

# Studies of Ciprofloxacin Encapsulation on Alginate/Pectin Matrixes and Its Relationship with Biodisponibility

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**Abstract** Screening of ciprofloxacin (Cip) with selected biopolymers brings about 90% antibiotic interactions with a coacervate composed of alginate/high methoxylated pectin in 2:1 ratio. Fourier transform infrared spectroscopy analysis provides information about the nature of this interaction, revealing ionic and hydrophobic patterns among the molecules. Alginate/high methoxylated pectin gel microspheres developed by ionic gelation encapsulates  $46.8 \pm 5.0\%$  Cip. The gel matrix can release Cip in a sustained manner, releasing  $42.7 \pm 0.2\%$  in 2 h under simulated stomach pH conditions, and  $83.3 \pm 1.1\%$  Cip release in 80 mM phosphate at pH=7.40 (intestinal). The increase of sodium chloride from 50 to 200 mM implies a Cip release from  $69.0 \pm 1.5\%$  to  $95.1 \pm 3.6\%$  respectively in 2 h. Scanning electron microscopy revealed the cohesive effect of HM pectin over alginate molecules on the microsphere surface. Those results guarantee all Cip contained in the alginate/HM pectin microspheres could be released in an established kinetic profile along the gastrointestinal tract, avoiding the Cip undesirable side effects during absorption.

**Keywords** Pectin · Alginate · Biopolymer blends · Ciprofloxacin · Controlled release

## Introduction

Ciprofloxacin (Cip) is the fifth largest generic antibiotic produced in the world keeping 24% of the therapeutic prescription market (about US \$2.3 million). Cip (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) belongs to the fluoroquinolone antibiotic family, a wide class of antibiotics with broad antibacterial spectrum [1]. Cip is currently used in many infections such as clinical respiratory, urinary tract, and

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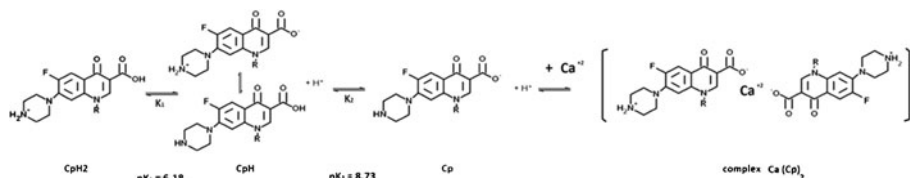
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intestinal, intra-abdominal, gynecologic, infections of bones and joints and systemic treatment used either in human and veterinary diseases [2]. However, Cip administration is commonly associated with many undesirable and diverse physiological side effects that involve many organs [3]. Many of the reported problems could be associated to a poor drug biodisponibility related to Cip low solubility in physiological media. Cip is a “sticky” drug interacting with many molecules from membrane bilayers to proteins such as glucose transporter type 1 and BSA among others [4–6]. In addition, Cip stacking phenomena can surpass drug solubility and becoming highly toxic [7].

From the structural point of view, the Cip molecule contains two ionizable groups: a carboxylic side chain at C-3 ( $pK_a=6.16$ ), and a piperazinyl group at C-7 ( $pK_a=8.62$ ) [8], which are able to form a zwitterionic structure in the pH range of 6.16 to 8.62 (Fig. 1). In addition, Cip molecule posses a polarizable C-4 keto group in the quinolone ring. The C-3 carboxylate group is able to form intra- and/or inter-molecular H-bridge with the carbonyl group in C-4 position. Based on the presence of the ionic groups and the quinolone aromatic ring (hydrophobic motif), the performance of Cip is complex and strongly influenced by environmental physico-chemical conditions.

In oral administration, Cip is dissolved in the stomach in about 2 h [9], and consequently the biodisponibility of the antibiotic associated to the intestinal uptake will be determined by the residence time of drug, physicochemical and biological properties, dose, and drug formulation among other relevant factors. In a recent work, the levels of Cip in ill patients determined in plasma were under the required dose because of the complex pharmacokinetic of the antibiotic [10].

In order to avoid undesirable secondary effects, reduced antibiotic concentration and activity by stacking, controlled release techniques can be a feasible alternative for drug delivery. Alleviation of Cip secondary toxic effects in the body and increase antibiotic efficiency and decreasing the chances of developing antibiotic-resistant mechanisms are the main advantages of controlled release systems. Particularly, the challenges of oral delivery are of specific interest in order to reduce the cost and potential health risks related to parenteral administration of drugs. Matrices for drug delivery are regularly using synthetic polymers, which can cause adverse side effect by product of its partial and/or total polymer degradation. Other options as drug carriers are liposomes, but most of them are unstable, difficult to determine the concentration of the loaded cargo, and unpredictable drug-release kinetics. On the other side, biopolymeric-based formulations are non-toxic, safe, can be tailored and targeted, posses well-known loading capabilities, high stability, and well-established release kinetics. Among biopolymers, alginates are regularly used in living systems for different purposes [11]. Alginate is a linear polysaccharide of  $\beta$ -D-mannuronic (M) and  $\alpha$ -L-guluronic (G) acids found in algae and some bacteria that can be gelled by multivalent cations making a gel structure know as *egg-box*. Besides, alginate gels are unstable in presence of cation chelating agents such as phosphate and/or competing non-gelling cations such as sodium or potassium, which are present in biological fluids. Additionally, alginate gels are sensitive to shear and desiccation.



**Fig. 1** Cip speciation at different pH values and displacement of equilibrium to Cip–calcium complex formation at alkaline pH

Pectins are water-soluble polysaccharides present in the plant cell wall. The use of pectins as matrix in oral drug delivery devices was proposed [12]. Pectins are not degradable by intestinal enzymes but degradable by the intestinal flora, which is an advantage for cargo-sensitive molecules to avoid hydrolytic process (e.g., in proteins) being excellent carriers for intestinal drug delivery. Pectins are composed of linear polysaccharides of partially methoxylated poly- $\alpha$ -(1,4)-D-galacturonic acids. The esterification degree (ED) of pectins has strong influence on biopolymer properties. Pectins can be grouped into low methoxylated (LM) with ED below of 40%, medium methoxylated (MM) with ED range between 40–60%, and high methoxylated pectins (HM) with ED higher than 60%. LM and MM pectins can be gelled by multivalent cations, meanwhile HM only by acid pH and in presence of solutes [13]. Besides the differences among pectin interaction with ions, the pectin  $pK_a$ s are ranging from 3.5 to 4.1 [14].

Biopolymer blends is an alternative strategy for molecular- controlled release to increase formulation stability, the amount of cargo drug in the matrix and to target specific organs. Besides, interactions among the cargo and other formulation components should be determined. Alginates and pectins showed interesting synergistic properties forming mixed gels that lead to a microstructure very different from that of biopolymers alone. The ability of alginates to make strong and insoluble gels with calcium ions are use to produce microspheres [15]. However, gel formation of alginate with calcium ions is so instantaneous that it extrusion of molecules cargo could occur. Additionally, alginate gel networks are susceptible to disintegration in the presence of excess monovalent ions,  $Ca^{2+}$ -chelating agents and harsh chemical environments. Some authors have reported that alginates can form strong complexes with other natural polyelectrolytes such as pectin (also a polyuronate) by undergoing chain-chain association and forming hydrogels upon addition of multivalent cations (e.g.,  $Ca^{2+}$ ) [16]. The gel blend showed an enhanced mechanical and chemical stability compared with alginate beads [17]. In this way, the nature of the pectin with different ED could possibly affect the network density and the properties of gel microspheres [18] and concomitantly modify the release profile of Cip.

The aim of the present work is to study the encapsulation of Cip in biopolymeric blend gel matrices for oral drug delivery. In order to get the rational of Cip encapsulation on gel matrix, studies of the Cip microspeciation under different pH conditions, and the interaction with pectin containing different ED was analyzed. Determination of the molecular interactions among Cip and the matrices were analyzed and established by Fourier transform infrared spectroscopy (FTIR)s. Gelling conditions to optimize the Cip encapsulation were studied. Kinetic experiments of Cip release from microspheres were determined in vitro by simulating gastric and intestinal environments. Scanning electron microscopy of gel microspheres was performed to determine bead morphologies and swelling under different experimental conditions.

## Materials and Methods

### Materials

Cip (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) was purchased from Sigma-Aldrich (St. Louis, Mo). Low-viscosity sodium alginate ( $MW_{av}=120$  kDa) was a gift from Monsanto (Buenos Aires, Argentina). LM (ED, 33%,  $MW_{av}=156$  kDa), MM (ED, 55.3%,  $MW_{av}=158$  kDa), and HM (ED, 74%,  $MW_{av}=160$  kDa) pectins were kindly provided by C.P. Kelco (Buenos Aires, Argentina).

## Determination of Cip and Biopolymers Interactions

In order to select the appropriate biopolymer to be blend with alginate, interaction assays between different biopolymers with Cip were carried out. Solutions of 2.0–3.0% (w/v) alginate and 1.0% (w/v) pectin (LMP, MMP, or HMP) were used to make different biopolymer blends. Four hundred ten microliters of each biopolymer solution was mixed with 90  $\mu$ l of Cip solution (100  $\mu$ g/ml) and stirred for 1 h at room temperature. Later, 1.0 ml of cold ethanol (96.0%) was added to precipitate the biopolymer complex [19]. The resulting suspension was centrifuged at 10,000 $\times$ g. for 10 min. Remaining Cip in the supernatant was determined and then the percentage of Cip binding was calculated.

Equilibrium constant of Cip biopolymers were determined considering 1:1 ratio between the antibiotic and each polymer at pH 4.0 and 25 °C. Quantitation of free Cip at equilibrium was measured as mentioned before.

## Vibrational Spectroscopic Analysis

Free Cip and Cip-biopolymer blend complexes were analyzed by ATR-FTIR at the SMIS beamline (Soleil, French National Synchrotron Facility, France). Additionally, samples were analyzed by FTIR (JASCO FT/IR-4200). Pellets were prepared by mixing the samples at 5.0% (w/w) with potassium bromide (Pike technologies) and scanned with background correction at 256 number scan, against a high-energy ceramic source and DLATGS detector.

## Preparation of Alginate and Alginate/Pectin-Based Microspheres Containing Cip

Aqueous solutions containing 36.0  $\mu$ g/ml of Cip and 2.0% (w/v) alginate were adjusted at different pH values (4.0, 5.0, and 6.2). Alternatively, biopolymer blends containing the antibiotic were made with 1.0% or 2.0% (w/v) alginate and 1.0% or 2.0% (w/v) HM pectin. Microspheres were prepared by jet technique, dropping 2.0 ml of alginate solutions in a 500-mM  $\text{CaCl}_2$  solutions, previously adjusted to the respective pH values, under gently stirring in an ice-cooled bath for 20 min. Later, the microspheres were washed with ultrapure water and matured  $\text{CaCl}_2$  solution. Furthermore, the beads were dried on cellulose paper (Whatman # 1) at room temperature for 5 min.

## Measurement and Calculation of the Cip Encapsulation Percentage

Cip in solution were spectrophotometrically quantified at the maximum absorption peak ranging from 270 to 277 nm (depending on pH values) with appropriate calibration curves in the linear range. Encapsulated Cip was determined by the difference between total and the remaining antibiotic concentration in the supernatant after microsphere formation and calculated as follows:

$$\text{Encaps(\%)} = \frac{(Q_0 - (\text{Cr} \times V)) \times 100}{Q_0} \quad (1)$$

In where,  $Q_0$  is initial amount of Cip (in micrograms), Cr is concentration of Cip in the filtered solution (in micrograms per milliliter), and  $V$  is volume of filtrated solution (in milliliters).

Additionally, the cargo of gel microspheres were evaluated by weighting 100 mg and incubating them in 2.0 ml of 100 mM phosphate buffer solution at pH=7.40 for 1 h (until total gel microsphere dissolution). The Cip content in the supernatant was assayed after centrifugation.

## Scanning Electron Microscopy

Microspheres were freeze-dried during 72 h before scanning electron microscopy (SEM) analysis. Furthermore, samples were prepared by sputtering the surface with gold using a Balzers SCD 030 metalizer obtaining a layer thickness between 15–20 nm. Microsphere surfaces and morphologies were observed using Philips SEM 505 (Rochester, USA), and processed by an image digitalizer program (Soft Imaging System ADDA II (SIS)).

## Release of Cip from Microspheres

### *Release at Simulated Gastric and Intestinal Conditions*

Cip release was evaluated in simulated gastric fluid and simulated intestinal fluid following the US Pharmacopeia without enzymes (USPCCE 2004). In simulated gastric fluid experiments 350.0 mg of microspheres were weighted and incubated in 50 mM KCl/HCl buffer solution (pH=1.20) at 37 °C and 100 rpm. Samples were taken at different times and Cip was measured at the maximum absorbance wavelength in this buffer ( $\lambda=277$  nm). In order to keep a constant vial volume of 1.0 ml of fresh media was refilled at each sample point.

Similar experiments simulating intestinal environment using microspheres at pH 7.4 media in 10- to 100-mM phosphate buffers were performed as mentioned before. The Cip release was determined after 2 h of incubation.

### Effect of Ionic Strength on Cip Release from the Microspheres

The effect of ionic strength on the release of Cip from the microspheres was evaluated with different amounts of NaCl (from 50 to 200 mM) in 10-mM phosphate buffer solution (pH=7.40). Cip release was determined in 350 mg of Cip-loaded microsphere samples incubated at 37 °C for 2 h.

## Results and Discussion

Cip is an amphoteric quinolone containing two proton-binding sites at carboxyl group and amine group (N4') from the piperazinyl ring, which  $pK_a$ s are 6.18 and 8.73, respectively [20]. Cip molecules exist in solution as four microspecies, according to the degree of deprotonation of the ionizable groups, namely positive ( $CpH_2^+$ ), zwitterionic and neutral ( $CpH$ ) and negative forms ( $Cp^-$ ) as is represented in Fig. 1. Abundance of Cip carboxyl group species in the protonated state as function of pH was estimated using the equilibrium constants. When pH is approaching to 6.0, the proportion of Cip molecules with the carboxyl group ionized became higher (Table 1), being 50.7% at pH=6.20. Cip UV–vis scanning showed three absorption maximums at UV and changes in the electronic structure of Cip generate shifts in those peaks around pH 6, indicating the relevant effect of carboxyl group in Cip molecule. Deprotonation of piperazyl group does not modify the electronic distribution of the Cip molecule, because is not conjugated with the aromatic ring.

Considering ionotropic gelation procedure for the selected biopolymers, interaction among the cargo, Cip and calcium ion was analyzed. In previous work, an increase of antibiotic solubility by complex formation between divalent cations and Cip toward the C3 and C4 interactions was reported [21]. The interaction of Cip and calcium ion at two pH values is shown in Table 2. No changes on the maximum wavelengths at acid pH (1.2) were

**Table 1** Speciation of ciprofloxacin carboxyl group in protonated or ionized form at different pH values

	pH	$F(\text{COOH})$	$F(\text{COO}^-)$
	1.20	0.999	0.001
	4.00	0.994	0.006
	5.00	0.939	0.061
Calculated from Sun <i>et al.</i> [20]	6.20	0.493	0.507
$F(X)$ fraction of ciprofloxacin molecules with carboxyl group in protonated or ionized form	7.40	0.056	0.944
	12.00	0.001	0.999

observed, suggesting no interaction between the protonated carboxyl group and the divalent calcium ion occurs. On the contrary, at pH 12 and based on Table 1 results, 99.9% of carboxyl group of Cip molecule is deprotonated and 3-nm shift were detected in all absorption maxims, suggesting an ionic interaction between calcium ions and Cip, as it can be expected.

For controlled release purposes, alginate-divalent ions crosslinking is providing an excellent matrix for drugs to be targeted to the intestine where the alkaline pH will disintegrate the gel network slowly and the complex formation between Cip and calcium. The increase in calcium concentration during ionotropic polymer gelation makes a stronger gel network, due to a thicker “egg-box” model between alginate chains and divalent ions [22]. Besides, encapsulation of Cip in the pH 4.0 to 5.0 range does not show significant differences ( $p > 0.05$ ). However, Cip on alginate gels showed a decreasing encapsulation trend at pH 6.2 and with the increase of calcium ion concentration. This fact could be attributed to the interaction of the carboxylate ion of Cip with calcium in solution. Another factor is the equilibrium displacement between ionized and non-ionized Cip species produced by complex formation making Cip more soluble in aqueous environment rather than in alginate gel matrix. Both situations leads to a decrease the amount of Cip entrapped into the gel (see Tables 1 and 3; Fig. 1).

According to the results of Table 3, Cip encapsulation at pH=4.0 is desirable for the enhanced antibiotic stability, and because of the highest encapsulation percentage obtained:  $46.4 \pm 1.7\%$  in presence of 500 mM  $\text{Ca}^{+2}$ . Additionally at pH 4.0, calcium–Cip complex formation is less relevant (Table 2), and Cip would be remain inside the gel matrix providing a reliable release profile and decreasing the solubility toward stomach media by the calcium ion. Thus, when Cip loaded microspheres reach first portion of intestine, the drug will be released around its isoelectric point as a neutral and/or zwitterionic molecule, which directly affect absorption through epithelial cell wall (where hydrophobic molecules have predilection to come across) and passage toward bacteria cytoplasm through the cell membrane.

**Table 2** Shift on absorption spectrum of ciprofloxacin at different pH values due to interaction with calcium

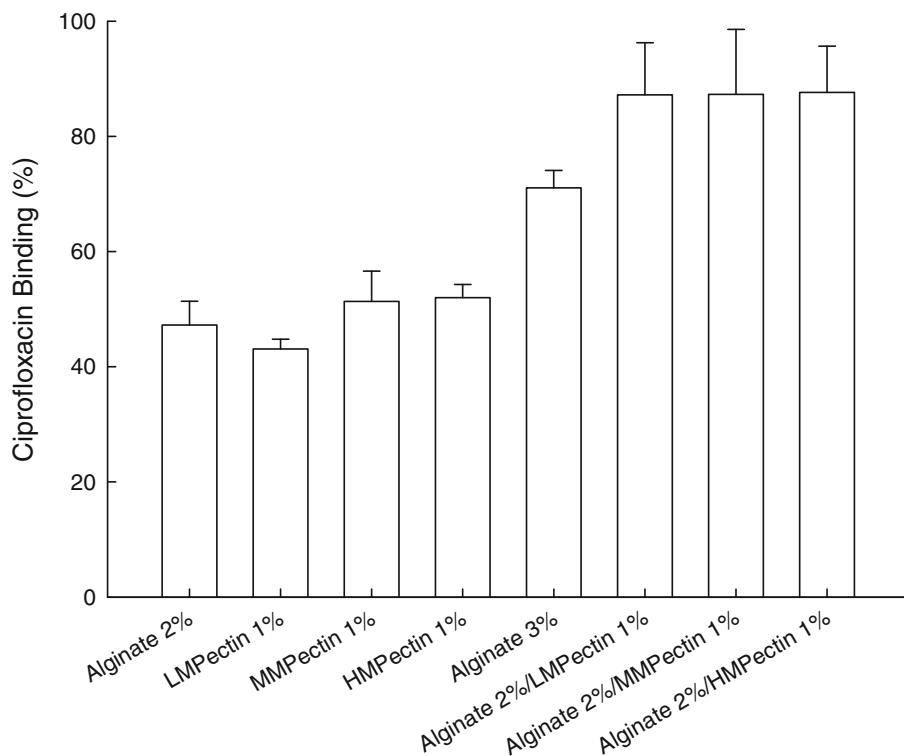
pH	Maximum wavelengths (nm)		
	$\lambda_1$	$\lambda_2$	$\lambda_3$
1.20	277	314	330
1.20+ $\text{Ca}^{+2}$	277	314	330
12.00	271	323	333
12.00+ $\text{Ca}^{+2}$	274	320	330

**Table 3** Effect of pH and Ca(II) concentration on ciprofloxacin encapsulation on alginate microspheres

Ca <sup>+2</sup> (mM)	Ciprofloxacin encapsulation (%) at pH		
	4.0	5.0	6.2
100	42.0±0.6	44.8±0.9	40.0±1.8
250	45.3±3.1	42.1±2.0	38.1±1.9
500	46.4±1.7*	42.9±2.9	37.2±3.7*
750	46.8±2.5	42.1±1.8	37.8±0.2

\* $p<0.01$ , significant differences

Figure 2 showed the interaction between Cip with pectins of different methoxylated degrees and their blends with alginate at pH 4.0. The Cip binding to alginate increased 1.5 times (from 47.2% to 71.1%) when the alginate concentration rose from 2.0% to 3.0%. The data suggest that Cip–alginate interaction is mainly through electrostatic forces, because at pH 4.0, alginate possesses all carboxyl groups ionized displaying negative charge in the molecule. On the other hand, Cip is positively charged (if  $\text{pH} < \text{pK}_a = 6.1$ ) with both ionic groups (carboxyl and amine) in the protonated state. However, Cip interactions with LM, MM, and HM pectins displayed more strong interaction than alginate. 1.0% pectins, instead of 2.0% alginate, displayed an interaction degree with Cip around 50%. Significant differences between LM pectin ( $43.1 \pm 1.7\%$ ) and HM pectin ( $52.0 \pm 2.3\%$ ) were found ( $p < 0.01$ ).

**Fig. 2** Interaction between Cip and methoxylated pectins and their alginate blends: alginate, low methoxyl pectin (ED, 33.0%), medium methoxyl pectin (ED, 55.3%), and high methoxyl pectin (ED, 74.0 %)

**Table 4** Determination of equilibrium constants of biopolymers and ciprofloxacin at pH 4.0 and 25°

Polymers	$K_{eq}$ (mM <sup>-1</sup> )
Alginate	5.42
LM pectin	10.6
MM pectin	14.5
HM pectin	16.1

The differences could be explained by the presence of more than one type of interaction, meaning ionic interaction, with Cip, and may be associated to hydrophobic pectin pockets (see below). Thus, HM pectin would be a good candidate to be blend with alginate. Despite alginate–pectin blends have showed no significant differences ( $p=0.05$ ) in the interaction with Cip between themselves. They had important differences compared with 3.0% alginate ( $p=0.05$ ), showing high interaction (around 90%), which justify the use of pectin (especially HM pectin) to be blend with 2.0% alginate.

Interactions between Cip and all tested biopolymers are highly favored based on the values of the equilibrium constants (Table 4). The  $K_{eq}$  for all tested polymers are in the same range, though  $K_{eq}$  for Cip–pectins are two to three times higher than  $K_{eq}$  of Cip–alginate. Additionally, the  $K_{eq}$  for Cip–pectins increases as the pectin methoxylation degree suggesting a relevant role of hydrophobic interaction between Cip and the methoxylated pockets of the pectin molecule (Table 4).

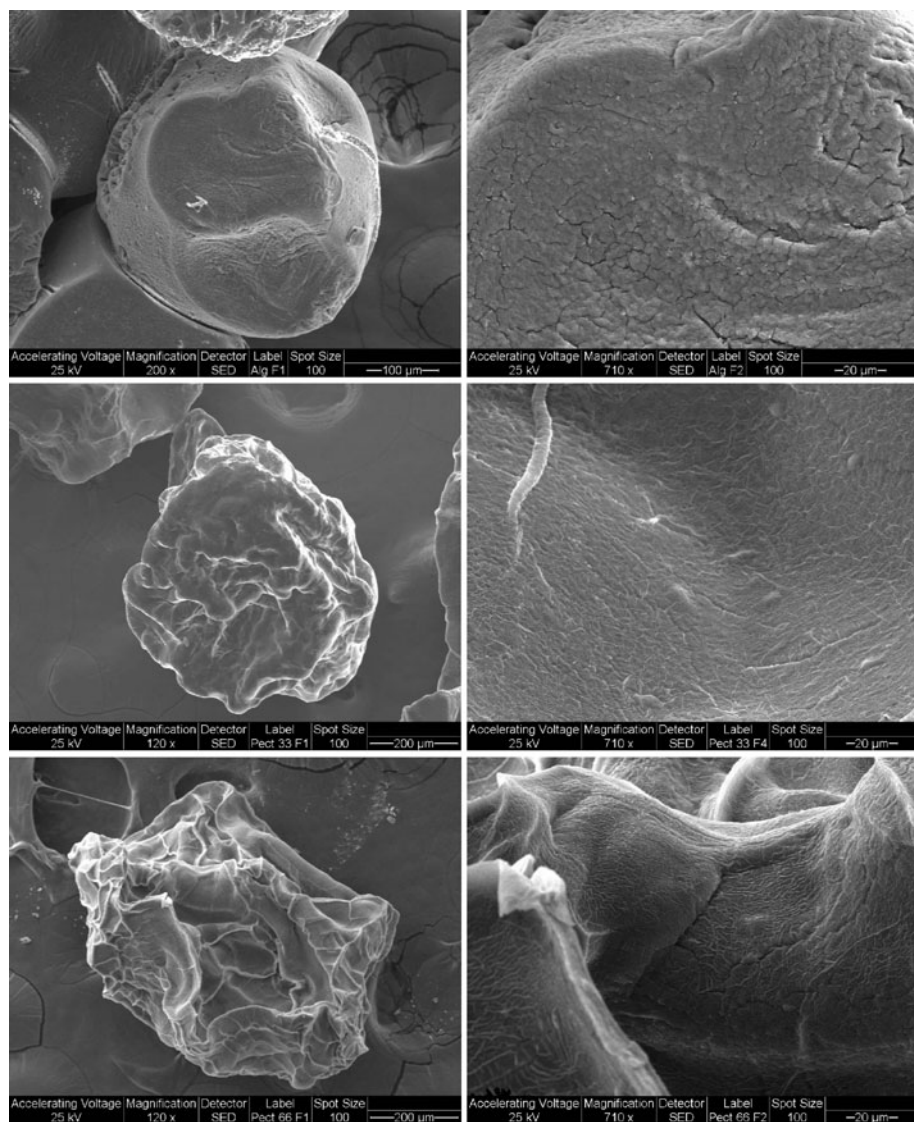
Table 5 is displaying the relevant assignments of Cip and HM pectin peaks provided by FTIR and ATR-FTIR (Soleil, French National Synchrotron Facility, France) [23, 24]. Characteristic peak of Cip carboxyl group shifted to a higher value from 1,709 to 1,725 cm<sup>-1</sup>. In addition, the band associated to the protonation of amine group in the piperazine moiety is shifted 4 cm<sup>-1</sup>, indicating ionic interaction between Cip and HM pectin. Moreover, the carboxyl group of HM pectin shifted from 1,609 to 1,626 cm<sup>-1</sup>. Another kind of interaction was elucidated when bands associated to the aromatic ring of the quinolone and the methyl esters groups of HM pectin shifted from 1,743 to 1,726 cm<sup>-1</sup>, suggesting a possibly hydrophobic interaction made by the pectin pockets composed by methoxylated

**Table 5** FTIR band positions (cm<sup>-1</sup>) and assignments for ciprofloxacin (Cip) molecule, HM pectin (solid state), and formulation Cip/HM pectin at pH=4.0

Wavenumbers (cm <sup>-1</sup> )			Assignments
Cip	HM pectin	Formulation	
1,709	–	1,725	$\nu$ ( $\phi$ -COOH)
1,625	–	1,630	$\nu$ ( $\phi$ =O) and phenyl breathing modes
1,552	–	1,542	N–H flexion piperazyl
1,518	–	1,507	N–H flexion piperazyl
1,385	–	1,389	Protonation of amine group in the piperazine moiety
1,343	–	1,339	(–R–CO–R) aromatic flexion
987	–	–	–CH bend and phenyl
–	1,743	1,726	$\nu$ (–COOCH <sub>3</sub> )
–	1,609	1,626	$\nu$ (–COOH)

carboxylates. On the other side, hydrogen bonds can contributed to strength the interaction between Cip and HM pectins and water molecules coordinating them [25].

As HM pectin was selected to form blends with alginate, different proportions of both biopolymers in the coacervate were analyzed by SEM. SEM images of alginate microspheres showed a rigid structure with appreciable spherical structure but displaying cracks on the bead surface (Fig. 3a, b). Incorporation of 1.0% HM pectin to alginate makes cohesive effect giving a smother surface with a less rigid structure (Fig. 3c, d). Increase in HM pectin content on the blend resulted in production of microspheres with no symmetry



**Fig. 3** SEM images of alginate and HM pectin blends: **a, b** 2.0% alginate, **c, d** 2.0% alginate/1.0% HM pectin, and **e, f** 1.0% alginate/2.0% HM pectin

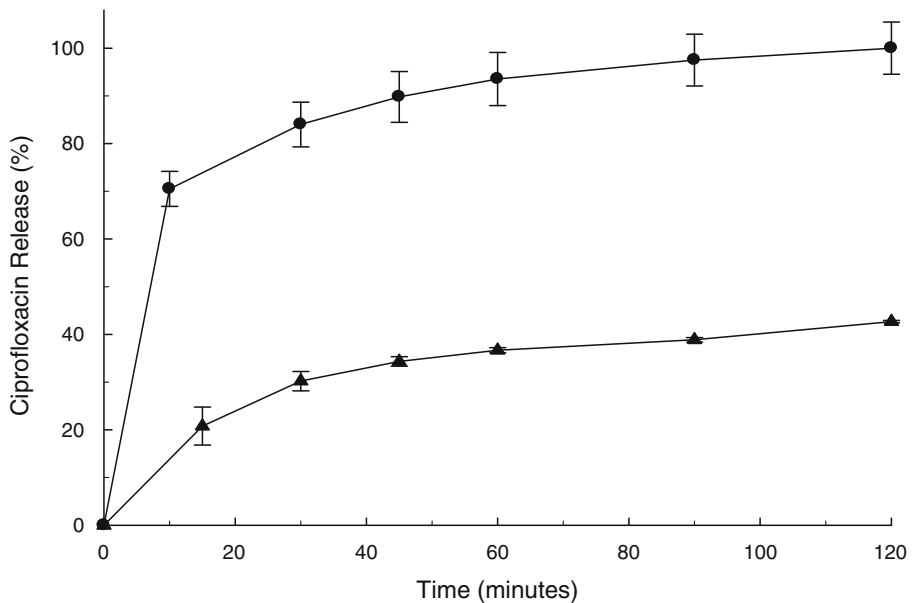
and folds on the surface (Fig. 3e, f). HM pectin–alginate blend showed fastest gel formation kinetics, indicating a strong synergism between alginate with low  $M/G$  ratio and pectin with a high methoxylation degree.

The entrapment efficiency of Cip within alginate/HM pectin blends (ratio, 2:1 and 1:2) microspheres was found in the order of 50% ( $46.8 \pm 5.0\%$  and  $49.6 \pm 0.9\%$ , respectively). No significant differences ( $p > 0.05$ ) of Cip encapsulation capabilities for both coacervates and alginate matrix were found. These results are indicating that almost the same amount of antibiotic is entrapped in all tested matrices during the ionotropic gelation process.

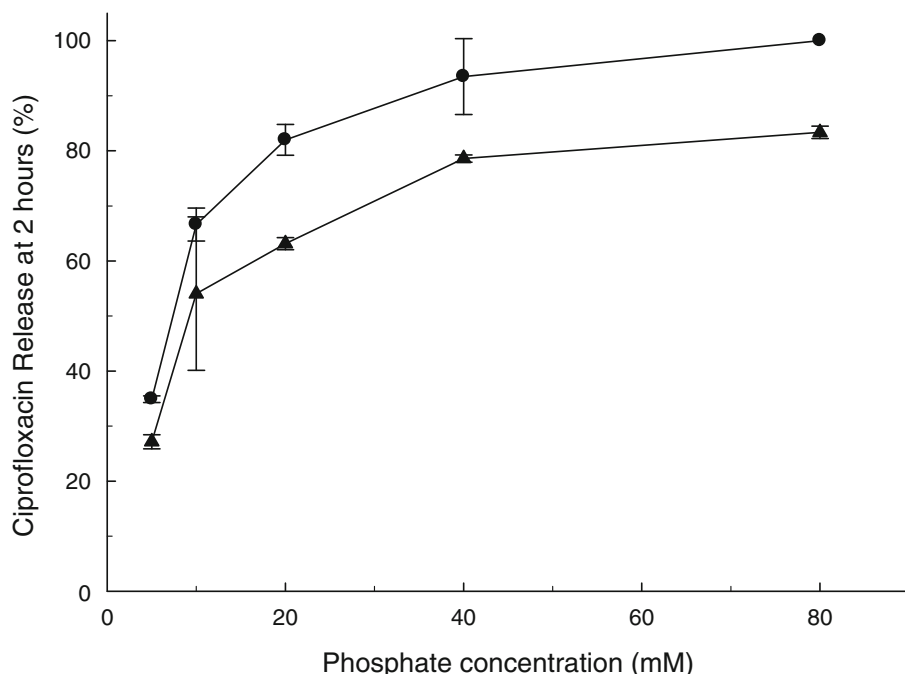
Screening of kinetic release from the different formulations was done at pH=1.20 and 30 °C in order to select the final blend composition. The 2.0% alginate/1.0% HM pectin blend showed a 34.2% Cip release after 3 h of incubation which was lower compared with the other formulations ( $p = 0.01$ ; data not shown).

Figure 4 displayed the Cip kinetic release from alginate and alginate/HM pectin blend (ratio, 2:1) gel microspheres in simulated gastric media at pH=1.20 at 37 °C. Alginate/HM pectin microspheres released  $42.7 \pm 0.2\%$  of the initial drug in 2 h, almost 60% less than alginate formulation. In this way, the antibiotic is released at slow rate and consequently high drug amounts will be able to reach intestine, where Cip has the best absorption properties [26].

In the intestine, not only the slight alkaline pH (around 7.4) is affecting the microsphere swelling but also presence of quelating activities of phosphates are enhancing the Cip release from the beads. Calcium-phosphate complex formation is favored since the  $K_{ps}$  of  $(Ca_3(PO_4)_2) \approx 2.07 \times 10^{-33}$  [27]. In vitro intestinal simulated environmental conditions showed a Cip release from the coacervate gel matrix of  $27.2 \pm 1.3\%$  to  $83.3 \pm 1.1\%$  in presence of 5 to 80 mM phosphate at 37 °C for 2 h (Fig. 5). These values are showing a decrease of Cip release of about 20% from the gel blend compared with the alginate ones.



**Fig. 4** Release kinetic of Cip from 2.0% alginate (circles) and 2.0% alginate/1.0 % HM pectin (triangles) gel microspheres at pH=1.20 and 37 °C



**Fig. 5** Effect of phosphate concentration on Cip release from gel microspheres of 2.0% alginate (circles) and 2.0% alginate/1.0% HM pectin (triangles) at pH=7.40 in 2 h

Stability of alginate gel matrices was also affected by the presence of Na(I) ions, which displaced Ca(II) present in the gel network. However, The Cip release from alginate/HM pectin gel coacervates were reduced from  $66.6 \pm 3.0\%$  to  $54.1 \pm 5.1\%$  (18.8 reduction ratio) compared with the alginate gel matrix (ionic strength of 11.3 mM). Besides, at sodium concentrations higher than 90 mM, no differences were observed on Cip release in both type of matrices under the same experimental conditions. Besides the presence of phosphate, Na(I) and K(I) in human fluids, at the alkaline pH of the intestine, generally higher than 7.0, repulsive effect of carboxylate ions of alginate can cause an increase in the matrix relaxation [28].

According to the showed results, combination of phosphate and sodium ions and alkaline pH values in the intestinal fluid would enhance the release of Cip from the microspheres to the intestine lumen. In addition, Cip equilibrium at alkaline pH was displaced to neutral and zwitterionic species, leading to a best assimilation and permeation through intestinal epithelium and bacterial cell membrane.

Dehydration of alginate beads generated changes in the particle size but keeping spherical morphology. However, alginate gel rehydration process did not allow recovering the initial morphology (data not shown), possibly due to modifications of the crosslinking mediated by hydrogen bonds in the sites occupied by water molecules inside the biopolymeric network [29]. Incorporation of HM pectin on alginate gave rise to production of less spherical beads, which was observed in all the hydration states.

The swelling degree was considerable higher for alginate microspheres containing HM pectin, with a difference of around 190% with alginate microspheres at 15 min and more than 300% at 30 min. However, both gel matrices showed no significant differences ( $p=0.05$ ) in the percentage of dehydration— $88.2 \pm 2.4\%$  and  $90.2 \pm 0.9\%$  for alginate and alginate/HM pectin,

respectively. These results, in accordance with the images from Fig. 3, suggest that HM pectin generate changes on alginate gel network structure and the increase of water absorption capacity. In addition, HM pectin would be generating new interactions in the polymerized alginate and even in the presence of Cip.

## Conclusions

Alginate/HM pectin microspheres containing Cip were prepared by ionotropic gelation using calcium as crosslinker under acid conditions. Different composition of biopolymer blends were tested by interactions assays, equilibrium constant, SEM images, and release studies. The coacervate blend with desirable properties was alginate/HM pectin (2:1 ratio). Interactions between the components were established by FTIR analysis. Alginate/HM pectin microspheres release at simulated gastric pH in 2 h, only 42.72% of the initial Cip encapsulated. Cip release in simulated intestinal pH with different concentrations of phosphate and sodium ions produced a fast antibiotic release associated with the matrix disaggregation.

Commercial available formulations commonly solubilize 30% of Cip content in the gastrointestinal tract in 2 h [21]. The low release leads to problems associate to a low biodisponibility, leaving most of the drug stacked and not assimilated by the organism with toxic consequences. The system developed in the present work based on alginate/HM pectin provides better conditions for Cip oral delivery, not only because reduction in the antibiotic release in simulated gastric conditions, but also for releases most of Cip content in intestinal lumen, improving its biodisponibility and decreasing the Cip toxicity.

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