Reacetylated Chitosan/Cashew Gum Gel: Preliminary Study for Potential Utilization as Drug Release Matrix

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Received 21 February 2005; accepted 19 April 2005
DOI 10.1002/app.22099
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The aim of this work was to characterize and investigate the potential of chitosan/cashew gum (CH/CG) gel for the controlled release of pilocarpine hydrochloride. Gels were prepared by reacetylation of CH with acetic anhydride, characterized by infrared spectroscopy, thermal analysis, and X-ray diffraction. Swellings in water and in phosphate buffers were also investigated. The release of pilocarpine for CH and CH/CG gels was shown to be similar in the first 100 min, where about 60% of the pilocarpine was released. After this time, addition of CG to the gels decreases pilocarpine release rate in the medium. CH gel release was found to be dependent on pH values, a non-Fickian mechanism was observed for the release at pH 2 and 7.4, while at pH 9.8, a Fickian (diffusion) mechanism took place. On the other hand the release of pilocarpine in CH/CG matrix occurred by Fickian mechanism, independent of the pH value. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 99: 326–334, 2006

Key words: cashew gum; chitosan; pilocarpine; drug delivery systems; gels

INTRODUCTION

Polysaccharides and their derivatives represent a class of polymeric materials largely used in systems for controlled drug delivery, as well as in pharmaceutical formulations. The research of polysaccharide formulation for the drug delivery system has increased in recent years due mainly to their excellent biocompatibility and biodegradable properties.[1–4]

Chitosan (CH) is a biopolymer obtained by the deacetylation of chitin. Because of its cationic character, as well as gel- and film-forming properties, CH gels and also CH-polysaccharide systems are becoming very important in controlled delivery systems, which among other features provide desired concentration of drug at the absorption site and reduction of the dosage frequency.[5]

Chemical modification of CH results in the formation of gels, which exhibit physicochemical features suitable to drug delivery systems. The high aqueous solubility of CH restricts the utility of CH for gastric drug delivery or oral administration. Reacetylated CH microspheres showed a controlled water swelling capacity and gelified at acidic pH, resulting in prolonged release of the encapsulated antibiotics.[6]

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Journal of Applied Polymer Science, Vol. 99, 326–334 (2006)

Interaction of CH with other polysaccharides such as carboxymethyl cellulose,[7] alginate,[8–10] carrageenan,[11] hyaluronic acid,[12] pectin,[13] and acacia[14] promoted the formation of polyelectrolyte complexes through the interaction between amine group of CH and anionic groups of those polysaccharides having carboxylate or sulfate groups. Those complexes were reported to be pH- and ion-sensitive.[4,12,15] Alginate and CH systems have been widely investigated for the controlled drug delivery such as the release of tramadol hydrochloride, the drug liberation being found to be dependent on the pH of the medium. The release mechanism indicates an anomalous drug transport.[16] The same drug mechanism was also observed for the release of diltiazem.[17] Metaclopramide hydrochloride showed controlled release behavior when embedded in a CH/alginate matrix, following a first order release mechanism.[18]

Microcapsules of CH–alginate were also used to study the release of ketoprofen. The results showed that high molar mass and high CH concentration reduced the release of the drug and gave controlled release in the intestinal fluid.[19] The release of insulin by CH and sodium hyaluronate matrices demonstrated that the drug release was markedly influenced by both polymer mixing ratio and the total pellet weight.[20]

Complexes of pectin/CH and acacia/CH were used to investigate the release of chloropromazine-HCl. The

drug release was dependent on the polymer gel properties and also on the drug-polymer interaction.[14]

Cashew gum (CG) is an exudate polysaccharide from *Anacardium occidentale* trees. The composition of the Brazilian polysaccharide was investigated, whereby it was shown that CG is rich in β-D-galactose (72%) but the presences of α-D-glucose (14%), arabinose (4.6%), rhamnose (3.2%), and glucuronic acid (4.7%) were also detected.[21]

CG can be industrially produced as a by-product of Brazilian cashewnut industry and has attracted attention from academia because of its potential use in chemical and pharmaceutical industries.[22]

CH and CG physical gels have being prepared by the interaction of polysaccharides in acidic pH and reacetylation of free amine groups. Swelling of these hydrogels in water diminishes sharply when the CH/CG ratio increases. It was also found that maximal swelling was observed at pH 2 and pH 10.[22]

The aim of this work was to characterize and investigate the potential of CH/CG gels for the controlled release systems using pilocarpine hydrochloride as a model drug.

EXPERIMENTAL

Materials

CH was obtained from shrimp shells (deacetylation degree, 85% and molar mass $M_v = 1.5\times 10^5$ g/mol). CG ($M_v = 1.1\times 10^5$ g/mol) was collected as a natural exudate from native *A. occidentale* trees in Ceará-Brazil and purified as described.[21] Ground gum (5 g) was dissolved in distilled water (100 mL) and 0.5 g of NaCl was added. The pH of the solution was adjusted to 6.0. The solution was filtered and precipitated with ethanol. The gum was redissolved in water, dialyzed against distilled water and reprecipited with ethanol (yield, 70%). Pilocarpine hydrochloride ($C_{11}H_{16}N_2O_2$.HCl) (Merck) and the other reagents were of analytical grade or equivalent and were used as purchased.

Preparation of reacetylated CH and CH/CG gels

The gels were prepared as described by Paula et al.,[22] with modifications. Briefly, CH (2 g) was dissolved in 100 mL of a 1% acetic acid solution by stirring it at room temperature. Acetic anhydride (50 mL) was added and the mixture was left at room temperature overnight, until gelation was completed. The gel was then exhaustively washed with distilled water, dialyzed against water, and freeze-dried. For CH/CG gel preparations, CH was dissolved in 1% acetic acid solution and added to CG aqueous solution, the mixture being stirred until complete dissolution. The ratios of CH/CG used for gel preparations were 2 : 1 and 1 : 2 (w/w). After filtration, CH/CG gels were reacetylated, washed, dialyzed, and freeze-dried. Powder forms of gels were classified by sieving through 80 mesh sieves.

For the swelling experiments, samples (~0.1 g) were placed in 10-mL test tubes and swollen in about 5.0 mL of water or phosphate buffer solutions pH 2.0, 7.4, and 9.8 (ionic strength $I = 0.2$). After reaching equilibrium (~48 h) the gels were dried and accurately weighted. All samples were run in duplicate. The degree of swelling (Q) was calculated from the ratio of the mass of water in the gel to the mass of dried gel.[23,24]

FTIR measurement

IR spectra were obtained using a SHIMADZU FTIR-8300 spectrometer under dry air, at room temperature, using KBr pellets, which were prepared by thoroughly mixing KBr and dry gels and made into pellets.

X-ray diffraction

X-ray powder diffraction patterns of lyophilized gel samples were obtained using a Philips X-Part Pro instrument equipped with a θ—θ goniometer, under the following operating conditions: 30 kV and 30 mA, with Cu Kα radiation tube. The diffraction patterns were determined over a range of diffraction angle $2\theta = 3$–$60°$.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) curves were obtained using a SHIMADZU DSC-50 instrument. All runs were performed with 5 mg of finely powdered samples in an aluminum pan, under N_2 atmosphere, with gas flowing at 50 mL min^{-1}.

Thermal analysis

Thermogravimetric analysis (TG) were carried out with a Shimadzu–TGA–50 thermobalance. All TG runs were performed with 10± 0.1 mg of finely powdered samples in a platinum crucible, under N_2 atmosphere, with gas flowing at 50 mL min^{-1}.

In vitro drug release

A mixture of dried gel and pilocarpine hydrochloride (10 : 1, w/w) were kneaded in a mortar for homogenization. The mixture was placed in a cellulose membrane and 5 mL of buffer solution was added for gel swelling, after complete buffer absorption, the cellulose bag placed in a glass beaker was immersed in a 180 mL of desired buffer solution and incubated at 37°C with stirring at 100 rpm. At an appropriated interval of time, 1 mL of the sample was withdrawn,

diluted to 5 mL and pilocarpine content in the medium was determined by UV-visible spectroscopy (Ultrospec 2000 Pharmacia Biotec) at 214 nm. An equal volume of buffer solution was added back to maintain the constant volume.

RESULTS AND DISCUSSION

Gel formation

CH/CG interaction is likely to be formed by the reaction of amine groups of CH and carboxylate groups of CG as suggested by Paula et al.[22]:

$$CG—COO^- + CH—NH_3^+ \rightarrow CG—COO^{-+}NH_3—CH$$

As the interaction between the opposite charge is not strong because of the low content of carboxylate groups in CG, precipitation of polyelectrolyte complex was not observed, as found for the interaction of CH with alginate,[8–10] carboxymethylcellulose,[7] and hyaluronic acid.[12] Reacetylation of free amine group of CH was necessary for inducing the gel formation and the ability to swell in acid medium.[6,22]

The CH or CH/CG gels were therefore obtained by reacetylation of free amine groups. The degree of acetylation (DA) of CH/CG and CH gels was determined by IR spectroscopy method as proposed by Moore and Roberts,[25] whereby the percentage of the amine group acetylated is given by: $A_{1655}/A_{3450} \times 100/1.33$, where A_{1655} and A_{3450} are the absorbances of amine I and hydroxyl band, respectively. The 1.33 value is the ratio for a fully *N*-acetylated CH. Figure 1 shows IR spectra of samples, whereby an average DA of 68.2± 2.4 for CH/CG gels was found for all the prepared samples.

X-ray diffraction

In Figure 2(a) is shown the X-ray powder diffraction patterns of CH. The sample presented a strong diffraction at 2θ of 10.3° which is due to hydrated crystal. Amorphous CH does not show any diffraction, but exhibits a broad peak at 2θ of around 20°.

After reacetylation, sample gels showed diffraction patterns of chitin in dry state,[26] showing a very strong reflection at 2θ of 9.2° [Fig. 2(b)]. This is characteristic of the (020) diffraction of α-chitin form, an anhydrous crystal of chitin.[27,28] By increasing the CG concentration, the diffraction at 2θ of 9.2° decreases in intensity, this could be a consequence of the increase of amorphous region due the CG in the structure of the gel. The crystalline degree *X* was calculated according to the eq. (1) (Hermans–Weidinger equation):[29]

$$X\,(\%) = \left\{ K \times \frac{I_c}{(I_c + I_a)} \right\} \times 100 \qquad (1)$$

where I_c is the integrated intensity of (110) diffraction peak, I_a is the integrated intensity of amorphous peak and *K* is a modulus having a value from 1.1 to 0.8. The *X* values were obtained (using the eq. (1)) for the samples CH powder (hereafter denoted as CH), CH gel, CH/CG 2 : 1, and CH/CG 1 : 2, and are listed in Table I. These results reveal that an increase in CG concentration leads to a decrease in the gel crystalline degree, in other words, the CG is changing the structure of (110) crystallographic plane.

Differential scanning calorimetry

Figure 3 shows the transitions detected in the thermograms of CH, CH gel, and CH/CG gels. The first thermal event registered in all the samples was a wide endothermic peak centered in the range 36–129°C. Polysaccharides have a strong affinity for water and in the solid state these macromolecules may have disordered structures that can be easily hydrated.[30] Close examination of thermograms in Figure 3 reveals that there are differences in the area and position of peak temperature of endothermic events, indicating that these macromolecules differ in their water holding capacity and strength of water–polymer interaction. The fact that ΔH increases when CG is added to the gel could be related to the large number of polar groups due to the presence of CG in the gel.

The second main thermal event registered was a wide exothermic peak due to polysaccharide degradation. This peak was shifted to higher temperature concomitantly, with a decrease in the peak area, after CH reacetylation. Curves for gel samples are similar to those obtained for chitin, as observed by Kittur et al.[30]

Thermogravimetry analysis

Figure 4 shows thermogravimetric curves for CH, CG, and CH/CG gels. CH and its gels show a two-step decomposition pattern, the first one being assigned to water loss and the second step to polysaccharide degradation. It has been reported that chitin and CH decomposition mechanisms result in formic and acetic acids and low molar mass fatty acids as the main degradation products.[31]

CG decomposes in three steps (probably due to its highly branched structure), as described by Silva et al.[32] Hereby, it is noticed that water evaporation occurs in the range 25–200°C, and also that the main polysaccharide degradation was in the range 270–320°C, the maximum being at 307°C.

Regarding the gel samples, it can be seen that for reacetylated gels the onset of decomposition temperature is around 256.0°C, whereas CH gels show higher temperatures (around 261.4°C). On the other hand, the final decomposition temperature was found to be roughly the same for CH/CG and CH gels, i.e.,

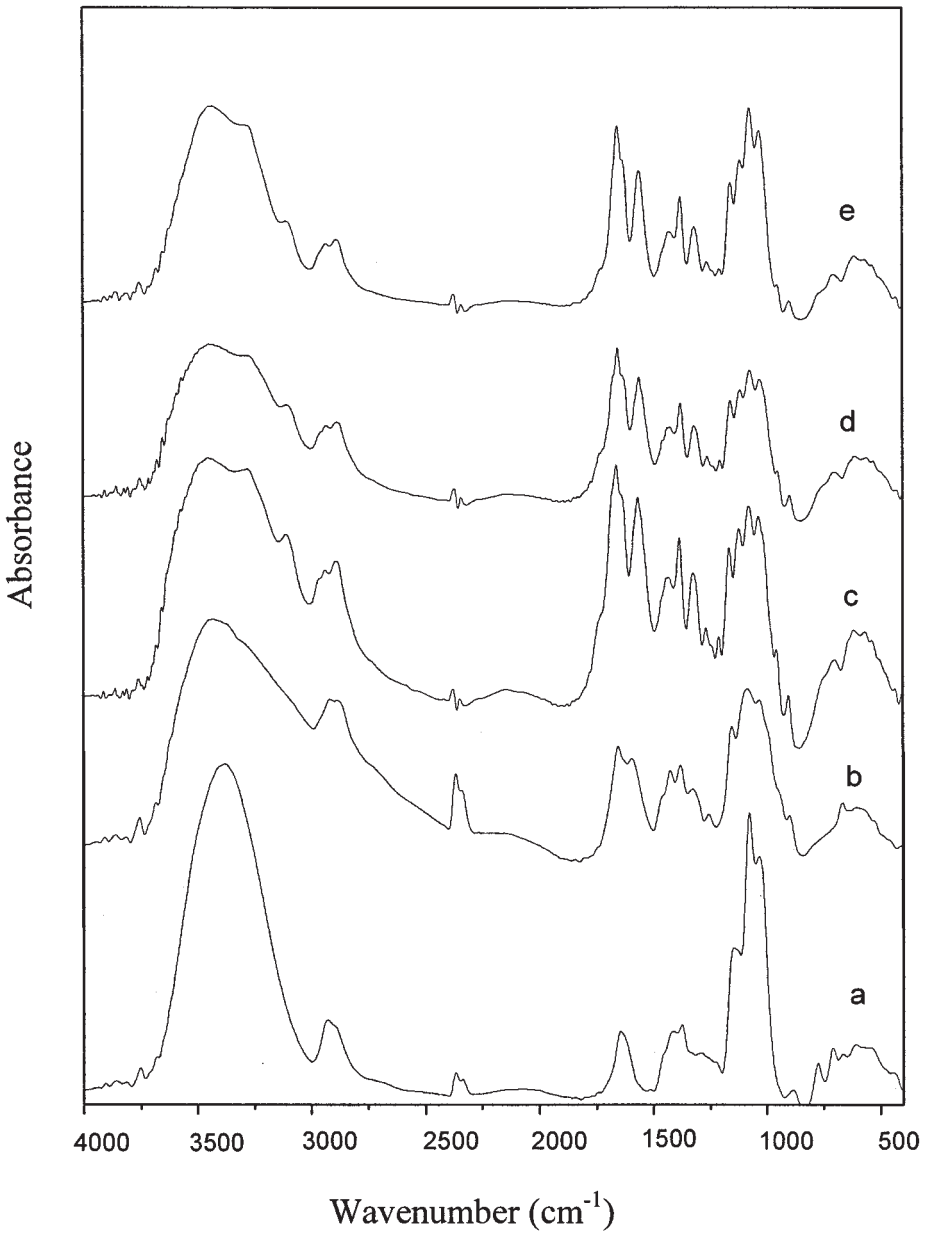

Figure 1 Infrared spectrum of (a) CG, (b) CH, (c) CH gel, (d) CH/CG 2:1, and (e) CH/CG 1 : 2.

365.5°C and 363.9°C, respectively. CH/CG gels presented larger amount of residues than did CH gel. The DTG peak temperature for CH, CH gel, CH/CG 2 : 1, and CH/CG 1 : 2 gels were 306.5°C, 344.0°C, 348.2°C, and 350.2°C, respectively. A slight increase of DTG peak temperature was observed when CG in the gel composition was augmented.

Swelling studies

Swelling experiments were carried out with CH and CH/CG gels. Figure 5 shows the swelling of CH and CH/CG gels in water and at different pH buffer solutions. The swelling degree of CH gel in water is in the range observed in the previous work,[22] which seems to indicate that the swelling behavior of CH gel is not strongly dependent on DA (one should takes into consideration that in the previous work the average DA was 47.6± 2.4). Addition of CG to CH leads to a slight increase in water absorption.

In phosphate buffer solutions, the swelling degree diminishes sharply. The decrease in Q value at same pH was smaller than that previously reported by Paula et al.[22] These results may be explained as being due to the different ion kind and ionic strength used in the present work (phosphate buffers, $I = 0.2$). The presence of phosphate in the swelling medium seems to strongly affect the swelling behavior of CH and

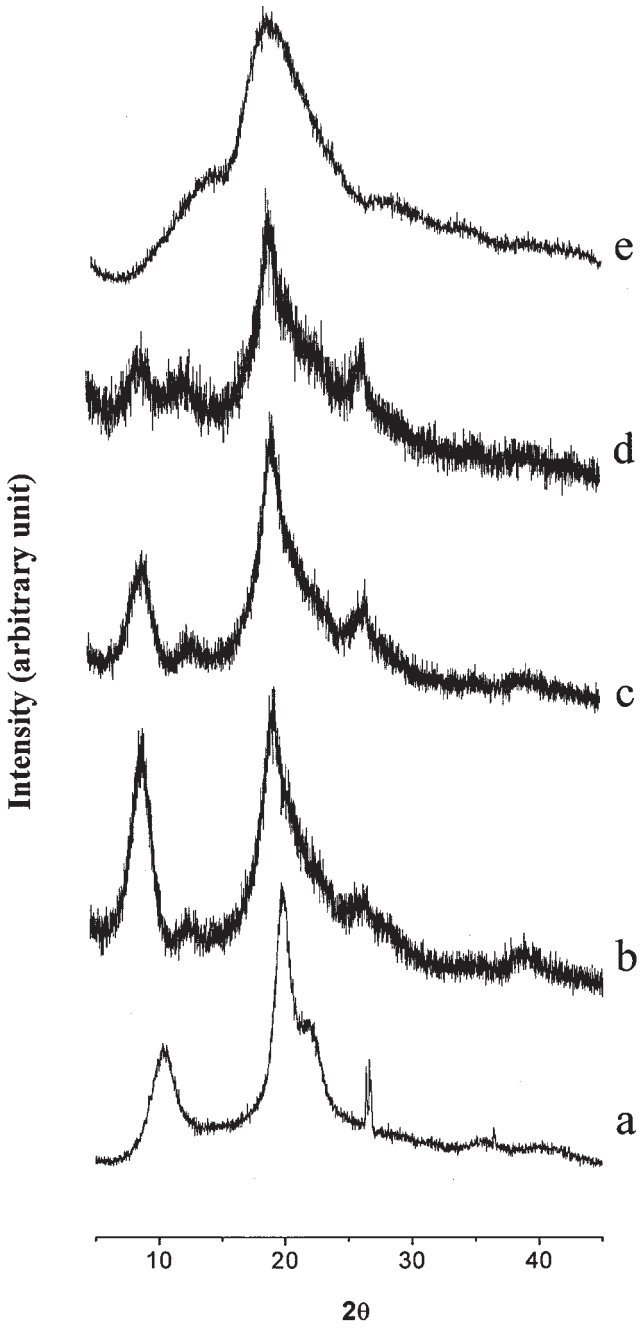

Figure 2 X-Ray diffraction patterns of (a) CH, (b) CH gel, (c) CH/CG 2 : 1, (d) CH/CG 1 : 2, and (e) CG.

CH/CG hydrogels, which is likely due to the formation of crosslinking region between CH and anions that could lead to a decrease in swelling. Crosslinked matrices of CH by anions as sulfate, citrate, and tripolyphosphate have been reported. The gels formed do not employ toxic crosslinked agents for their preparation and complexes were found to be useful in pharmaceutical industry as drug release systems.[15,33–36]

Paula et al.[22] have shown that, for CH/CG reacetylated gel, swelling is maximal at pH 2 and 10, and

TABLE I
Degree of Crystallinity for CH and Reacetylated Gels

	Degree of crystallinity			
Angle	CH	CH gel	CH:CG 2:1	CH:CG 1:2
19.7(110)	57	—	—	—
19.2(110)	—	36.4	33.1	19.5

reaches the minimum values at around pH 5. At low pH, high water absorption can be explained by the fact that CH amine groups are protonated (NH_3^+), which favors chain expansion. In addition to that, CG carboxyl groups in alkaline medium are present in the

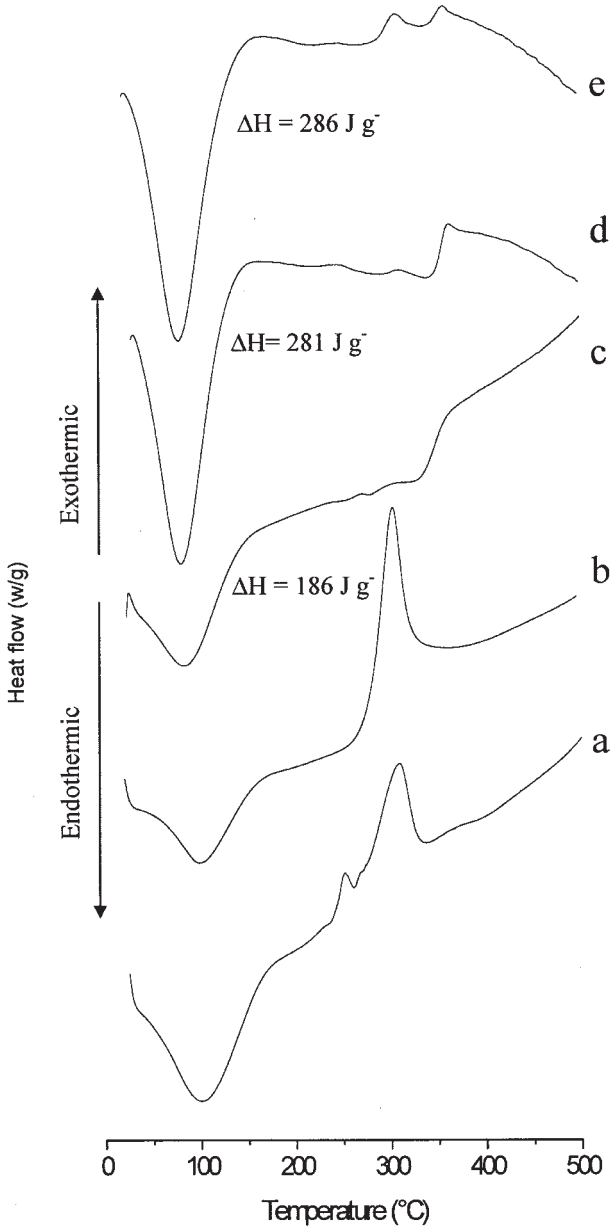

Figure 3 DSC curves of (a) CG, (b) CH, (c) CH gel, (d) CH/CG 2 : 1, and (e) CH/CG 1 : 2.

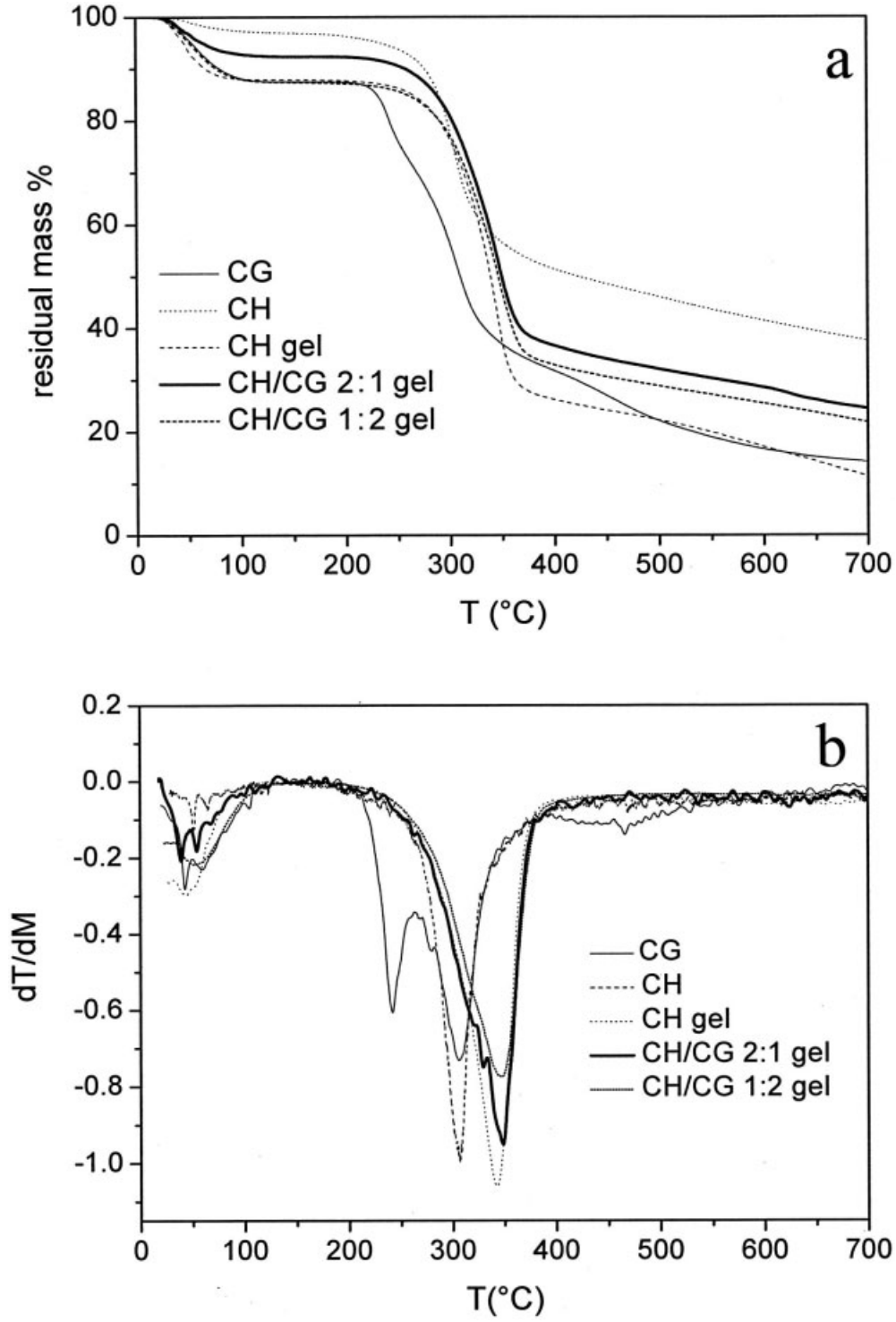

Figure 4 (a) TGA curves and (b) DTG curves.

carboxylated form, which also favors chain elongation. In the present work, this general behavior was also observed, however, it was less pronounced. The concentration of CG in the gel also affects the swelling degree in the phosphate buffers, whereby increasing CG concentration provokes a significant decrease of swelling degree (Fig. 5).

In vitro drug release of pilocarpine hydrochloride

Figure 6 shows the release profile of pilocarpine hydrochloride by CH and CH/CG gels at different pH values. The release is similar for all the three gels in the first 100 min, where about 60% of the drug was released. After this time, the presence of CG on the gel

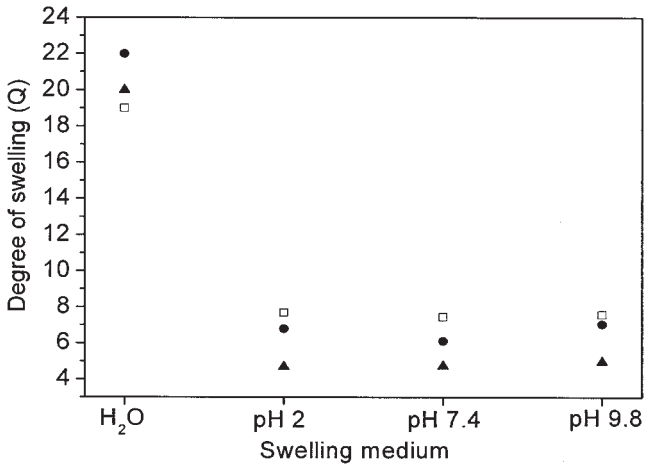

Figure 5 Swelling of CH and CH/CG gels in water and at different pH buffer solutions. The symbols represent CH gel (□), CH/CG 2 : 1 gel (●), and CH/CG 1 : 2 gel (▲).

matrices (CH/CG 2:1 and 1:2 samples) decreases pilocarpine release rate in the medium. This effect can be better observed in Figure 7, which shows the release of drug from the gels at 180 min at 2.0, 7.4, and 9.8 pH values. The release of pilocarpine hydrochloride at pH 2.0 was higher for CH gel than for CH/CG gels. At this pH the free amine groups in CH gel are protonated and provoke gel expansion, which may be responsible for the high release rate. The presence of CG carboxylate group in CH/CG gels, which interacts with amine group of CH, decreases the number of free amine groups, leading to less chain expansion and ultimately to a smaller pilocarpine hydrochloride release. It can also be depicted from Figure 7 that CH/CG 1 : 2 gel shows a decrease of around 12% in the pilocarpine hydrochloride release at pH 2.0, in comparison with that of CH gel. Reacetylated CH microspheres have been investigated for the controlled release of active antimicrobial agents on gastric mucosa, as this matrix does not dissolve on gastric fluid.[6] CH/CG gels were found to be stable at pH 2.0 and were more efficient in sustaining the drug than did the CH gel, therefore, these matrices could be suggested as a vehicle for controlled delivery of antibiotics for the treatment of gastric ulcers in a similar way, as done by Portero *et al*.[6]

By increasing the pH value to 9.8, a decreased drug release is observed for CH and CH/CG gels. This seems to be related to the decrease in pilocarpine solubility, as the nitrogen group of pilocarpine hydrochloride is not charged at high pH value. At pH 7.4 an increase of drug release is observed for CH/CG 2:1 and 1 : 2 gels, in comparison with the release rate at pH 2.0 and 9.8. This behavior seems to be due to a competing phenomenon between the drug–polymer interaction and matrix swelling. Gupta et al.[37] showed that the solubility and basicity/acidity of the drug can affect the release profile, depending on the pH of the dissolution medium. For carrageenan tablets a faster release of theophilline in pH 1.2 was observed because of its properties of acting as a base at pH < 2, while for sodium salicylate the release rate was higher due to its high solubility in basic solution.[35] Although a controlled release profile has been observed, most of the drug was released within 4 h. To increase the drug controlled release, the gel matrix could be crosslinked

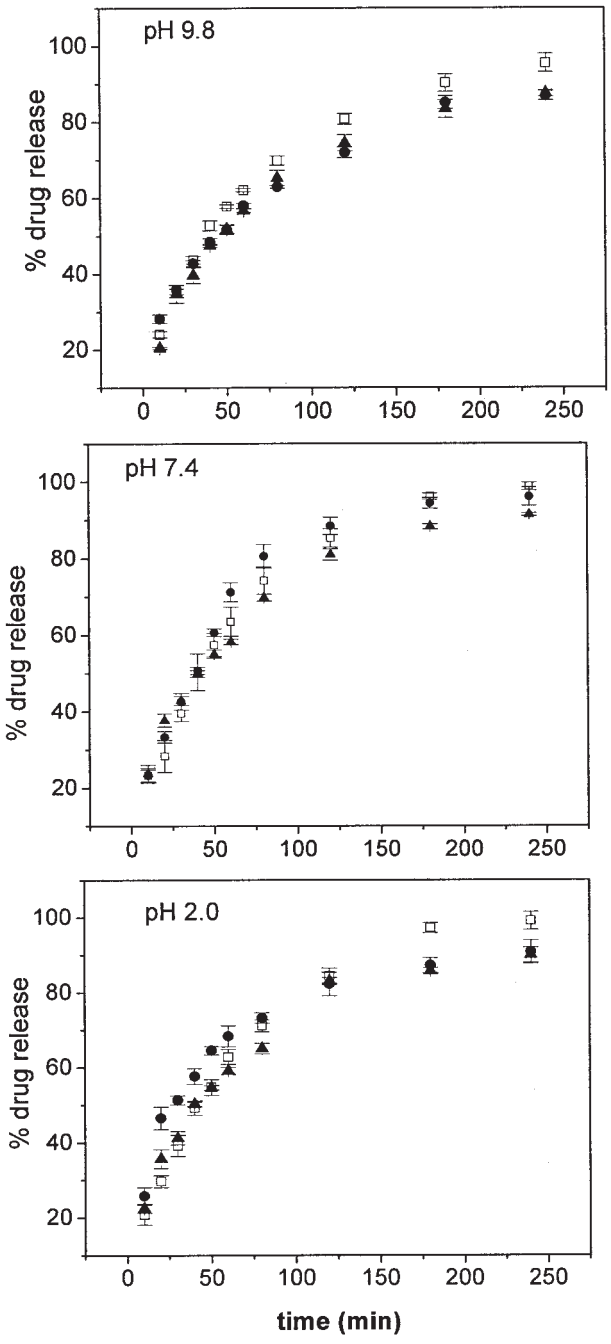

Figure 6 Release of Pilocarpine at different pH medium having ionic strength of 0.2 at 37°C. The symbols represent CH gel (□), CH/CG 2 : 1 gel (●), and CH/CG 1 : 2 gel (▲).

so as to create a "high density" polymer matrix, which would result in sustained release the drug in gels with different compositions.

The drug release data can be treated by a semiempirical equation proposed by Ritger and Peppas[38] for swellable polymeric matrix systems:

$$M_t/M_\infty = Kt^n \quad (2)$$

In this equation M_t/M_∞ denotes the fraction of drug release, t is the release time and K represents a constant characteristic of the system.[38] The diffusional exponent n is an indication of the mechanism of drug release and takes values depending on the geometry of release devise. A Fickian system (case I transport) is described by the diffusional phenomena, while the case II transport is characterized by a relaxation constant. Non-Fickian system is described by diffusion and relaxation phenomena. A Fickian diffusion can assume n values of 0.5 in thin films, 0.45 for cylindrical samples, and 0.43 for spherical samples. Anomalous (non-Fickian) transport assumes n values as being $0.5 < n < 1$, $0.45 < n < 0.89$, and $0.43 < n < 0.85$, respectively, for thin films, cylindrical and spherical systems. Values of n equal to 1, 0.89, and 0.85 are characteristic of case II transport for thin films, cylindrical and spherical systems, respectively.[38]

The swellable CH and CH/CG gels show spherical forms, as observed by microscopy. Table II shows the diffusional exponent n for CH and CH/CG gels at different pH values. The release of pilocarpine from CH gel is found to be dependent on pH values, a non-Fickian mechanism being observed for the release at pH 2 and 7.4, while at pH 9.8, a Fickian (diffusion) mechanism took placed. Several swellable systems

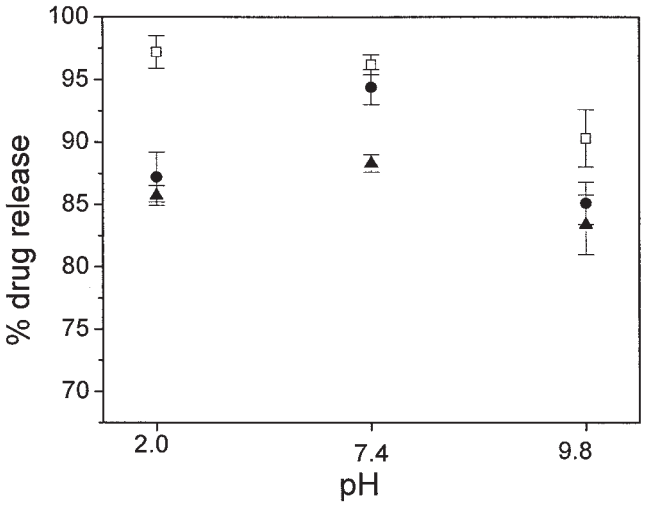

Figure 7 Drug release at 180 min from CH and CH/CG gels in different dissolution media. The symbols represent CH gel (□), CH/CG 2 : 1 gel (●), and CH/CG 1 : 2 gel (▲).

TABLE II
Kinetic Data for CH/CG Hydrogels at Different pH Values

	n values			*k* values		
pH	CH gel	CH/CG 2 : 1	CH/CG 1 : 2	CH gel	CH/CG 2 : 1	CH/CG 1 : 2
2.0	0.52	0.40	0.44	2.6	2.0	2.4
7.4	0.50	0.43	0.40	1.9	2.3	2.1
9.8	0.44	0.40	0.45	2.3	2.1	2.4

containg polysaccharides show release by diffusion mechanism.[39,40] The liberation of pilocarpine in CH/CG matrix occurred by Fickian mechanism, independent of the pH value. Release of pilocarpine from the matrix of PVA/xantan gum, PVA/hyaluronic acid, and PVA/hydroxypropylmethyl cellulose also showed release mechanisms via diffusion phenomena (Fickian), while the release from PVA/carrageenan matrix occurred by non-Fickian mechanism.[39] Fickian mechanism was also observed for the release of verapanil hydrocloride from polyacrylamide/guar matrix.[40] Release of endomethacin from polyacrilamide-grafted CH followed a non-Fickian trend and the diffusion was relaxation controlled.[41]

The authors are thankful to Fundação Coordenação de Aperfeiçoamento do Pessoal de Ensino Superior (CAPES-Brazil), Fundação Cearense de Amparo à Pesquisa (FUNCAP-Ce-Brazil), and Fundação Cearense de Amparo `a Pesquisa (FUNCAP-Ce-Brazil).

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