release (k_R) for each pH by plotting the term in the left of eq. (4) as function of time (t):

$$\frac{\alpha}{2} \times \ln\left(\frac{F_L - 2F_L Fr_{\max} + Fr_{\max}}{Fr_{\max} - F_L}\right) = k_R t \tag{4}$$

where, F_L is the fraction of solute released at time t.

The values of α and k_R are presented in Table I. These values could be correlated with the pH of the swelling medium and

Applied Polymer SCIENCE

Table I. Values of α and k_R Calculated from the Fr_{max} of CS and TH

Solute released	pH	Fr_{max}	α	k_R (10^{-2} h^{-1})
CS	2	0.13	0.120	0.390
CS	8	0.58	1.248	6.011
TH	2	0.87	5.619	26.45
TH	8	0.56	1.186	10.37

the affinity of solute for the medium. This meant that when in contact with pH 8 buffer, the CHI/CS/TH hydrogel had higher values of α compared to those obtained at pH 2 for CS release. On the other hand, for TH release, when in contact with the pH 2 buffer, the CHI/CS/TH hydrogel had higher values of α compared to those obtained at pH 8. In accordance with the predetermined conditions, higher values of α implied that the solute had more affinity for the solvent than the device in which it was inserted. Therefore, the values of α and k_R may quantitatively explain the high affinity of CS for the swelling medium at pH 8 and the high affinity of TH for the swelling medium at pH 2.

Mechanical Properties

In this study, the forces from the swollen hydrogel samples were used to evaluate the force required to deform 10% of the sample (Figure 4) and E (Figure 5). These two parameters were evaluated for the swollen hydrogel with the variation of two factors: the pH of the swelling media and the immersion time.

According to the data presented in Figure 4, we observed that the samples that swelled at extremes of pH showed higher rigidity; this increased the force necessary to deform the swollen samples. As discussed previously, samples swollen at pH 2 preserve their polymeric networks in an entangled and highly stable state. This prevented the chain removal and the consequent network expansion and prevented a great amount of liquid from diffusing inward in the hydrogel network. Thus, to deform the hydrogel, it was necessary to apply a higher force. Furthermore,

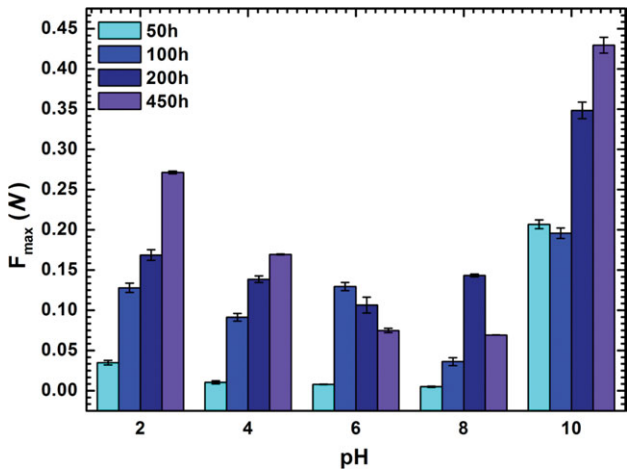

Figure 4. Maximum force necessary to deform 10% of the swollen hydrogel samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

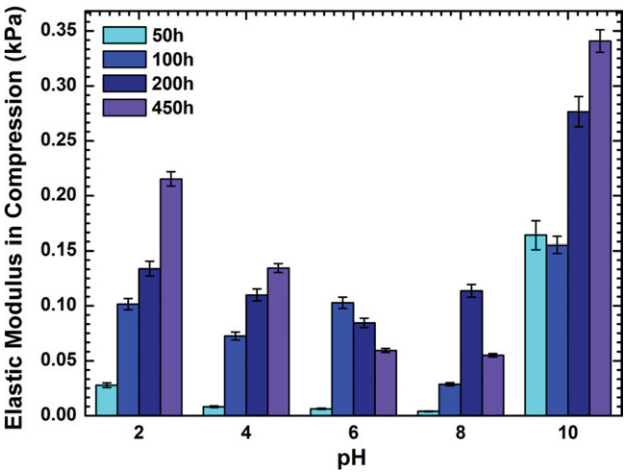

Figure 5. E values calculated for the swollen hydrogels samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for long immersion times, the hydrogel network got rigid, and this increased the maximum force evaluated. For the samples swollen in a pH range of 4–8, we evaluated a decrease in the maximum force values. In this pH range, the strong electrostatic interactions among the protonated amino groups from CHI and the polyanions from CS no longer existed. Thus, the imbalance between positive and negative charges destabilized the hydrogel network and allowed a great amount to be absorbed. Water acted as a plasticizer, which lowered the tensile strength; the higher the degree of swelling was, the lower the tensile strength of the wet hydrogel was.[39] On the other hand, long immersion times contributed to the decrease in the maximum force necessary to deform the swollen hydrogel samples. At pH 10, the maximum force values increased again. Fajardo et al.[37] suggested that when CHI/CS polyionic complexes are immersed in a medium with pH $\geq$ 10, self-rearrangement in the polymer network can occur. This rearrangement occurs because of the large amount of OH^- anions that neutralizes the $-NH_3^+$ groups from CHI. The nonreleased CS chains had mobility and moved close to the CHI chains. This approach allows the formation of hydrogen bonds among the $-NH_2$ groups on CHI and $-OSO_3^-$ and $-COO^-$ groups on nonreleased CS. Also, hydrogen bonds among the CHI chains are formed. Thus, the polymer network regained stability and ceased to release CS. The chain approach and the formation of hydrogen bonds promote the formation of ordered regions within the polymer network. These ordered regions act as support against mechanical compression of the samples, so we observed an increase in the maximum force values for the samples swollen at pH 10.

From the maximum force data, E for each swollen hydrogel sample was calculated. The results are shown in Figure 5.

The samples swollen at pH 2 and 10 exhibited the highest E values. These results corroborated the maximum force results (Figure 4) because E was directly proportional to them, and the reasons given earlier for this behavior applied in this case. The results allowed us to infer that the materials formed in this study could be tailored before use to improve their E parameters and

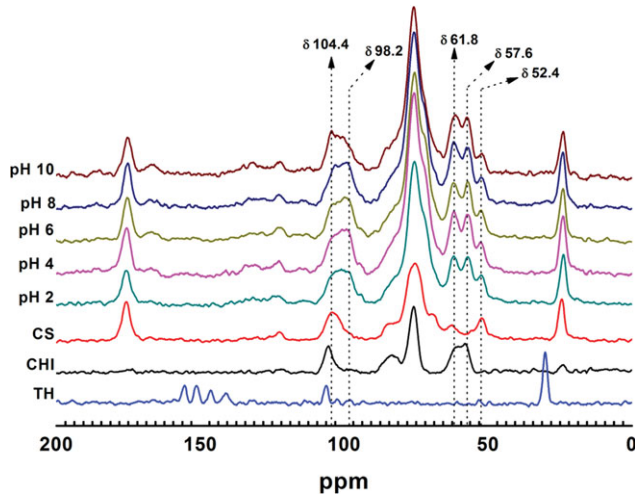

Figure 6. Solid-state ^{13}C-NMR spectra of the pure TH, CHI, and CS and hydrogel samples (75.5 MHz) previously swollen under different pH conditions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

could then be applied under conditions where better mechanical properties are required. Another interesting note is that the best values of *E* were obtained for samples swollen at pH 10.

Solid-State ^{13}C-NMR and WAXS Analysis

The solid-state ^{13}C-NMR spectra of the samples previously swollen under different pH conditions for 450 h and the spectra of the pure components, CHI, CS, and TH, are shown in Figure 6.

Comparing the solid-state ^{13}C-NMR spectra of the hydrogel samples swollen in the pH range 2–10, we observed no significant changes. However, we observed in the spectra of the samples that the resonance signal at $\delta = 52.4$ ppm, referent to a carbon of the glycopyranose ring bonding to the amide group (C—N) on the CS chain, decreased in intensity when the samples were swollen in pH conditions. The increase in pH also caused an increase in the intensity of resonance signals at $\delta =$ 57.6 and 61.8 ppm. These signals indicated the carbon of the glycopyranose ring bonded to the amino group (C—N) and the carbon from the acetyl group bonded to amine, respectively, on the CHI chains. These variations in the signals intensity could be interpreted as a change in the hydrogel networks due to the CS release, especially for the samples swollen at high pH. In addition, we observed in the spectrum of the sample swollen at pH 10 that the signal in the region between δ values of 95 and 105 ppm shifted to near $\delta = 104.4$ ppm. This signal was assigned to the C—O bonding that formed the glycopyranose ring on CHI. Again, this was consistent with the fact that CS release caused a change in the initial hydrogel stoichiometry. The polymer network that formed the hydrogel after their swelling under basic conditions became richer in CHI. Therefore, the resonance signals relating to CHI became more evident.

The characterization of the changes promoted in the hydrogel structure due to the swelling process in buffer solutions with different pH values for 450 h was performed with the WAXS technique. The WAXS profiles of the swollen hydrogel samples are shown in Figure 7.

Despite the fact that no WAXS analysis was performed for pure CHI and CS, it is known that only CHI has a crystalline structure.[12] This crystalline structure is formed by hydrogen bonds among the CHI chains.[12] After the complexation reaction with CS, such hydrogen bonds were broken and replaced by electrostatic interactions among the CHI chains and the CS chains. Thus, the WAXS profiles of the formed material must not have had any diffraction signal referent to the hydrogel crystallinity. According to the WAXS profiles of each sample, only the hydrogel sample previously swollen at pH 10 showed clear diffraction peaks at $2\theta = 43.9$ and 64.4°. Tests were also carried out at shorter immersion times, and no diffraction peaks were observed. Other researchers have discussed that the complexes formed by CHI and CS when swollen in media at pH $\geq$ 6 undergo a self-rearrangement process in the polyionic chains, and crystalline regions are formed in their polymer networks.[37,38] However, in this case, this rearrangement phenomenon was only observed for the sample swollen at pH 10. This different behavior could be explained by the presence of the drug, TH, entrapped within the hydrogel network. The TH prevented the CS nonreleased chains from moving close to the CHI chains. This restrained hydrogen-bond formation among the —NH_2 groups from CHI and the polyanions on CS. The hydrogen bonds were responsible for the formation of crystalline regions within the hydrogel polymer network. However, when the samples were swollen at high pH, a larger fraction of CS was released, and a smaller fraction of TH was also released (see Figure 2). This reduced the chain density within the polymer network and allowed the displacement of the nonreleased CS chains. This displacement, coupled with the approach of nonreleased CS chains with CHI chains and the rearrangement of these chains, facilitated the formation of hydrogen bonds and the consequent formation of crystalline regions, as observed in the WAXS profile analysis, and we found that the presence of a solute incorporated into the hydrogel matrix affected the self-rearrangement.

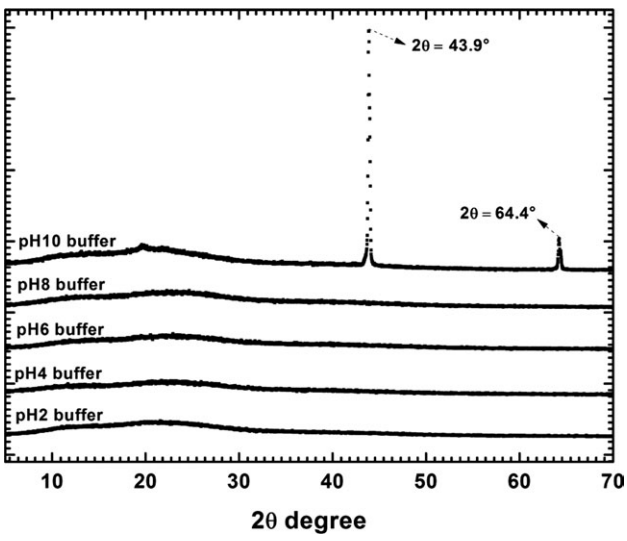

Figure 7. WAXS profiles of the hydrogel samples previously swollen under different pH conditions.

CONCLUSIONS

In this study, a TH-loaded CHI/CS hydrogel was formed to act as a potential device for controlled drug delivery. The hydrogel formation and TH loading were characterized by FTIR spectroscopy. *In vitro* CS- and TH-release studies were performed at pH 2 and 8. According to the obtained data, it was clear that both CS and TH release had a strong dependence on pH. When the hydrogel samples were swollen under acid conditions, a high fraction of TH was released ($\approx$ 0.87), whereas the fraction of CS released was equal to 0.13. At pH 8, the fractions of TH and CS released from the hydrogel samples were very close, about 0.57. In addition, the variation of the pH of the swelling media also affected the mechanical and structural properties of the CHI/CS/TH hydrogels. When the hydrogel samples were swollen at extreme pHs (highly acidic or basic conditions), we observed an increase in the force required to deform the samples and, therefore, in the *E* values calculated for the swollen hydrogel samples. The variation of the pH of the swelling medium favored the reorganization of the CHI chains and the unreleased CS chains. This reorganization also promoted the formation of ordered regions within the polymer network as observed for the hydrogel sample swollen at pH 10 and as observed by WAXS. All of these results will contribute to the development of a new device for controlled TH release and will thus ensure the efficacy of this drug, reduce its undesirable collateral effects, and contribute to the reduction of the number of daily doses that need to be administered.

REFERENCES

1. Elzatahry, A. A.; Eldin, M. S. M.; Soliman, E. A.; Hassan, E. *J. Appl. Polym. Sci.* **2001,** *111*, 2452.
2. Renard, D.; Robert, P.; Lavenant, L.; Melcion, D.; Popineau, Y.; Guéguen, J.; Duclairoir, C.; Nakache, E.; Sanchez, C.; Schmitt, C. *Int. J. Pharm.* **2002,** *242*, 163.
3. Cascone, M. G.; Maltinti, S. *J. Mater. Sci. Mater. Med.* **1999,** *10*, 301.
4. Hennink, W. E.; van Nostrum, C. F. *Adv. Drug Delivery Rev.* **2002,** *54*, 13.
5. Berger, J.; Reist, M.; Maye, J. M.; Felt, O.; Peppas, N. A.; Gurny, R. *Eur. J. Pharm. Biopharm.* **2004,** *57*, 19.
6. Oviedo, I. R.; Mendez, N. A. N.; Gomez, M. P. G.; Rodriguez, H. C.; Martinez, A. R. *Int. J. Polym. Mater.* **2008,** *57*, 1095.
7. Chen, W. B.; Wang, L. F.; Chen, J. S.; Fan, S. Y. *J. Biomed. Mater. Res. A.* **2005,** *75*, 128.
8. Nakajima, A.; Shinoda, K. *J. Colloid Interface Sci.* **1976,** *55*, 126.
9. Aranaz, I.; Mengíbar, M.; Harris, R.; Paños, I.; Miralle, B.; Acosta, N.; Galed, G., Heras, Á. *Curr. Chem. Biol.* **2009,** *3*, 203.
10. Enescu, D.; Olteanu, C. E. *Chem. Eng. Commun.* **2008,** *195*, 1269.
11. Chourasia, M. K.; Jain, S. K.: *J. Delivery Target Ther. Ag.* **2004,** *11*, 129.
12. Pillai, C. K. S.; Paul, W.; Sharma, C. P. *Prog. Polym. Sci.* **2009,** *34*, 641.
13. Denuziere, A.; Ferrier, D.; Damour, O.; Domard, A. *Biomaterials* **1998,** *19*, 1275.
14. Amrutkar, J. R.; Gattani, S. G. *AAPS PharmSciTech.* **2009,** *10*, 670.
15. Galtrey, C. M.; Fawcett, J. W. *Brain Res. Rev.* **2007,** *54*, 1.
16. Iovu, M.; Dumais, G.; Souich, P. *Osteoarthr. Cartilage* **2008,** *16*, 14.
17. Bruyere, O.; Reginster, J. Y. *Drug. Ag.* **2007,** *24*, 573.
18. Sui, W.; Huang, L.; Wang, J.; Bo, Q. *Colloids Surf. B.* **2008,** *65*, 69.
19. Ganza-Gonzáles, A.; Anguiano-Igea, S.; Otero-Espinar, F. J.; Blanco Méndez, J. *Eur. J. Pharm. Biopharm.* **1999,** *48*, 149.
20. Al-Kahtani, A. A.; Sherigara, B. S. *J. Mater. Sci. Mater. Med.* **2009,** *20*, 1437.
21. Finnerty, J. P.; Lee, C.; Wilson, S.; Madden, J.; Djukanovic, R.; Holgate, S. T. *Eur. Resp. J.* **1999,** *9*, 1672.
22. Zhang, W. F.; Chen, X. G.; Li, P. W.; He, Q. Z.; Zhou, H. Y. *J. Mater. Sci. Mater. Med.* **2008,** *19*, 305.
23. Mellstrand, T.; Svedmyr, N.; Fagerstorm, P. O. *Eur. J. Respir. Dis.* **1980,** *61*, 54.
24. Mi, F. L.; Her, N. L.; Kuan, C. Y.; Wong, T. B. I.; Shyu, S. S. *J. Appl. Polym. Sci.* **1997,** *66*, 2495.
25. Miyazaki, Y.; Yakou, S.; Nagai, T.; Takayama, K. *Drug Dev. Ind. Pharm.* **2003,** *29*, 795.
26. Mao, S.; Shuai, X.; Unger, F.; Simon, M.; Bi, D.; Kissel, T. *Int. J. Pharm.* **2004,** *28*, 45.
27. Wasteson, A. *Biochem. J.* **1971,** *122*, 477.
28. Guilherme, M. R.; Fajardo, A. R.; Moia, T. A.; Kunita, M. H.; Gonçalves, M. C.; Rubira, A. F.; Tambourgi, E. B. *Eur. Polym. J.* **2010,** *46*, 1465.
29. Thunemann, A. F.; Muller, M.; Dautzenberg, H.; Joanny, J. F. O.; Lowne, H. In Polyelectrolytes with Defined Molecular Architecture; Springer-Verlag: Berlin, **2004; Vol.** *116*, p 113.
30. Larsson, B.; Nilsson, M.; Tjalve, H. *Biochem. Pharmacol.* **1981,** *30*, 2963.
31. Rosca, C.; Popa, M. I.; Lisa, G.; Chitanu, G. C. *Carbohydr. Polym.* **2005,** *62*, 35.
32. Da Róz, A. L.; Leite, F. L.; Pereiro, L. V.; Nascente, P. A. P.; Zucolotto, V.; Oliveira, O. N. J.; Carvalho, A. J. F. *Carbohydr. Polym.* **2010,** *80*, 65.
33. Cabassi, F.; Casu, B.; Perlin, A. S. *Carbohydr. Polym.* **1978,** *63*, 1.
34. Blout, E. R.; Fields, M. *J. Am. Chem. Soc.* **1950,** *72*, 479.
35. Krayukhina, M. A.; Samoilova, N. A.; Yamskov, I. A. *Russ. Chem. Rev.* **2007,** *77*, 799.
36. Piai, J. F.; Lopes, L. C.; Fajardo, A. R.; Rubira, A. F.; Muniz, E. C. *J. Mol. Liq.* **2010,** *156*, 28.
37. Fajardo, A. R.; Lopes, L. C.; Valente, A. J. M.; Rubira, A. F.; Muniz, E. C. *Colloid Polym. Sci.* **2011,** *289*, 1739.
38. Fajardo, A. R.; Piai, J. F.; Rubira, A. F.; Muniz, E. C. *Carbohydr. Polym.* **2010,** *80*, 934.
39. Feng, X.; Pelton, R.; Leduc, M. *Ind. Eng. Chem. Res.* **2006,** *45*, 6665.
40. Reis, A. V.; Guilherme, M. R.; Rubira, A. F.; Muniz, E. C. *J. Colloid Interface Sci.* **2007,** *310*, 128.