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Chitosan–carboxymethylcellulose interpolymer complexes for gastric-specific delivery of clarithromycin

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ABSTRACT

Chitosan (CS) and carboxymethylcellulose (CMC) sodium interpolymer complexes were formed using the novel method "tablets-in-capsule" for stomach drug delivery. The aim of this study was to investigate the influence of the molecular weight (M.wt.) of CS and the proportion CS/CMC on physical properties and clarithromycin (CAM) release.

Swelling was dependent on CS M.wt., on medium pH and on proportion polymer/polymer. The higher M.wt., the major presence of protonated amined moieties that can be ionized and modify the swelling/eroding and dissolution medium uptake capacity. Swelling kinetics at pH 1.2, were close to Fickian diffusion, whereas at pH 4.2 were non-Fickian. Furthermore, dissolution medium uptake capacity can be adjusted to an exponential equation at pH 1.2 ($r^2 \geq 0.96$), and to a linear equation at pH 4.2 ($r^2 \geq 0.99$), showing that at low pHs the repulsion among ionized chains is higher.

CAM release rates have shown to be dependent on pH and on polymer proportion. At pH 1.2, the fastest profile was obtained when using high M.wt. CS. Drug diffusion was Fickian, so the process is governed by swelling/eroding. Whereas at pH 4.2, CAM release followed zero-order kinetics with no influence on M.wt. So, release is controlled by CAM low solubility.

1. Introduction

It is well known that *Helicobacter pylori* is the main cause of gastritis, gastroduodenal ulcer and gastric cancer. Therefore, its eradication is a prerequisite for curing duodenal and gastric diseases (Suzuki et al., 2006). In vivo, *H. pylori* is located at the adherent mucus layer, living both within and beneath it. It is also adhered to the gastric epithelial cells (Bardonnet et al., 2006). First-line therapy is a combination of a gastric protector with two antibiotics selected among amoxicillin, clarithromycin (CAM) or metronidazol for 7–14 days. But these regimens do not have a complete eradication (Di Mario et al., 2006). A possible alternative to enhance *H. pylori* eradication would be the administration of gastroretentive systems that can maintain higher levels of antibiotics for longer periods of time (Chun et al., 2005; De la Torre et al., 2005).

A novel system called "tablets-in-capsule" has been developed by Li and Zhu (2004) and consisted of the combination of mini-matrices in side a hard gelatine capsule to obtain different drug delivery systems. The simplicity of this method, as well as the easiness of administration to patients and the wide range of opportunities from changes on the composition of the matrices were factors that helped us to select this system as an alternative to enhance *H. pylori* eradication.

Hydrophilic swellable polymers, like alginates, carrageens, celluloses or chitosan (CS), have been widely used as rate controlling agents in matrices (Conti et al., 2007a,b; Li and Zhu, 2004; Nerurkar et al., 2005; Verhoeven et al., 2006). Among several available polymers, CS and some celluloses have been reported to have good mucoadhesive and swellable properties (Majithiya and Murthy, 2005; Park et al., 2006) which allow these polymers to be potential candidates to achieve site-specific gastric delivery. There are several studies about using CS IPCs to develop stomach controlled systems (Bardonnet et al., 2006; Conway, 2005). So, it would be of interest to study the factors that control the physical properties of CS IPCs as gastroretentive swellable systems like swelling, eroding or release rates (Kavanagh and Corrigan, 2004; Puttipipatkhachorn et al., 2001).

Nevertheless, fewer studies have considered how the molecular weight of CS could affect physical properties. The presence of pendant groups within IPCs, which can be ionized, increases with the molecular weight of polymers and could have relevant influences. In fact, similar studies about molecular weights of polymers have been reported for other hydrophilic polymers like hydrox-

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ypropylmethylcellulose (Kavanagh and Corrigan, 2004) or alginates (Mladenovska et al., 2007).

This paper is aimed at evaluating the different electrostatic interactions among cationic moieties from CS and carboxylic moieties from carboxymethylcellulose (CMC) sodium, which lead to obtain polyionic hydrogels to achieve stomach controlled antibiotic delivery for the treatment of *H. pylori*. The main objectives of this investigation were to study (I) the influence of CS molecular weight on physico-chemical properties (swelling, eroding and drug release), (II) the effects of the pH of the medium, as well as to evaluate (III) the proportion of CS and CMC within polyionic networks.

2. Materials and methods

2.1. Materials

Low molecular-weighted chitosan (CS low M.wt.) (55 mPa s) was supplied by Fluka (Spain), whereas chitosan medium molecular-weighted (CS med. M.wt.) (284 mPa s) and chitosan high molecular-weighted (CS high M.wt.) (1406 mPa s) were manufactured by Sigma–Aldrich (Spain), and all of them supplied with a minimum degree of acetylation of 75%. CMC sodium salt (high viscosity, range from 1500 to 2500 mPa s) was supplied by BDS (Lutterworth, UK) and CAM was provided by Normon (Spain). All other chemicals were at least of pharmaceutical grade and used without further purification.

2.2. Preparation of delivery drug systems

The novel method called "tablets-in-capsule system" (Li and Zhu, 2004) is based on the combination of different mini-tablets to obtain drug delivery systems. Following this method, we prepared different formulations which consisted on mini-matrices (6 mm in diameter) inside a hard gelatine capsule. In order to study the influence of the molecular weight of CS, we prepared polyelectrolyte complexes in the ratio 40:60 (w/w) CAM:[CS–CMC], where the proportion of CS and CMC was 25:75 (w/w), henceforth designated as 40:[25:75]. Furthermore, to evaluate the influence of the proportion of CS and CMC we prepared systems with ratios of 40:60 and 80:20 (w/w) CAM:[CS–CMC], where the proportion of CS and CMC was 75:25 and 25:75 (w/w), henceforth designated as 40:[75:25], 80:[25:75] and 80:[75:25], respectively.

The systems were prepared as follow. Firstly, drug and polymers were sieved through 0.45 mm of mesh and then mixed homogeneously. Afterwards they were directly compressed to get mini-matrices of 6 mm in diameter and weight of 50 mg. Ten of these mini-matrices were placed into a gelatine capsule (no. 0) and they constituted the final system.

2.3. Scanning electron microscopy (SEM) studies

Freeze-dried samples were mounted on an aluminium sample mount. After coating with a thin layer of gold–palladium, the hydrogel samples were analysed with a Jeol® 6400 SEM. All micrographs were the product of secondary electron imaging used for surface morphology identification at different magnifications and an accelerating voltage of 20 kV.

The studied surfaces were from complexes 40:[25:75] containing different CS M.wt at 30 min and 1 h at pH 1.2, while at pH 4.2 were at 1 and 4 h. All samples were lyophilized just after taken them from the dissolution media.

2.4. Swelling and eroding studies

The method used was based on studies of Kavanagh and Corrigan (2004). An USP Apparatus 2 (paddle) dissolution bath (Vankel® VK 700) was used set to 37 °C. The rotational speed was 50 rpm and 250 mL of dissolution medium was measured in each vessel. Each system was weighted (W_i) and then placed in each vessel. The experiment consisted of allowing the system to dissolve in the media at the listed conditions for certain time periods, specifically at 15, 30, 45, 60, 90, 120, 240 and 360 min. After vacuum-filtration to drain the excess of medium, the remaining system was weighted to determine its wet weight (W_w). The system was then dried to constant weight (W_d) in an oven at 37 °C. Each determination at each point was carried out in quadruplicate and the error bars on the graphs represented the standard deviation.

The indicators calculated were, firstly, the relative swelling (Kavanagh and Corrigan, 2004) which is calculated as follows:

$$\text{Relative swelling} = \frac{W_w}{W_i} \quad (1)$$

To investigate more precisely the relative swelling mechanism in the hydrogel, the initial swelling data were fitted to Eq. (2) for $W_t/W_\infty < 0.6$ (Lee et al., 1997; Sakiyama et al., 1999) as follows:

$$\frac{W_t}{W_\infty} = K_1 t^n \quad (2)$$

where K_1 (h^{-1}) is a characteristic constant of the hydrogel and n is a characteristic exponent related to the mode of transport of the penetrant.

Secondly, the dissolution medium uptake per unit polymer remaining was calculated in order to determine the rate of hydratation considering what had been eroded and dissolved from the system.

$$\text{Dissolution medium uptake per polymer remaining} = \frac{(W_w - W_d)}{W_d} \quad (3)$$

These values were then fitted to a square root equation, obtaining the rate of dissolution medium uptake per unit polymer remaining.

Finally, data of dry weight is used to calculate the relative erosion, as an indicator of the erosion and dissolution of the system.

$$\text{Relative erosion} = \frac{W_d}{W_i} \quad (4)$$

These values were fitted to the cube root relationship as outlined by Kavanagh and Corrigan (2004) to determine the apparent polymer erosion rate constant K_2 (h^{-1}).

$$\left(\frac{W_d}{W_i}\right)^{1/3} = 1 - K_2 t \quad (5)$$

2.5. In vitro drug release and in vitro drug stability

These studies were carried out in a dissolution bath (Vankel® VK 700). An USP Apparatus 2 (paddle) was set up at 37 °C, with a rotational speed of 50 rpm and 500 mL of dissolution medium were measured into each vessel.

The system was weight and then placed in each vessel. At times of 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min, a sample of 5 mL was withdrawn and was filtered through a 0.45 μm filter (Acrodisc® HPVL 0.45 μm).

The quantity of CAM was determined by HPLC method, consisting of a UV detector (Jasco UV-1575 Intelligent UV/VIS Detector), a pump (Jasco PU-1580 Intelligent HPLC pump), a degasser (Jasco DG-2080-53) and an automatic injector (Gilson® 231 XL Sampling

Injector). The selected wavelength was 210 nm. A C18 column was used (ACE® 5 C18), a 4.6-mm × 15-cm column with a particle size of 5 μm, and temperature was maintained constant at approximately 60 °C (Pickering Laboratories CHX700 Column Temperature Controller). The flow rate was 1.0 mL per minute. The mobile phase consisted of a mixture of methanol and 0.079 M monobasic potassium phosphate (650:350, v/v), and pH was adjusted to 4.5 with phosphoric acid. The cumulative amount of CAM released from the system was determined from the appropriated calibration curve. Each determination at each time point was performed in triplicate and the error bars on the graphs represented the standard deviation.

The dissolution media tested were HCl 0.1N (pH 1.2) and acetate buffer (pH 4.2) prepared as described in USP 30–NF 25 (2007). As CAM is extensively degraded at pH 1.2 (Erah et al., 1997; Nakagawa et al., 1992), a solution of NaOH was added to samples obtained from HCl medium so as to increase their pH and therefore prevent further degradation (Chun et al., 2005).

To investigate more precisely the effect of polyionic complex formation on the release of CAM, results were analysed according to a zero-order kinetics (Eq. (6)) or to Ritger–Peppas equation (Eq. (7)) (Ritger and Peppas, 1987) for $M_t/M_\infty < 0.6$, which are expressed by the following equations:

$$\frac{M_t}{M_\infty} = K_d t \quad (6)$$

$$\frac{M_t}{M_\infty} = K_1 t^n \quad (7)$$

where M_t/M is the fractional drug released at time t (h), K_d (h^{-1}) is the kinetic dissolution constant, n is a diffusional exponent and K_1 (h^{-1}) is release constant.

2.6. Statistics analysis

All statistics were obtained with Statgraphics® *Plus*, version 5.1. Data were analysis using a *T*-test for comparison between two different groups. For three or more different groups, ANOVA was used followed by a least significant differences method. Results were considered statically significant at $p < 0.05$. All data are presented as mean values with standard deviation (mean ± S.D.).

3. Results and discussion

3.1. SEM characterization

The mini-matrices formed by CS and CMC have shown to form pores on their surface in both media tested. Figs. 1 and 2 show the scanning electron micrographs of 40:[25:75] (w/w) mini-matrices' surface at different times, which have been lyophilized, in order to study the influence of molecular weight of CS and the pH of the medium within the interpolymeric network. A freeze-drying process was used to prepare the samples due to it is shorter and it will help to prevent further degradation of CAM within the interpolymer network. Morphological changes could appear in the structure surfaces and they would be of similar magnitude in all samples.

At pH 1.2 (Fig. 1), the pore size is dependent on the molecular weight of CS. Low M.wt. CS presents a pore size of about 40 μm, while high M.wt. has a pore size larger, about 50 μm at initial time (30 min). So, this is due to the presence of more pendant chains within the interpolymeric network that causes more repulsion among chains and larger swelling degree. It can be observed in the micrographs c and d (Fig. 1) which show structures with a great penetration of the medium into the system with pores that form connections (channels) with the interior of the structure. This

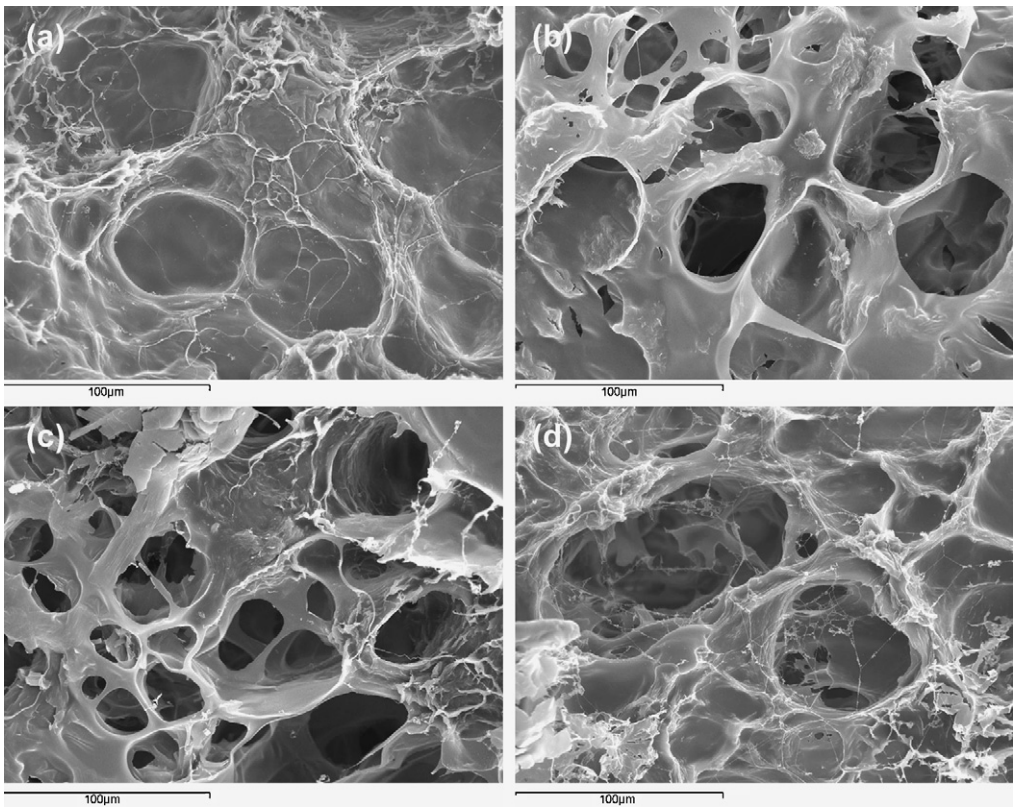

Fig. 1. Scanning electron micrographs of CAM:[CS:CMC] 40:[25:75] (w/w) complexes. The first two micrographs depict top view of mini-matrices at 30 min and pH 1.2 with low M.wt. CS (a) and high M.wt. CS (b). The other two micrographs outline surfaces at 1 h and pH 1.2: low M.wt. CS (c) and high M.wt. CS (d).

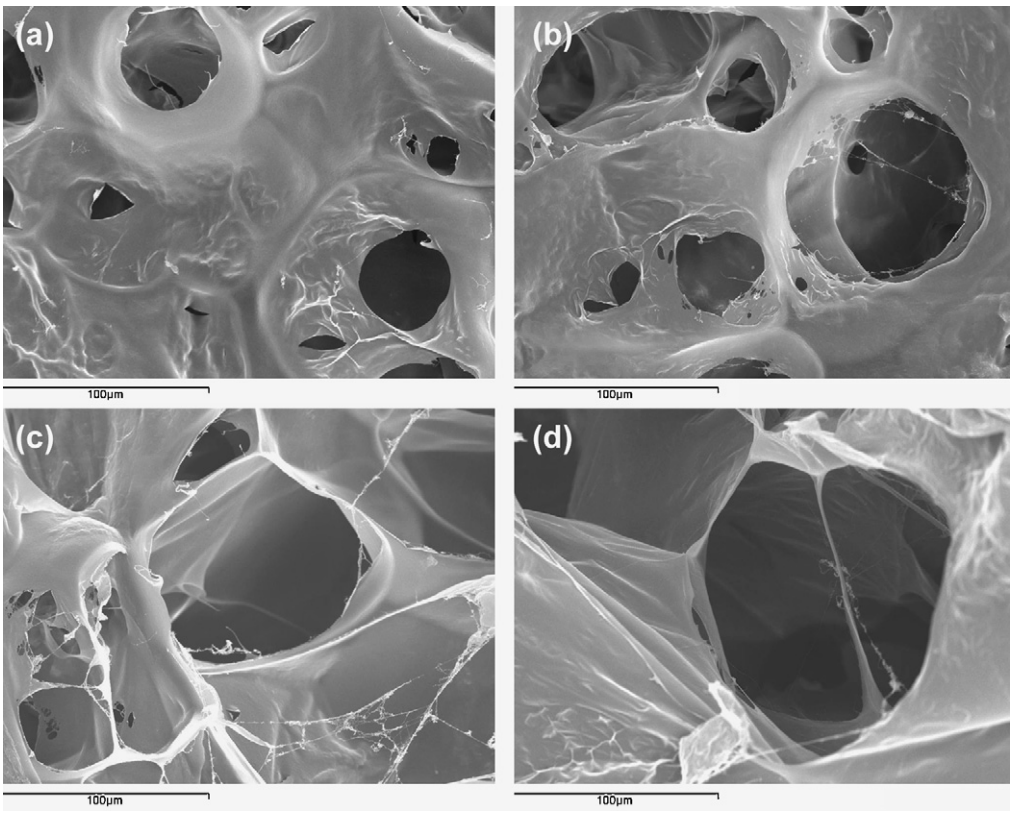

Fig. 2. Scanning electron micrographs of CAM:[CS:CMC] 40:[25:75] (w/w) complexes. The first two micrographs depict top view of mini-matrices at 1 h and pH 4.2 with low M.wt. CS (a) and high M.wt. CS (b). The other two micrographs outline surfaces at 4 h and pH 4.2: low M.wt. CS (c) and high M.wt. CS (d).

can be related to the erosion process which plays an important role at this pH. Similar porous surfaces have been reported before in lyophilized hydrogels comprised of CS (De la Torre et al., 2003; Foda et al., 2004).

At pH 4.2 (Fig. 2), the micrographs also show porous surfaces. The results are similar to those obtained at pH 1.2, where the largest pore size is obtained when high M.wt. CS is used. At initial time (1 h), low M.wt. CS forms pores of about 50 μm, whereas high M.wt. CS has pores of about 80 μm. At 4 h the pore size is larger, with values of 75 and 120 μm for low and high M.wt. CS, respectively.

These differences in the size surface pores is in accordance with other authors (Foda et al., 2004) who stated that cross-linked high M.wt. CS sponges form larger pores than cross-linked low M.wt. ones. It can be explained by the more presence of pendant chains within the interpolymeric network which causes more repulsion among chains and larger swelling degree (Chellat et al., 2000; De la Torre et al., 2003; Mladenovska et al., 2007; Van Tomme et al., 2006).

The micrographs c and d (Fig. 2) outline surfaces after 4 h in the dissolution medium at pH 4.2 showing the low erosion process at this pH. This could be attributed to the low penetration into the network which it is related to drug release, and reflects the important role of the erosion.

So, differences in pore size and dissolution medium uptake capacity were crucial factors for the more delayed drug release rate from the systems (Foda et al., 2004).

3.2. Swelling and eroding properties

3.2.1. The effect of CS molecular weight

Fig. 3 shows the relative swelling of CAM:[CS:CMC] 40:[25:75] (w/w) with different M.wt. CS. Changes in networks as a result of electrostatic interaction between polymeric chains were studied.

In terms of relative swelling, the graphs show higher relative swelling at pH 4.2, compared with pH 1.2. At both pHs there were no differences between low and medium M.wt. CS. Whereas, for high M.wt. CS, the profile obtained is the highest. These data

Table 1
Estimated parameters obtained from fitting relative swelling experimental data to Eq. (2) and from fitting dissolution medium uptake per unit polymer remaining (DMPR) to a linear equation ($y = ax(h^{1/2}) \pm b$) for pH 1.2 and to an exponential equation ($y = be^{ax(h^{1/2})}$) for pH 4.2 ($n = 4$)

CAM:CS:CMC complexes		pH 1.2				pH 4.2			
% (w/w)	Type of CS	Relative swelling		DMPR		Relative swelling		DMPR	
		n (±S.D.)	a (±S.D.) $h^{1/2}$	b (±S.D.)	r^2	n (±S.D.)	a (±S.D.) $h^{1/2}$	b (±S.D.)	r^2
40:[25:75]	Low M.wt.	0.5089 (±0.037)	1.498 (±0.0099)	0.4501 (±0.0484)	0.9759	0.6165 (±0.019)	3.2601 (±0.0322)	1.8531 (±0.2799)	0.9954
40:[25:75]	Med M.wt.	0.5051 (±0.0619)	1.3629 (±0.008)	0.5884 (±0.0569)	0.9949	0.6391 (±0.071)	3.1518 (±0.0235)	1.6178 (±0.2929)	0.9969
40:[25:75]	High M.wt.	0.5177 (±0.004)	1.5495 (±0.0098)	0.7899 (±0.0918)	0.9628	0.6283 (±0.0335)	3.5198 (±0.0679)	1.2381 (±0.4347)	0.9926
40:[75:25]	High M.wt.	–	–	–	–	0.7591 (±0.008)	6.6249 (±0.3510)	2.8401 (±0.0101)	0.9857
80:[25:75]	High M.wt.	–	–	–	–	0.2913 (±0.0072)	2.1527 (±0.0904)	1.4622 (±0.3735)	0.9051
80:[75:25]	High M.wt.	–	–	–	–	0.2079 (±0.0008)	6.7406 (±1.3191)	−0.2354 (±0.1208)	0.9698

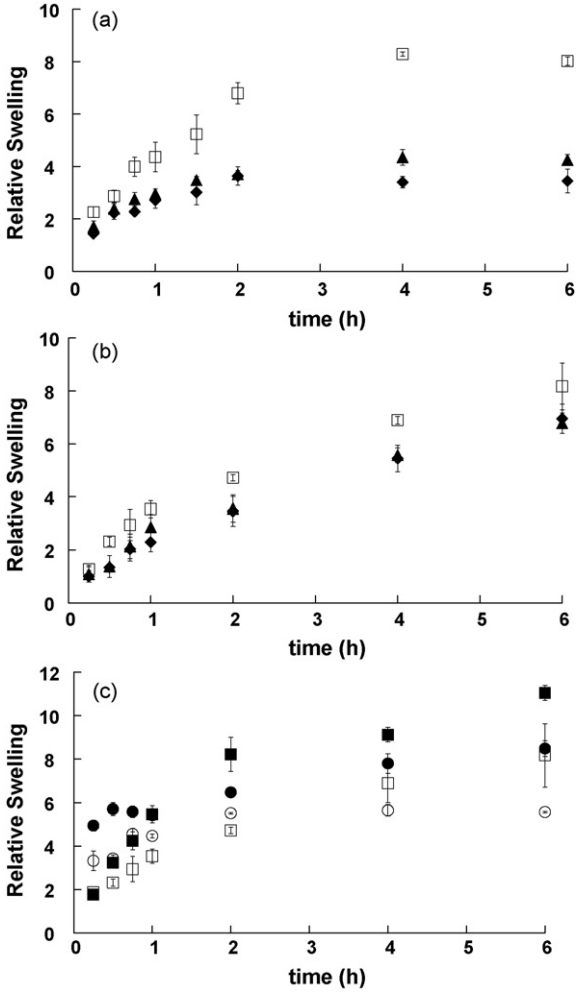

Fig. 3. Relative swelling at 37 °C, at pH 1.2 (a) and pH 4.2 (b) for CAM:[CS:CMC] 40:[25:75] (w/w) complexes with low M.wt. CS (♦), medium M.wt. CS (▲) and high M.wt. CS (□), (n = 4). (c) Relative swelling at 37 °C and pH 4.2 for complexes with high M.wt. CS in the ratio CAM:[CS:CMC] (w/w) 80:[75:25] (●), 80:[25:75] (○), 40:[75:25] (■) and 40:[25:75] (□), (n = 4).

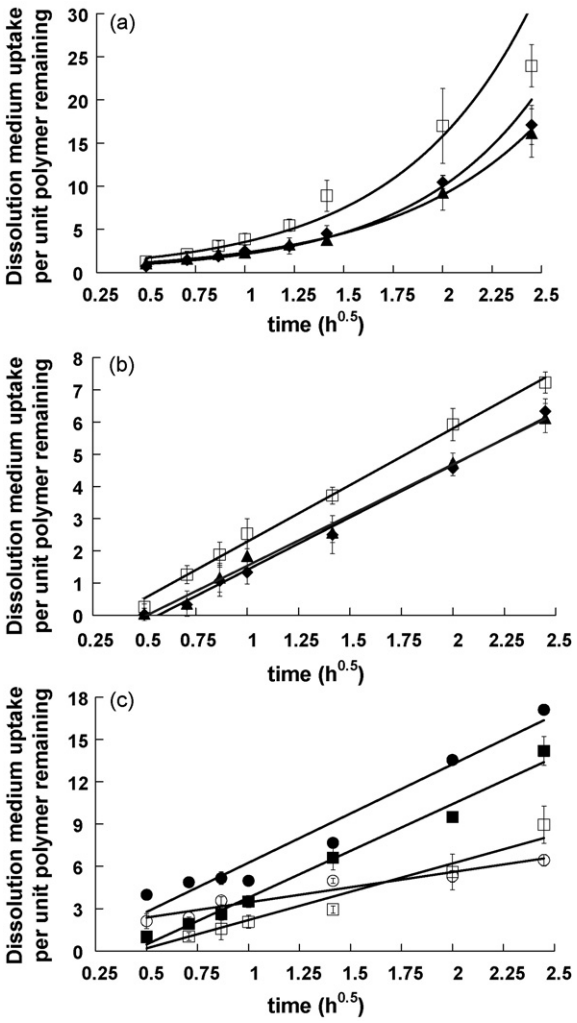

Fig. 4. Dissolution medium per unit polymer remaining profiles at 37 °C, at pH 1.2 (a) and pH 4.2 (b) for CAM:[CS:CMC] 40:[25:75] (w/w) complexes with low M.wt. CS (♦), medium M.wt. CS (▲) and high M.wt. CS (□), (n = 4). (c) Dissolution medium uptake per unit polymer remaining profiles at 37 °C and pH 4.2 for complexes with high M.wt. CS in the ratio CAM:[CS:CMC] (w/w) 80:[75:25] (●), 80:[25:75] (○), 40:[75:25] (■) and 40:[25:75] (□). (—) Fitted experimental data, (n = 4).

show that the higher molecular weight, the higher relative swelling behaviour. To investigate the swelling kinetics of these systems the n exponent were calculated and listed in Table 1.

Lee et al. (1997) distinguished three classes of diffusion according to the relative rates of diffusion and polymer relaxation. The first is Fickian diffusion (n = 0.5), in which the rate of diffusion is much smaller than the rate of relaxation. In this case, the system is controlled by diffusion. The second case is Case II (n = 1.0), where the diffusion process is much faster than the relaxation. The controlling step is the velocity of an advancing front, which forms the boundary between swollen hydrogel and glassy core. The third case is non-Fickian diffusion (n = 0.5–1.0), which describes those cases where the diffusion and relaxation rates are comparable.

The n values of the systems (high, medium and low M.wt.) were 0.5177, 0.5051 and 0.5089 at pH 1.2, with r^2 higher than 0.98 in all cases. The results indicate that the transport mechanism is close to Fickian diffusion. So, at pH 1.2 the systems are controlled by diffusion. But at pH 4.2 the n values were (high, medium and low M.wt.) 0.6283, 0.6391 and 0.6165, with r^2 higher than 0.93 in all cases. Transport, in all systems at this pH, was non-Fickian diffusion with both diffusion- and relaxation-controlled systems. In both cases, there were no statistically significant differences (p-values >0.05) among molecular weight of CS. Some authors have reported before Fickian and non-Fickian swelling kinetics for polyelectrolyte complexes comprised of CS and sodium alginate, dextran sulfate or sodium tripolyphosphate (Lee et al., 1997; Lin et al., 2005; Sakiyama et al., 1999).

However, if relative swelling is corrected by the eroding process of the system, calculated as the dissolution medium uptake per unit polymer remaining (Kavanagh and Corrigan, 2004), the results are different (Fig. 4). It was observed an influence of the molecular weight of CS within the interpolymeric network. The dissolution medium uptake capacity increased with the molecular weight of CS, with a maximum data of dissolution medium uptake per unit polymer remaining for 40:[25:75] (w/w) high M.wt. CS of 23.9 (±2.4) and 7.2 (±0.3) at pH 1.2 and 4.2, respectively. Similar results were reported by Kavanagh and Corrigan (2004) with hydroxypropylmethylcellulose of different molecular weights. This behaviour could be attributed to a greater presence of ionized groups (cationic groups) due to presence of more pendant groups with high M.wt. CS. At higher pHs the degree of ionization is lower and the moieties from CS are involved in hydrogen bonding, so at these pHs the network is in a collapsed conformation. Meanwhile, at lower pH values, the number of ionized cationic groups increases

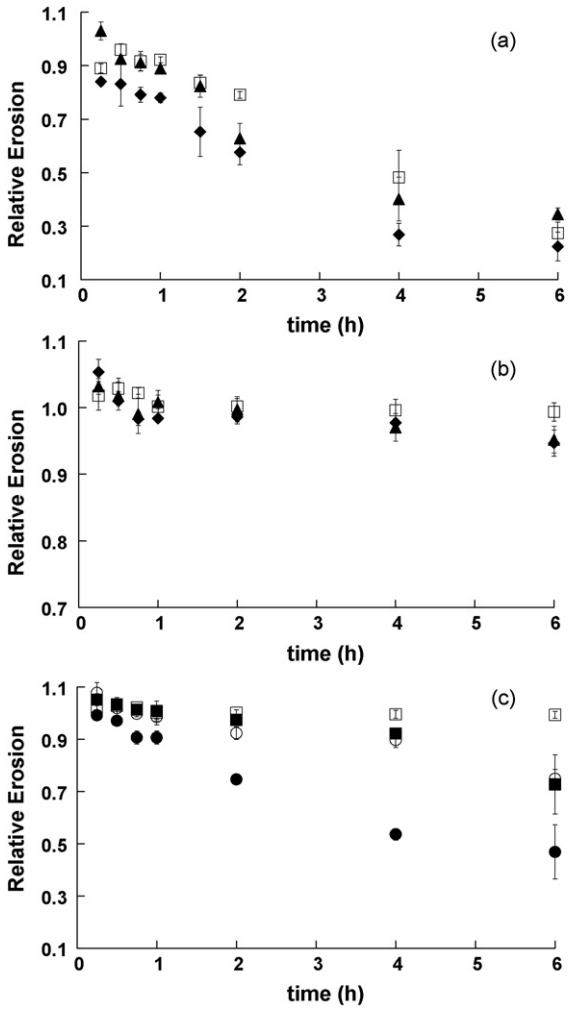

Fig. 5. Relative erosion at 37 °C, at pH 1.2 (a) and pH 4.2 (b) for CAM:[CS:CMC] 40:[25:75] (w/w) complexes with low M.wt. CS (♦), medium M.wt. CS (▲) and high M.wt. CS (□), (n = 4). (c) Relative erosion at 37 °C and pH 4.2 for complexes with high M.wt. CS in the ratio CAM:[CS:CMC] (w/w) 80:[75:25] (●), 80:[25:75] (○), 40:[75:25] (■) and 40:[25:75] (□), (n = 4).

and these moieties do not participated in hydrogen bonding. Consistently, the complex expands. Similar polyionic interactions have been observed between CS and other polymers like polyacrylic acid (De la Torre et al., 2003), charged dextran hydrogels (Van Tomme et al., 2006), and alginates (Mladenovska et al., 2007).

Moreover, dissolution medium uptake per polymer experimental data can be adjusted to different kinetics (Table 1). At pH 1.2, all the systems follow an exponential equation, where r^2 are higher than 0.96 in all cases. Meanwhile, at pH 4.2 rates are adjusted to a linear equation, where r^2 are higher than 0.99 in all cases. The statistical study of the exponent a has shown that there are significant differences between high M.wt. CS and low and medium M.wt. CS, with p-values of 0.0043 and 0.038 for pH 1.2 and 4.2, respectively. The fact that at pH 4.2 the swelling kinetics can be adjusted to linear equations reflects that at final times there is a minor entrance of dissolution medium because of a smaller expansion of the IPCs owing to the lower presence of ionized pendant groups from CS and CMC.

In the case of relative erosion, we observed an important influence of the pH of the medium (Fig. 5). At pH 4.2 the erosion process is much lower than at pH 1.2. These eroding profiles are related to slow release profiles obtained at pH 4.2, due to the lower swelling degree and the fact that CAM is poorly soluble at this pH. The solubility of CAM highly depends on the pH of the medium because it is a weak base compound with a pK_a = 8.76 (Nakagawa et al., 1992). The relationship between the pH and the solubility can be explained by the Henderson–Hasselbalch's equation. So, its solubility increases with a decreasing pH of the dissolution medium. It is expected that drug release will be highly influenced by the eroding process. At pH 1.2, higher eroding process is observed. At initial times (until 1 h) the systems presented low relative erosions which were mainly attributed to the time that systems need to absorb the dissolution medium. However, at middle times (from 1 to 4 h) the erosion process is increased as shown by high slope values in the graph, attributed to repulsion among polymers chains. The apparent polymer erosion rate constants (K_2) were calculated for this interval (Table 2). The molecular weight of CS does not have any influence on the eroding kinetics because there were no statistically significant differences (p-value = 0.1317). Finally, for periods of time longer than 4 h, erosion profiles are slower, with steady relative erosion values that can be attributed to the lower presence of CAM within the interpolymer network structure. Similar bioerosion results had been previously observed for other chitosan–xanthan polyionic complexes (Chellat et al., 2000) and carboxymethylated-chitin hydrogels (Loke et al., 2000).

These results confirm that CS–CMC complexes are pH-sensitive due to the presence of cationic and anionic pendant groups that are involved in the physical interaction (hydrogen bonding) between polymers.

3.2.2. The effect of the polymer proportion

The effect of the proportion of CS and CMC within the network in the swelling properties is depicted in Figs. 3c and 4c. For relative swelling data, the exponents (n) were calculated and listed in Table 1.

When the proportion of drug/polymer is 40:[25:75] and 40:[75:25] (w/w), the exponent n has values between 0.5 and 1.0, meaning that swelling mechanism is a non-Fickian in which diffusion and relaxation are comparables.

Non-Fickian mechanisms for relative swelling have been observed by other authors in polyelectrolyte complexes comprised of CS (Lee et al., 1997; Lin et al., 2005; Sakiyama et al., 1999). But, for 80:[25:75] and 80:[75:25] (w/w), the n values indicate an anomalous behaviour, because n is <0.5. These results suggest the need of further investigations to study more precisely the effect of the proportion drug/polymer within polyionic complexes.

However, for all complexes, the dissolution medium uptake capacity have shown a linear behaviour at pH 4.2 (Table 1) with a determination coefficients (r^2) higher than 0.9. The greatest swelling is obtained with the highest ratio of CS, like complexes 40:[75:25] and 80:[75:25] (w/w). This can be explained by a higher presence of cationic moieties in the network, that at this pH are partially ionized and these groups do not participated in hydrogen bonding, thus the structure expands more. Comparing their linear equations, there are statistically significant differences (p-value <0.0001).

In the case of relative eroding, also the highest erosion process is observed when a high CS proportion is within the network.

The eroding rates in all cases can be adjusted to a linear equation (Table 2), with r^2 higher than 0.93 in all cases. Statistically, there are significant differences with p-value lower than 0.0001. The fact that the formulations with a high proportion of CS, such as 40:[75:25] and 80:[75:25] (w/w), can be more easily erosionable is explained by partial ionization of cationic moieties from CS which leads to a greater expansion of the network and, consistently, a faster CAM release. Similar results have been confirmed also in other polyionic hydrogels, like CS-polyacrylic acid (De la Torre et

Table 2
Estimated parameters obtained from fitting the apparent polymer erosion experimental data to a linear equation ($y = n - K_2x(h^{-1})$) at 37 °C and pH 4.2 from CAM:[CS:CMC] (w/w) interpolymer complexes ($n = 4$)

pH 4.2	40:[75:25]	40:[25:75]	80:[75:25]	80:[25:75]
K_2 (±S.D.) h^{-1}	0.0110 (±0.007)	0.0086 (±0.0004)	0.0401 (±0.0083)	0.0192 (±0.0010)
n (±S.D.)	1.0156 (±0.0524)	1.0202 (±0.0051)	0.9985 (±0.0110)	1.0159 (±0.0077)
r^2	0.9888	0.9314	0.9665	0.9631

al., 2003), charged dextran hydrogels (Van Tomme et al., 2006), and CS-alginates (Mladenovska et al., 2007).

3.3. *In vitro* drug release

3.3.1. *CAM stability*

Some authors have reported that CAM is quite unstable in acidic solutions (Erah et al., 1997; Nakagawa et al., 1992). In this study we compared the stability of CAM at 37 °C in two different pHs: 1.2 and 4.2. The degradation profile at pH 1.2 appeared to follow a pseudo-first-order kinetic (data not shown). The degradation rate constant (K_d) and half-life ($t_{1/2}$) were calculated, and the results were: 11.38 (±0.48) (h^{-1}) and 3.65 (±0.015) (min), respectively. Similar kinetics have been previously reported as pseudo-first-order and first-order kinetics (Chun et al., 2005; Nakagawa et al., 1992), and also the half-life obtained in these studies at similar pH was low, with a value of 10.4 min. However, at pH 4.2, CAM has demonstrated to be quite stable for 24 h, where there was still 96 (±2.14) (%) of remaining CAM. These results are in accordance with Nagawaka et al. (1992) who reported that CAM was stable for pHs above 3. These results confirm the need of using CAM in combination with gastro-protectors that can increase gastric pH above values of 3, such as proton pump inhibitors (PPIs). PPIs suppress gastric acid and can maintain the pH with a common median 24-h of 4.2 (Stedman and Barclay, 2000).

3.3.2. *The effect of CS molecular weight*

Fig. 6 shows the release profile of CAM from CS–CMC complexes. As CAM is degraded at pH 1.2, release data at this pH have been corrected by degradation in order to compare release rates. To investigate more precisely the effect of the interpolymeric complex formation on release of CAM at pH 1.2, results were analysed according to Ritger–Peppas equation (Eq. (7)) (Ritger and Peppas, 1987). In all cases, n values were higher than 0.5 (Table 3). These non-Fickian behaviours may suggest that CAM released from interpolymeric networks is controlled by a combination of drug diffusion and swelling processes. There was a significant difference (p-value = 0.0001) between release profiles depending on the molecular weight of CS. The fastest profile is obtained with the high M.wt. CS. This can be explained by the fact that high M.wt. CS leads to higher values of dissolution medium uptake per polymer remaining. But no significant differences were obtained between the diffusional exponent (n) of low and medium M.wt. chitosan complexes. This is further supported by the differences observed in the eroding process, suggesting the importance of the polymer erosion in drug release (Conti et al., 2007b; Lim Soo et al., 2008; Mladenovska et al., 2007). So, release of CAM from these systems is explained by a multiple mechanism: diffusion through the swollen system, eroding process and dissolution process.

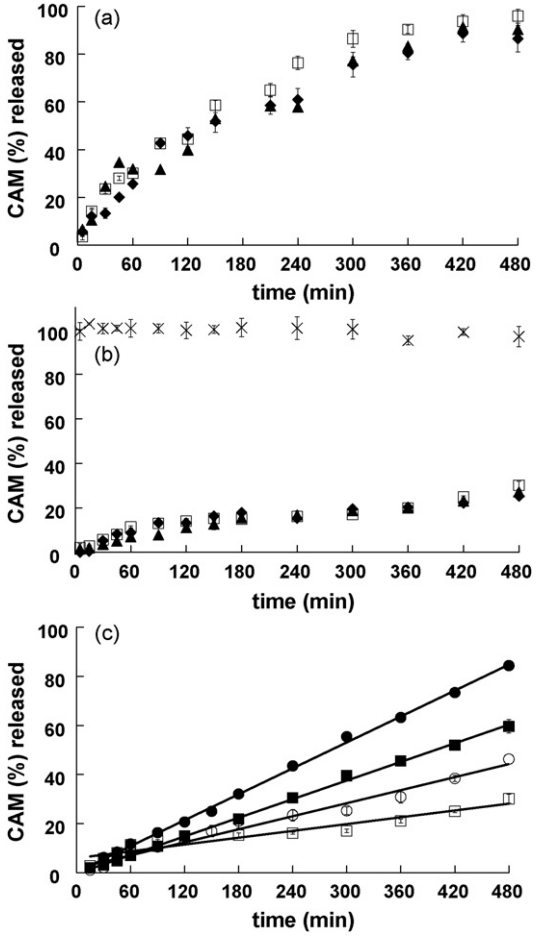

Fig. 6. *In vitro* release of CAM at 37 °C, at pH 1.2 (a) (where the data are corrected by degradation) and pH 4.2 (b) from CAM:[CS:CMC] 40:[25:75] (w/w) complexes with low M.wt. CS (♦), medium M.wt. CS (▲) and high M.wt. CS (□) and CAM raw material (×), ($n = 3$). (c) *In vitro* release of CAM at 4.2 from complexes with high M.wt. CS in the ratio CAM:[CS:CMC] (w/w) 80:[75:25] (●), 80:[25:75] (○), 40:[75:25] (■) and 40:[25:75] (□). (—) Fitted experimental data, ($n = 3$).

Importantly, the mini-matrices prepared permitted an efficient interpenetration of CAM within the structure of the interpolymeric

Table 3
Estimated parameters obtained from fitting drug release experimental data to zero-order model (Eq. (6)) and to Ridger–Peppas model (Eq. (7)) $n = 3$

CAM:CS:CMC complexes		pH 1.2			pH 4.2	
% (w/w)	Type of CS	n (±S.D.)	K_1 (±S.D.) h^{-1}	r^2	K_d (±S.D.) h^{-1}	r^2
40:[25:75]	Low M.wt.	0.5401 (±0.0484)	1.498 (±0.0099)	0.9759	0.0472 (±0.0241)	0.9174
40:[25:75]	Med M.wt.	0.5884 (±0.0569)	1.3629 (±0.008)	0.9949	0.0509 (±0.0073)	0.9634
40:[25:75]	High M.wt.	0.7899 (±0.0918)	1.5495 (±0.0098)	0.9628	0.0492 (±0.0120)	0.9082
40:[75:25]	High M.wt.	–	–	–	0.0886 (±0.0025)	0.9785
80:[25:75]	High M.wt.	–	–	–	0.1761 (±0.0019)	0.9985
80:[75:25]	High M.wt.	–	–	–	2.1527 (±0.0904)	0.9051

network. This can be justified by the low initial burst shown on the release profiles (De la Torre et al., 2003; Lim Soo et al., 2008). All systems presented suitable controlled-release profiles for oral devices. At this pH (1.2), for initial times (15 min) the amount of CAM released was low, about less than 15%. These results show proper release in early stages for controlled-release devices comprised of CS (Patel and Patel, 2007). A value of 50% was obtained at approximately 3 h, and finally, the maximum was reached at about 7 h.

However, at pH 4.2, drug release can be adjusted to zero-order kinetics (Eq. (6)) with r^2 higher than 0.90 in all cases (Table 3). The fact that we have achieved order-zero kinetics is very important, because typically these kinetics are the most desirable for extended-release dosage forms in order to maintain steady state plasma profiles (Shah, 1988). The systems 40:[25:75] showed slower release profiles than at pH 1.2, with only 20% of CAM released at 6 h (Fig. 6b). These profiles are independent of the molecular weight of CS because the statistical study showed that there were no significant differences (p-value = 0.4020), as already shown by Mladenovska et al. (2007).

These results show that the release rate of CAM depends on the pH of the medium. The slow release rate of CAM at pH 4.2 appears to result of its low solubility at this pH. Therefore, at this pH the diffusion process could not play as much an important role in the release of CAM as the dissolution and the erosion processes do.

3.3.3. *The effect of the polymer proportion*

The effect of the proportion of CS and CMC within the interpolymer network on drug release at pH 4.2 is depicted in Fig. 6c. All profiles can be adjusted to zero-order kinetics (Eq. (6)) with r^2 higher than 0.90 (Table 3). These zero-order kinetics coincide with their dissolution medium uptake capacity and erosion behaviour. So, swelling and erosion processes play an important role in the release of CAM. The highest profiles are obtained from systems with high CS proportion, such as 40:[75:25] and 80:[75:25] (w/w), and also, they were the complexes with the highest swelling and erosion rates. These result are in accordance to the fact that the presence of more cationic moieties from CS which can be ionized and, consistently, major expansion of the systems and eroding process because less pendants groups are involved in hydrogen bonding. So, a greater released was obtained from these complexes (Chellat et al., 2000; De la Torre et al., 2003; Mladenovska et al., 2007).

The absent of burst effects in all profiles justified the efficient interpenetration of CAM within the network. At 2 h, the amount released is lower than 20%. And at 8 h the amount is 85 and 60% for 80:[75:25] and 40:[75:25] (w/w), respectively. However, for the complexes 40:[25:75] and 80:[25:75] (w/w), the CAM released is much lower, with less than 50% at 8 h.

In general, CAM release from polyionic complexes was controlled by the swelling/eroding process, where the higher degree of ionization, the higher the expansion of the network, and consistently, the higher erosion and the higher CAM released.

4. Conclusions

A controlled-release gastro-retentive system was obtained by the novel method “tablets-in-capsule”, where mini-matrices containing CAM and composed of CS and CMC in the proportion 80:[75:25] (w/w) have demonstrated to have suitable swelling properties and the most suitable drug release profile.

Swelling kinetics of CS–CMC complexes have shown to be dependent on the pH of the medium, on the molecular weight of CS and on the CS:CMC proportion, due to the presence of cationic moieties from CS within the interpolymeric network structure that can modify hydrogen bonding abilities. Furthermore, CAM release from CS–CMC complexes has shown to be dependent on the pH of the medium and on the proportion of CS:CMC. At pH 1.2, the release rate was faster when using high M.wt. CS as a result of major dissolution medium uptake capacity. While at ph 4.2, solubility of CAM has shown to play an important role on drug release. It was suggested that the pH of the medium, the erosion characteristics of the polymer, the pK_a of the drug and the polymer-polymer interaction were important factors governing the drug release patterns from CS–CMC complexes. All these factors have to be considered when designing and characterizing the physical properties for stomach site-specific delivery systems based on IPCs.

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