Effect of Sodium Hexametaphosphate Concentration on the Swelling and Controlled Drug Release Properties of Chitosan Hydrogels

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ABSTRACT: An ionically crosslinked polymer network composed of chitosan and sodium hexametaphosphate (SHMP) was synthesized to determine their swelling and ascorbic acid release kinetics at various SHMP concentrations. The chitosan/SHMP hydrogels were synthesized using an acetic acid aqueous solution (1% v/v). Ionization constants (pK_b) of the SHMP were obtained by potentiometric titration. The results show that the SHMP was hydrolyzed in acidic medium forming orthophosphate and trimetaphosphate. The swelling percentages were measured at different swelling media pH's; the higher swelling capacities were for the systems that were swollen in neutral solution. Also, it was studied the ionic crosslinking degree by turbidimetric titration, comparing the electrostatic interactions between the chitosan and the SHMP; the results shows that electrostatic interactions between the amine groups of the chitosan and the anionic groups of the SHMP are dependent of the swelling medium pH. The ascorbic acid diffusion inside the hydrogel follows the second law of Fick, and the diffusion coefficients were obtained for different SHMP concentrations.
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Key words: sodium hexametaphosphate; chitosan; hydrogels; swelling properties; drug delivery systems

INTRODUCTION

Chitosan is a copolymer of *N*-acetyl-glucosamine and *N*-glucosamine units distributed randomly or in blocks throughout the biopolymer chain. Chitosan has received a great deal of attention in the pharmaceutical field because of its promising properties.[1–3] Most of the drug delivery formulations based in chitosan (films, microspheres, nanoparticles, etc.) are prepared by chemical crosslinking with glutaraldehyde or glyoxal.[4] However, for the synthesis of completely biodegradable hydrogels the use of physical ionic crosslinking is preferred, such as electrostatic interactions.[5] Ionic crosslinking is a simple and clean process, in contrast with covalent crosslinking that requires a catalyst.[6] Yao et al.[7] reports the pectin/chitosan film preparation dissolving the polyelectrolyte complex in formic acid. Chuet et al.[8] synthesized xanthan/chitosan films using sodium chloride. The use of anions of low molecular weight for chitosan crosslinking has been reported by Shu et al.,[9] who prepared chitosan films crosslinked ionically with sodium tripolyphosphate by putting the chitosan film on a phosphate solution. Devika et al.[10] crosslinked chitosan nanoparticles ionically with sodium tripolyphosphate at low pH and by deprotonation mechanisms at higher pH. Chitosan is a polycation with chelating properties,[11] can react with groups negatively charged forming a network with ionic bonds between the cationic groups of the main polymer chain of the chitosan and the anionic groups. Formation of these links can be determined by infrared (IR) spectroscopy[11] or using turbidimetric titration.[12] The ionic crosslinking reaction in the chitosan is influenced by the size of the crosslinking agent molecule and the global charge of the chitosan and the crosslinking agent. Small-sized crosslinking agent molecules induce faster reactions because diffusion across the polymer chains is more simple.[13] The global charge density in the molecules depends on the p*K*'s of the molecules and the solution's pH during the reaction; the global charge of the chitosan and the anionic molecules must be high to allow the electrostatic interactions, and therefore, the forming of hydrogels.

The swelling of the chitosan hydrogels depends mainly on the protonation of the amine groups in the polymer chain, and the ionic crosslinking density in the network.[12] The ionic crosslinking density is affected by external conditions as the pH of the

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swelling media.[6,12] The drug release in these hydrogels is carried out by diffusion through its porous structure.[14–17] The physicochemical properties of the network and the drug loading method determine the drug release mechanism.[18] Loading of the drug in the hydrogel can be done either by absorption from a solution or by addition of the drug before the network formation, which results in the encapsulation of the drug once the network is formed. In the first case, the diffusion is the driving force; whereas in the second case, the delivery can be controlled by diffusion, hydrogel swelling, or interactions of drug-polymer.[18]

In this work, chitosan hydrogels crosslinked ionically using sodium hexametaphosphate (SHMP) were synthesized and the electrostatic interactions between the chitosan cationic charges and the SHMP anionic charges were studied. Hydrogel swelling as a function of the swelling media's pH and SHMP concentration were also investigated, as well as the release mechanism of ascorbic acid at different SHMP concentrations.

EXPERIMENTAL

Chitosan (99%) and SHMP from Aldrich Chemical were used as received. The chitosan deacetylation degree was 68%, the nominal molecular weight was 900,000 Da. Acetic acid (Sigma), hydrochloric acid (Sigma), sodium hydroxide (J. T. Baker), ascorbic acid (Aldrich Chemical), sodium chloride (J. T. Baker), and doubly distilled deionized water (Selectropura) were used as received.

Turbidimetric titration

The SHMP-chitosan interactions were studied by turbidimetric titration.[19] SHMP solution (0.01 g/L) and chitosan solution (0.2 g/L) were prepared and mixed; the resulting solution was adjusted to pH 1 with HCl 0.01*M*. The chitosan/hexametaphosphate solution was titrated a constant temperature of 25°C using different NaOH solutions ranging from 0.001 to 0.2*M*. The solution was stirred until a stable %*T* reading was obtained. An Orion 4-Star pH/conductivity meter was used to monitor the solution's pH. Transmittance was monitored with a Genesys 10 UV–vis spectrometer. The turbidity was reported as 100-%*T*, the transmittances readings were taken at wavelength of 420 nm. The time between transmittance readings was 2 min.

Potentiometric titration

Potentiometric titration was used to evaluate the chitosan ionization degree as a function of pH.[20] A 0.1% w/v chitosan solution was titrated under a nitrogen atmosphere at 25°C, using 0.1*M* NaOH with an Orion 4-Star pH/conductivity meter with a microburette with a 0.0001 mL sensitivity. The hexametaphosphate p*K*'s were determined from the slope in a neutralization chart for a 1% v/v hexametaphosphate aqueous solution that was adjusted to pH 1.0 using HCl 0.01*M* and titrated with NaOH 0.02*M*. Charge density as function of pH for the sodium trimetaphosphate and sodium ortophosphate was calculated using the p*K*'s and the modified Henderson–Hasselbalch equation:[21]

$$pH = pK_a + n \log \left(\alpha \Big/ (1 - \alpha) \right) \quad (1)$$

The pK_a is the dissociation constant for protonated amine groups, α is the degree of ionization. The parameter n is related to the polymer chains length, and in this case is equal to one because the results show that $pK_1 < pK_2 < pK_3$, for the orthophosphate and for the trimetaphosphate.[22]

Chitosan film formation

The chitosan films were made using the solvent evaporation technique. Chitosan was dissolved in acetic acid (1% w/v aqueous solution). The SHMP was dissolved in distilled water. Both solutions were stirred, and then the SHMP solution is added to the chitosan solution and thoroughly mixed. The final mix was poured in Petri dishes to form uniform thin films and was left at 50°C for 24 h. The formed membrane was removed from the plate after all the solvent evaporated. After washing with water for 10 min and drying at room temperature, a chitosan film about 3–5 mm thick with a surface area of 1 cm^2 was obtained. The concentration of the synthesized hydrogels was 2% w/w with 1, 3, and 5% w/w of hexametaphosphate related to chitosan.

Swelling behavior

The samples were immersed in four different swelling solutions: two acidic solutions (hydrochloric acid solutions, pH 1 and 4), a neutral medium (distilled water, pH 7), and a basic solution (sodium hydroxide solution, pH 9). The swelling solution's pH was measured with an Orion 4-Star pH/conductivity meter. As the hydrogel was swelling, the medium pH was changing; then the pH was adjusted with hydrochloric acid 0.1*M* or sodium hydroxide 0.1*M*. The swelling solutions ionic strength was adjusted to 0.01*M* with the addition of sodium chloride. Previously, weighed dry samples were placed in the swelling solution and the weight of the swollen samples was measured against time, once equilibrium was reached the excess surface water was removed

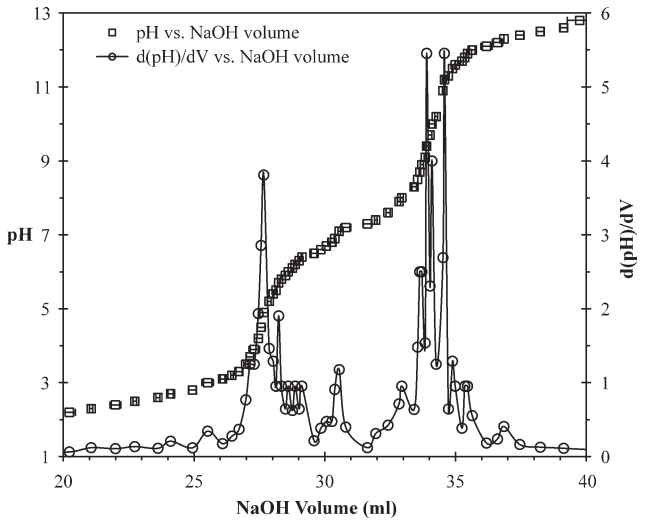

Figure 1 Potentiometric titration of sodium hexametaphosphate (SHMP) at 25°C.

using filter paper. The swelling (H) for each disk sample at any given time (t) was calculated by the following expression:

$$\%H = \left(\frac{w_t - w_0}{w_o}\right) 100 \quad (2)$$

Here w_t and w_0 represent the weights of the hydrogels at time t and of the xerogel, respectively.

Drug delivery measurements

Previously measured (weight and dimensions) xerogels were loaded with ascorbic acid by immersing them in a drug-saturated aqueous solution (333 g/L) until equilibrium was reached at room temperature. To study the drug delivery kinetics, the loaded xerogels were immersed in 200 mL of distilled water at 25°C, which was continuously stirred. To follow the delivery kinetics, solution samples were taken at different times to measure the ascorbic acid concentration in a Genesis 10 UV spectrophotomer with a wavelength of 258 nm. With the absorbance readings and the calibration curve for ascorbic acid, the released concentration (mg/L) was obtained.

RESULTS AND DISCUSSION

Chitosan is a weak polybase with amine (NH_2) and hydroxyl (OH^-) groups linked to the polymeric chain. In acidic media, the NH_2 groups are protonated to NH_3^+. The SHMP is a mixture of polymeric metaphosphates, which in acidic conditions are hydrolyzed to sodium trimetaphosphate and sodium orthophosphate.[23] Figure 1 shows the SHMP potentiometric titration with sodium hydroxide 0.1M, and can be observed that SHMP decomposes in two phosphates. From this figure, the pK's for the orthophosphate (pK_1 = 2.64, pK_2 = 7.27, and pK_3 = 11.72) and for the trimetaphosphate (pK_1 = 4.00, pK_2 = 6.10, and pK_3 = 10.00) were determined. If the pK_a for chitosan is $\sim$ 6.5,[13] then using the Henderson–Hasselbalch equation,[21] the charge density as a function of pH for the sodium trimetaphosphate and sodium orthophosphate, as well as the chitosan ionization degree as a pH function were obtained, the results are showed in Figure 2.

In Figure 2, it is observed that how the charge density in the phosphate anions and the chitosan ionization degree are controlled by the solution's pH. When the solution's pH changed from neutral to acid, the charge density of the phosphate anions decreased, whereas the chitosan ionization degree increased, which means the amines are protonated. On the other hand, increasing the solution's pH results in a decrease of the chitosan ionization degree, and therefore, the NH_3^+ groups are deprotonated, and the charge density on the phosphate anions increases. The ionic crosslinking of chitosan depends on the pH of the solution.

Figure 3 shows the changes in turbidity for the chitosan/SHMP solutions as a function of pH. It is noteworthy that at pH below 1.2, the solution is optically clear, due to the fact that chitosan is completely ionized, its NH_2 groups are fully protonated and the charge density of phosphates is very low. This suggests that the chitosan ionic crosslinking density is very low. At pH higher than 1.2, chitosan/SHMP solutions begin to show turbidity, increasing rapidly at about pH 3.1, mainly because of the phosphate groups beginning to ionize, which causes electrostatic interactions with the cationic

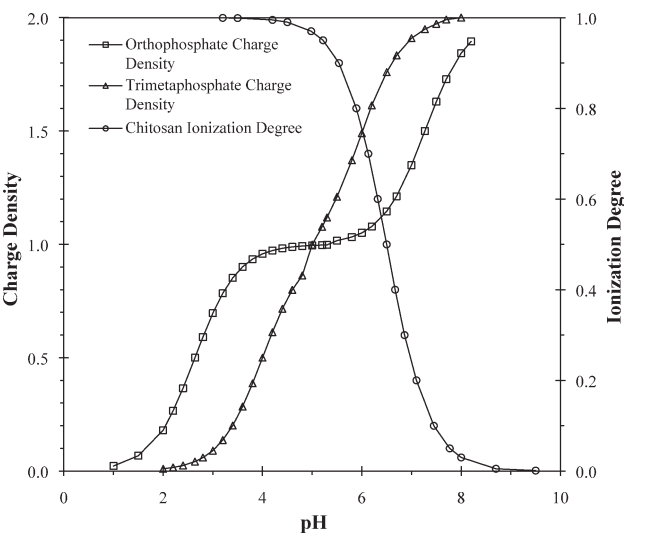

Figure 2 Charge density of the phosphate anions (orthophosphate and trimetaphosphate), as function of the pH; and chitosan ionization degree as a function of pH.

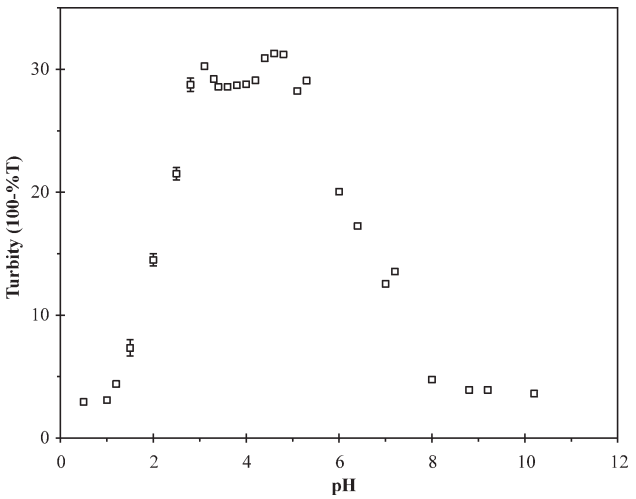

Figure 3 Turbidimetric titration of the chitosan/SHMP solutions. Transmittance (%*T*) readings taken at 420 nm (turbity is reported as 100-%*T*).

groups present in the chitosan chain, and thus, forming a chitosan/SHMP hydrogel ionically crosslinked.

At pH greater than 3.1, turbidity decreases and we can observe a peak in Figure 3. Afterwards turbidity slightly increases at a pH of about 4.8, forming another peak as can be seen in Figure 3. The reason for this is probably because the SHMP is hydrolyzed in acidic medium and forms orthophosphate and trimetaphosphate that have different p*K*'s. The first increase in turbidity is mainly generated by the interaction of the chitosan cations with the phosphate anions that are ionized in the first place. The second increase in turbidity is mainly generated by the electrostatic interaction of the chitosan cations with the phosphate anions and the anions generated by the trimetaphosphate at later stages of the process. This behavior suggests that the ionic crosslinking density in the chitosan hydrogel increases. At pH higher than 4.8, a decrease in turbidity of the solution is observed mainly due to solid precipitation and this behavior is observed until a pH of about 8 because of deprotonation of the chitosan NH_3^+ groups, thereby decreasing the chitosan ionization degree and causing chitosan to precipitate. Also a phase separation was observed, suggesting that the chitosan cationic groups are completely deprotonated and the electrostatic interactions between phosphate anions and chitosan were absent.

The swelling of the chitosan/SHMP hydrogels is mainly due to electrostatic repulsion between the NH_3^+ groups that are bonded to the chitosan polymer chain. Figure 4 shows the percentage of swelling of the chitosan/SHMP hydrogels versus the swelling solution pH at different SHMP concentrations. It can be seen that under acidic conditions, pH 1, the swelling percentage is high because of the electrostatic interactions between the cationic charges of chitosan. As the chitosan/SHMP hydrogel has very few ionic crosslinking, if the pH decreases the chitosan dissolves. When the pH of the solution is increased to 4, there is a decline in the hydrogel's swelling due to the fact that SHMP has a higher number of anionic charges and these interact with the NH_3^+ groups of the chitosan increasing the crosslinking and diminishing the ability to swell. This suggests that the hydrogel has higher ionic crosslinking density than that at pH 1.0. When increasing the pH of the solution to about 7, a significant increase in the swelling can be observed. This is probably due to the cationic groups of chitosan beginning to deprotonate causing a collapses of the hydrogel, manifested by precipitation. When increasing the pH of the solution to 9, there is a decline in the swelling of the hydrogel caused by the cationic groups of chitosan being completely deprotonated, and suggesting that the hydrogel swelling is due to the hydrogen bonds formed by hydroxyl groups attached to the chitosan's polymer chain.

Figure 4 shows that the hydrogel with 10% SHMP has a lower equilibrium swelling when compared with samples with 7 and 3% SHMP. This can be explained by the fact that if the SHMP concentration increases, then the crosslinking density also increases, which in turn causes a decrease in the swelling of the material, but improves network stability. For higher SHMP concentrations, there is a decrease in the NH_3^+ groups available in the polymer chain of chitosan, and thus, the swelling of the network is reduced.

For the study of drug delivery from the chitosan/SHMP hydrogels, ascorbic acid was selected as a convenient model drug due to its solubility in water, which allows a high loading of drug into the polymer matrix from concentrated solutions, and because its release into aqueous solutions can be followed by

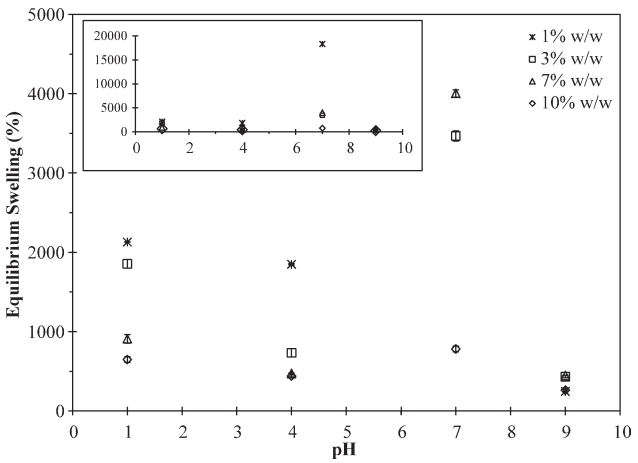

Figure 4 Equilibrium swelling (%) as function of swelling pH for chitosan/SHMP hydrogels at different SHMP concentrations.

UV spectroscopy. The swelling kinetics (fraction of solution uptake versus time) of hydrogels made with different SHMP concentrations is depicted in Figure 5. It can clearly be seen that the rate of release of ascorbic acid at low concentrations of SHMP is higher, meaning there is a decrease in the ionic crosslinking of chitosan/SHMP hydrogels. This suggests that drug release is mainly controlled by the porosity of the hydrogel.[16,17] And the drug release kinetics curves suggest Fickian diffusion-type.[24] The release data were analyzed using Fick's second law of diffusion:[25]

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2} \exp\left[\frac{-D_i(2n+1)^2\pi^2}{4l^2} t\right] \quad (3)$$

Where M_t/M_∞ is the fractional released at time t (F_t), n is an exponent, which depends on the geometry of the release system and called diffusional exponent, D_i is the apparent diffusion coefficient of ascorbic acid, and l is the original thickness of the sample. This equation can be simplified for short delivery times, that is to say for values of $0 \leq F_t \leq 002.60$, as follows:

$$F_t = \frac{M_t}{M_\infty} = 4kt^n \quad (4)$$

Where t is the release time, and k is a constant structural/geometric for a particular system, called release kinetic constant. The diffusional exponent describes the release mechanism and when $n = 0.5$, k is:

$$k = 4\left[\frac{D_i}{\pi l^2}\right]^{0.5} \quad (5)$$

The value of n, in eq. (4), was determined from slope of the release fraction (F_t) versus time log-log plot. The values of n shown in Table I indicate that the ascorbic acid release from the hydrogel follows a Fick's second law, Fickian diffusion, for all SHMP concentrations. This behavior suggests that the release process is controlled by diffusion. The values of n are used together with eq. (5) to calculate the release kinetic constant (k), which are also reported in Table I for different SHMP concentrations. The kinetic constants are lower when the SHMP concentration increases, and this fact suggests that the diffusion rate decreases when the amount of SHMP increases because of the decrease in size of the free spaces in the network. The diffusion coefficient (D_i) for ascorbic acid was determined using eq. (5), due to the fact that the value of n suggests a Fickian behavior. Table I shows the value of D_i for the different concentrations of SHMP. From Table I, we can see that the diffusion coefficients are inversely proportional to the SHMP concentration; ascorbic acid diffuses with difficulty into the hydrogels with higher concentrations of SHMP. A possible explanation is that when SHMP concentration increases, the density of ionic crosslinking in the chitosan increases, then the polymer network is more compact and less porous, and therefore, the release rate decreases. This behavior confirms that increasing the concentration of SHMP reduces the swelling in aqueous medium and the release rate.

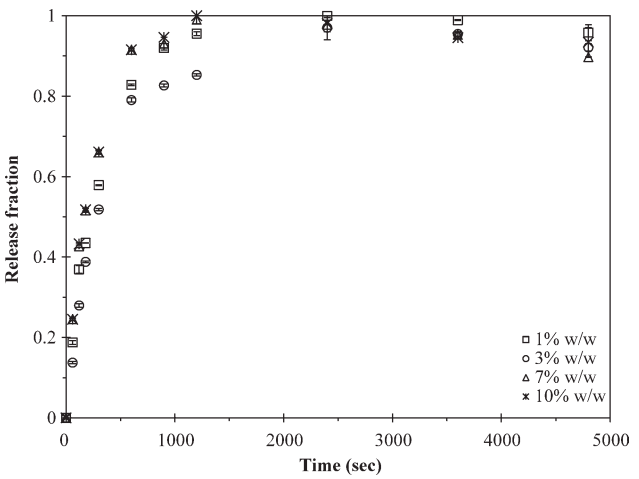

Figure 5 Ascorbic acid release fraction versus time for hydrogels with different SHMP concentrations at pH = 7 and 25°C.

TABLE I
Parameters k, n, and Diffusion coefficients (D_i) for the chitosan/SHMP Hydrogels at Different SHMP concentrations

SHMP concentration (w/w %)	k	n	D ($cm^2\ s^{-1}$)
1	0.0334	0.495	8.76×10^{-6}
3	0.0303	0.546	7.20×10^{-6}
5	0.0275	0.504	5.96×10^{-6}
7	0.0248	0.514	4.84×10^{-6}
10	0.0234	0.531	4.30×10^{-6}

CONCLUSIONS

The synthesis of a chitosan- and SHMP-based polymeric network was carried out to determine its swelling and ascorbic acid release kinetics as a function of SHMP concentration. It was found that SHMP hydrolyzes in acid medium in orthophophate and trimetaphosphate, and pK's were determined. The swelling kinetics was studied in three different swelling media: acidic, neutral, and basic. In aqueous media, a higher swelling capacity was observed. At pH lower than 1, it was shown that chitosan dissolves. At pH between 3 and 5, the hydrogel has a higher density of ionic crosslinking between the NH_3^+ groups and the anionic groups of the SHMP.

For pH higher than 6 but less than 8, the chitosan's NH_3^+ groups are deprotonated causing the chitosan to precipitate. At pH higher than 8, the groups attached to the polymer chain of chitosan form hydrogen bonds with water. The kinetics of the release of ascorbic acid in these hydrogels was also studied. The diffusional exponent (n) was obtained ant it was determined that the release follow the Fick's second law, suggesting the release mechanism of ascorbic acid is mainly controlled by diffusion. The diffusion coefficients for the hydrogels were also determined, and it was found that higher concentrations of SHMP induce a decrease in the value of the coefficients.

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