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Mucoadhesive beads of gellan gum/pectin intended to controlled delivery of drugs

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ABSTRACT

Gellan gum/pectin beads were prepared by ionotropic gelation, using Al^{3+} as crosslinker. High yield (92.76%) and entrapment efficiency (52.22–88.78%) were reached. Beads exhibited high circularity (0.730–0.849) and size between 728.95 and 924.56 μm. Particle size and circularity was increased by raising polymer and crosslinker concentrations. Polymers ratio did not influence beads properties. The materials stability and the absence of drug–polymers interactions were evidenced by thermal analysis and FTIR. The high beads mucoadhesiveness was evidenced by *in vitro* and *ex vivo* tests. The erosion of beads was greater in acid media while swelling was more pronounced in pH 7.4. Drug release was dependent on pH in which samples 11H1-3, 11H1-5 and 41H1-3 released only 34%, 20% and 22% of ketoprofen in pH 1.2, while in pH 7.4 the drug release was sustained up to 360 min. Korsmeyer–Peppas model demonstrated that drug release occurred according to super case-II transport.

1. Introduction

The research about drug delivery systems dates from the 1960s and the search for new materials and technologies that allow a constant innovation in this filed has grown expressively over the years. This evolution evidences the relevance of these systems in the pharmaceutical and biomedical field, since the design of different systems with specific and tailored properties is a rational approach to enhancing the biological performance of different drugs, solving problems related to instability and/or low absorption and bioavailability, making possible the oral administration of several molecules.

Targeting a drug to specific organs or tissues allows its release in the specific site of action and/or absorption, maximizing its therapeutic efficacy and reducing or even avoiding systemic side effects. The advantages also include simplification of posology and reduction of doses what also impact treatment compliance and costs. In fact, this approach can benefit the therapy of many diseases (Scholes et al., 1993; Torchilin, 2000).

In turn, the temporal control allows the drug release in pre-determined rates, keeping plasma concentrations within therapeutic levels for suitable periods, reducing the frequency of drug administration, what also leads to improved patient compliance, thus guaranteeing therapy success (Isha, Nimrata, Rana, & Surbhi, 2012; Pezzini, Silva, & Ferraz, 2007).

The particular interest in the development of new materials based on polysaccharides is due to their outstanding advantages as biodegradability, relative low cost, free availability and sustainability, besides their acceptance for human consumption once they are non-toxic (Kulkarni, Mangond, Mutalik, & Sa, 2011; Matricardi, Di Meo, Coviello, Hennink, & Alhaique, 2013). Moreover, these materials present particular structures, containing functional groups (carboxyl, hydroxyl and amino groups) that can be physically or chemically modified reaching a great range of properties that can be tailored according to specific needs (Bhardwaj, Kanwar, Lal, & Gupta, 2000; Carbinatto, de Castro, Cury, Magalhães, & Evangelista, 2012; Cui, Liu, & Xie, 2005; Cury, Castro, Klein, & Evangelista, 2009; Cury, de Castro, Klein, & Evangelista, 2009; Kurita, 2001; Meneguin, Cury, & Evangelista, 2014; Soares, Castro, Cury, & Evangelista, 2013).

In this way, these materials are highly attractive for application in the most diverse areas. In pharmaceutical field, particularly when designing drug delivery systems, important properties as swelling, gelling, drug entrapment, adhesiveness, biodegradability and dissolution can be modulated via physical or chemical modification of these biopolymers in order to succeed in this challenging task of complete control of drug release rates.

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The hydrophilic tridimensional polymer networks can also present the peculiar ability to adhere to the mucus layer that covers gastrointestinal mucosa, increasing the residence time in the site and establishing a closer contact with it, what can favor the drug permeation, enhancing its absorption and bioavailability (Hägerström, Paulsson, & Edsman, 2000; Morishita & Peppas, 2006).

Pectin, a natural polysaccharide found in the cell wall of several plant species (Souto-Maior, Reis, Pedreiro, & Cavalcanti, 2010), presents well-known mucoadhesive properties and the use of this versatile polymer has been extensively exploited isolated or associated to others natural or synthetic polymers in the designing of different drug delivery systems (Aydin & Akbuğa, 1996; Bigucci, Luppi, Monaco, Cerchiara, & Zecchi, 2009; Chambin, Dupuis, Champion, Voilley, & Pourcelot, 2006; Das, Chaudhury, & Ng, 2011; Kim, Park, Kim, & Cho, 2003; Liu, Fishman, Hicks, & Kende, 2005; Liu et al., 2004; Liu, Fishman, Kost, & Hicks, 2003; Maestrelli, Cirri, Corti, Mennini, & Mura, 2008; Prezotti, Meneguin, Evangelista, & Cury, 2012; Soares, Castro, Cury, & Evangelista, 2013; Sriamornsak, 1998; Thirawong, Kennedy, & Sriamornsak, 2008; Thirawong, Nunthanid, Puttipipatkhachorn, & Sriamornsak, 2007; Thirawong, Thongborisute, Takeuchi, & Sriamornsak, 2008).

In spite of pectin resistance to enzymes like amylase and protease in the stomach environment, being digested specifically by enzymes synthesized by colonic microbiota, its high solubility in water is a challenge that has to be overcome in order to achieve the best performance of this material, preventing premature drug release (Liu, Fishman, Kost, & Hicks, 2003). The cross-linking reaction is a rational approach to restrict its hydrophilicity as well as modify drug release patterns according to the crosslinking degree (Liu, Fishman, Kost, & Hicks, 2003; Sriamornsak, 1998).

Gellan gum is a linear anionic hydrophilic exopolysaccharide synthesized by *Sphingomonas paucimobilis* through aerobic fermentation (Morris, Nishinari, & Rinaudo, 2012; Prajapati, Jani, Zala, & Khutliwala, 2013). It is widely used in the food industry due to its gelling properties, besides being nontoxic, biocompatible and biodegradable (Picone & Cunha, 2013). Its ability of forming strong gels, even at very low concentrations, makes gellan gum a promising material for pharmaceutical application (Agnihotri, Jawalkar, & Aminabhavi, 2006; Kulkarni, Nagathan, Biradar, & Naikawadi, 2013; Narkar, Sher, & Pawar, 2010; Patil, Sharma, Nimbalkar, & Pawar, 2006). In addition, gellan gum presents important mucoadhesive properties (Lee, Park, & Robinson, 2000; Narkar, Sher, & Pawar, 2010; Salamat-Miller, Chittchang, & Johnston, 2005), and it has been widely used in the design of drug delivery systems (Cao et al., 2009; Kubo, Miyazaki, & Attwood, 2003; Mahajan & Gattani, 2009; Narkar, Sher, & Pawar, 2010; Rajinikanth & Mishra, 2007; Sanzgiri, Maschi, Crescenzi, Callegaro, Topp, & Stella, 1993).

Gellan gum ability to form hydrogel microcapsules in the presence of divalent cations, particularly Ca^{2+}, has been exploited for obtaining controlled release systems (Agnihotri, Jawalkar, & Aminabhavi, 2006; Babu, Sathigari, Kumar, & Pandit, 2010; Narkar, Sher, & Pawar, 2010; Patil, Sharma, Nimbalkar, & Pawar, 2006; Rajinikanth & Mishra, 2007).

Recently, Maiti, Ranjit, Mondol, Ray, and Sa (2011) evaluated the use of Al^{3+} in obtaining crosslinked beads of gellan gum for prolonged release of glipizide and demonstrated the effective reduction of release rates in acid media.

Blending different polymers with individual properties that can act with synergism in the final product is a strategic way to reach novel materials with modulated properties. These new polymer blends present advantages such as controlled drug delivery, modulated swelling/erosion, high drug loading capacity, besides its easy preparation avoiding the costs of synthesizing novel materials (Bajpai, Shukla, Bhanu, & Kankane, 2008; Soares, Castro, Cury, & Evangelista, 2013). Moreover, changing polymers ratio and crosslinking degree can originate different drug release profiles to achieve specific goals (Soares, Castro, Cury, & Evangelista, 2013).

In this paper, novel beads of gellan gum/pectin blends were prepared through ionotropic gelation technique using a trivalent cation (Al^{3+}) as crosslinking agent and ketoprofen as model drug. The influence of polymer, drug and cross-linker concentration as well as gellan gum:pectin ratio on beads preparation and properties was evaluated.

2. Materials and methods

2.1. Materials

Pectin (type LM-5206 CS) and gellan gum (type Kelcogel® CG-LA) were kindly provided by CP Kelco (Limeira, SP, Brazil). Mucin type II was purchased from Sigma Aldrich® (St. Louis, MO, USA), ketoprofen (batch #9072223) was from Zhejiang Jiuhzou Pharmaceutical Co. (Taizhou, China) and all other materials used were of analytical grade and obtained from commercial suppliers.

2.2. Beads preparation

Beads of gellan gum/pectin mixtures were prepared by ionotropic gelation technique. Briefly, aqueous dispersions at different concentrations (2 or 4%; w/v) of 1:1 and 4:1 gellan gum:pectin mixtures containing ketoprofen (0.5 or 1%; w/v) were prepared under magnetic stirring at 80 °C. These dispersions were dropped through flat-tipped needles (25 × 0.6 mm) into a cooled cation solution (Al^{3+}; 3% or 5%) under gentle magnetic stirring. The crosslinking reaction was kept by additional 20 min for strengthening of the beads. After that, they were separated by filtration, washed with distilled water, dried at room temperature and stored in a desiccator. Beads without drug were prepared as control.

Samples were labeled according to the gellan gum:pectin ratio (1:1 and 4:1 as 11 and 41, respectively), polymer concentration (L = 2% and H = 4%), drug concentration (0 = without drug; 0.5 = 0.5% and 1 = 1% of ketoprofen) and the crosslinking solution concentration (3 = 3% and 5 = 5% of $AlCl_3$).

2.3. Surface and internal morphology of beads

The surface and internal structure morphology were analyzed by field emission gun scanning electron microscopy (FEG-SEM) (JEOL JSM-7500F, Japan). In order to evaluate the internal structure, beads were frozen with liquid nitrogen and crushed. Beads were attached in the sample holder with a double-side adhesive tape and photomicrographs at various magnifications were taken.

2.4. Particle size analysis

The size and shape of beads were evaluated using a Leica MZ APO® stereoscope coupled to a Motic Images Advance 3.2 program. The circularity and the equivalent circle diameter of 100 beads of each formulation were analyzed. Beads were previously dried until constant weight and arranged in a Petri dish to capture the images.

Based on size distribution data, the Span index was also determined following Eq. (1), in order to evaluate the polydispersity of samples.

$$\text{Span} = (D_{90} - D_{10})/D_{50} \quad (1)$$

where D_{90}, D_{10} and D_{50} are the diameters (μm) determined for 90th, 10th and 50th percentile, respectively.

2.5. Percent yield values

The yield (%) of beads preparation process was calculated according to Eq. (2) (Banerjee et al., 2012).

$$\%\text{yield} = \left[(\text{mass of beads})/(\text{mass of drug} + \text{mass of polymer})\right] \times 100 \quad (2)$$

2.6. Drug entrapment efficiency

In order to ensure total extraction of the drug, a known mass of the beads was allowed to swell in phosphate buffer pH 7.4 and further stirred (11,000 rpm, 1 min). After centrifugation (2500 rpm; 5 min) to remove polymeric debris, the drug in the supernatant was quantified on an UV-spectrophotometer (Hewlett Packard-Kayak XA, USA) at 260 nm, in triplicate. Beads without drug were treated in the same manner and analyzed as control. The entrapment efficiency was estimated by Eq. (3):

$$\text{EE}\% = \left[(\text{experimental drug content})/(\text{theoretical drug content})\right] \times 100 \quad (3)$$

2.7. Evaluation of drug–polymer interactions

Fourier transform infrared (FTIR) spectra of isolated polymers, ketoprofen and beads with or without drug were recorded at room temperature with an IR Prestige 21-Shimadzu® spectrometer (Shimadzu, Japan) using KBr pressed discs to evaluate drug–polymer interactions. For each sample, 40 scans were recorded between 4000 and 400 cm^{-1}.

2.8. DSC

Differential scanning calorimetry (DSC) curves of beads, ketoprofen and polymers were registered in a *DSC 1 Star System* (Mettler Toledo®, Brazil) under nitrogen atmosphere (50 ml/min). The temperature range was 20–100 °C for the beads, 25–200 °C for the drug and −30–400 °C for the polymers. Runs were performed using an aluminum pan sealed with a central pin-hole in the lid containing about 1 mg of sample and an aluminum pan sealed with a central pin-hole in the lid was used as reference.

2.9. TG/DTG

The thermogravimetric (TG) and differential thermogravimetric (DTG) curves were recorded using a *TA SDT 2960* (TA Instruments, USA). Samples were heated at a heating rate of 10 °C/min, from 25 to 500 °C, under nitrogen atmosphere (flow rate of 100 ml/min), in open aluminum pans containing about 7 mg of sample.

2.10. Swelling and erosion evaluation

The swelling and erosion of beads were determined gravimetrically. An accurately weighed mass of beads was immersed in acidic media (0.1 N HCl pH 1.2) or phosphate buffer pH 7.4, during 2 h. After removal of samples, the excess of fluid was carefully removed from the surface with a filter paper. The swollen beads were weighted, dried until constant weight, and weighted again.

The swelling percentage was calculated using Eq. (4) (Banerjee et al., 2012):

$$\%\text{swelling} = \left[(m_{sb} - m_{fdb})/m_{fdb}\right] \times 100 \quad (4)$$

where m_{sb} = mass of swollen beads and m_{fdb} = final mass of dried beads.

The erosion that particles undergo when exposed to media with different pH values was obtained from Eq. (5) and represent the percentage of bead weight loss (Almeida & Almeida, 2004):

$$\%\text{erosion} = \left[(m_{idb} - m_{fdb})/m_{idb}\right] \times 100 \quad (5)$$

where m_{idb} = initial mass of dried beads and m_{fdb} = final mass of dried beads, obtained after drying the swollen particles. The tests were performed in triplicate.

2.11. In vitro mucoadhesion test

Beads (20 mg) were added to 10 ml of mucin aqueous solutions at different concentrations (50, 100, 150 and 200 μg/ml), vortexed and incubated in thermostatic bath at 37 °C for 1 h. Then, they were centrifuged at 3000 rpm (2 min) and the supernatant was used for the quantification of free mucin (749 nm), using a Lowry protein assay modified by Peterson (Dhawan, Singla, & Sinha, 2004; Peterson, 1977). For the determination of free mucin in the supernatant reagents from *Total Protein Kit*, *Micro Lowry*, *Peterson's Modification* (Sigma-Aldrich®, USA) were used.

Data from mucin adsorption were fitted with Freundlich model (Eq. (6)) that describes the adsorption isotherms (He, Davis, & Illum, 1998).

$$Q_e = k \times C_e^{\frac{1}{n}} \quad (6)$$

where Q_e is the amount of adsorbed mucin per mass of beads (mg/g) and C_e is the concentration of free mucin in the supernatant (mg/l), k is the Freundlich constant and represents the adsorption capacity and n is the constant that represents the adsorption intensity.

2.12. Ex vivo mucoadhesion test

The ex vivo mucoadhesion test was performed according to the procedure purposed by Rao and Buri (1989), with some modifications. Fresh porcine large intestine tissue was washed with saline solution (0.9%) and opened longitudinally. Pieces (4 cm × 4 cm) were attached to the inclined plastic support (30°) of the device. Beads (n = 30) were placed gently on the surface of the tissue and a 20 min contact time with mucous layer was allowed. After this, the tissue was rinsed with phosphate buffer pH 6.0 at a rate of 30 ml/min, for 5 min. The percentage of particles washed away was determined by counting the number of particles that remained attached to the tissue. The test was also performed with tissue in that the mucous layer was scraped off using a razor, as control.

The saline washing solution was centrifuged (3000 rpm, 2 min) and the free mucin in the supernatant was quantified using a Lowry protein assay modified by Peterson (item 2.11) in order to ensure that washing process did not promote the loss of mucous. The mucous of some pieces of tissues was scraped off and used as positive control for the presence of mucin.

2.13. In vitro drug release studies

Based on the best data of circularity, entrapment efficiency and mucoadhesion, the samples 11H1-3, 11H1-5, 41H1-3 and 41H1-5 were selected for the *in vitro* drug release tests.

Tests were performed sequentially in two different pH on a Hanson Dissolution Test Station SR8-Plus (Chastworth, USA) equipped with the USP apparatus I (basket) at 50 rpm and temperature of 37 ± 0.4 °C.

Solutions with different pH values were used to simulate the variation that occurs along the GIT. Initially, the test was carried out for 2 h in acid media without enzymes (900 ml of 0.1 N HCl—pH 1.2) added by sodium lauryl sulfate (0.75%) as surfactant. The second stage was conducted in simulated enteric medium (900 ml of

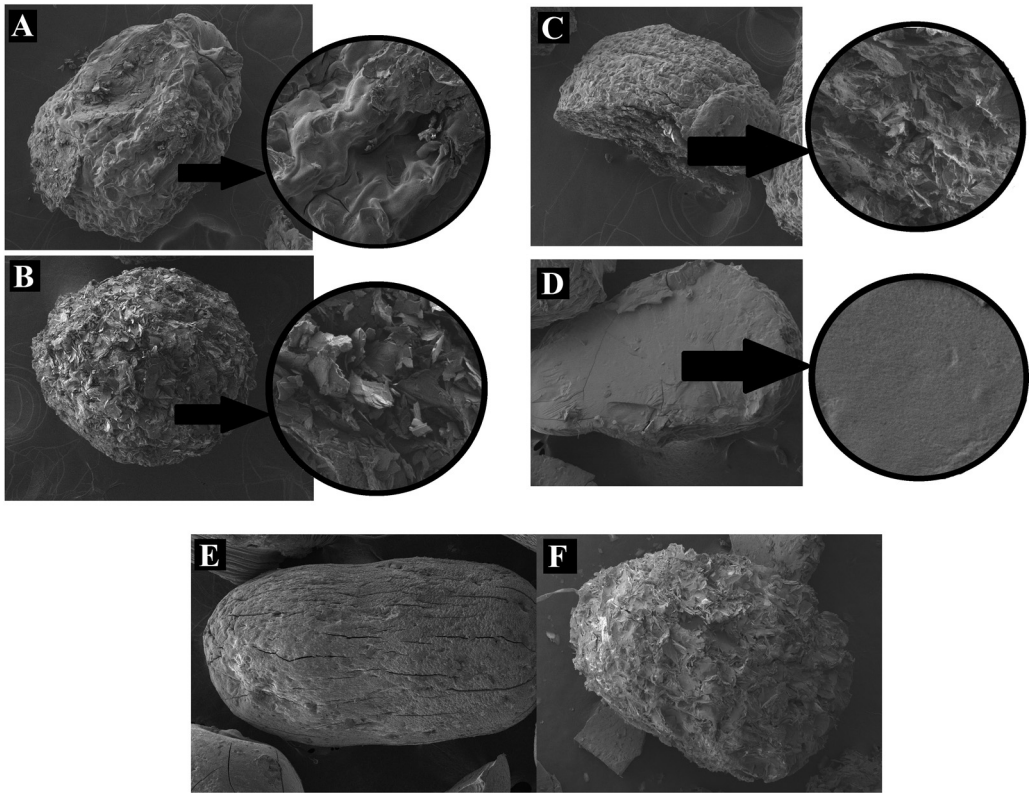

Fig. 1. Photomicrographs of samples (A) 11L0.5-3 and (B) 11H0.5-3 evidencing the highly irregular surface of beads (100× and 300×); (C) 11L1-3 and (D) 41L0-3 evidencing the internal structure of beads (100× and 1000×) and (E) 41H0-3 and (F) 41H1-3 evidencing the surface morphology and the irregularity caused by incorporation of drug (100×).

phosphate buffer solution pH 7.4) during 4 h. Aliquots were withdrawn at predetermined times and immediately replaced with fresh dissolution media to maintain the sink condition. The amount of ketoprofen released was quantified on a spectrophotometer at 258 nm and 260 nm for acid and enteric media, respectively. A mass of beads containing 150 mg of ketoprofen was tested in triplicate (USP, 2011).

2.14. Kinetics analysis of drug release

The mechanism of drug release was analyzed by fitting the dissolution data with different mathematical models (Korsmeyer–Peppas, Higuchi, First-order, Hixson–Crowell and Baker–Lonsdale) with Sigma Plot 10.0 software.

2.15. Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey test was used to evaluate significant differences. A significance level of 5% was adopted.

3. Results and discussion

3.1. Beads preparation

The effect of polymer concentration and gellan gum:pectin ratio on beads properties was evaluated in preliminary studies. Polymer concentrations higher than 2% (w/v) allowed obtaining particles of higher circularity, while 1:4 gellan gum:pectin ratio originated particles with tail deformation and low circularity (0.538).

The low viscosity of dispersions obtained with lower polymer concentrations (0.5, 1.0 and 1.5%) was not sufficient to overcome the impact and drag forces during the dropping so that non spherical beads were formed (Chan, Lee, Ravindra, & Poncelet, 2009; Seifert & Phillips, 1997). These samples were excluded from further studies.

3.2. Surface and internal morphology of beads

Photomicrographs (Fig. 1) revealed the highly irregular surface of the beads, as also observed by Maiti, Ranjit, Mondol, Ray, and Sa (2011) in a study with gellan gum beads containing glipizide.

Beads without drug presented a homogeneous polymeric matrix, however, the presence of ketoprofen originated highly irregular structures containing some pores, what indicates that the drug is inserted between polymer chains, expanding and disordering the network, which adopts a different structure (Fig. 1C and D).

Fig. 1E and F shows 41H0-3 and 41H1-3 samples photomicrographs, where it is possible to observe that the incorporation of drug led to greater surface irregularity, probably due to largest drug deposition in this region.

3.3. Particle size analysis

The particle size ranged from 728.95 to 924.56 μm (Table 1) with sample 11L0-3 presenting the smallest diameter ($p < 0.05$), what can be attributed to absence of drug and low polymer concentration (2%), since the amount of material present in the droplet influence the size of the bead (Zaniboni, Fell, & Collett, 1995).

When the drug was absent, the highest polymer concentration originated beads with the largest diameters ($p < 0.05$), because in these conditions the increased polymer mass and dispersion viscosity allowed the building of bigger droplets during extrusion process,

Table 1
Results of shape, size (μm), particle size distribution, yield (%) and entrapment efficiency (EE%) of gellan gum/pectin beads obtained through ionotropic gelation (mean ± SD); Freundlich parameters and Korsmeyer–Peppas coefficients from adsorption and release studies, respectively (r^2 = coefficient of determination).

Sample	Circularity	Particle size (μm)	Span	% yield	EE%	Freundlich isoterm parameters			Korsmeyer–Peppas release coefficients			
						k	$1/n$	r^2	pH 1.2		pH 7.4	
									n	r^2	n	r^2
11L0-3	0.734 ± 0.062	728.95 ± 34.88	0.12	86.39	–	–	–	–	–	–	–	–
11L0.5-3	0.770 ± 0.054	747.18 ± 56.05	0.19	86.43	52.22 ± 3.14	–	–	–	–	–	–	–
11L1-3	0.738 ± 0.049	838.59 ± 59.70	0.18	59.91	74.63 ± 8.68	–	–	–	–	–	–	–
11H0-3	0.730 ± 0.052	877.53 ± 27.97	0.08	84.66	–	0.327	1.550	0.997	–	–	–	–
11H0.5-3	0.827 ± 0.026	793.49 ± 25.48	0.08	80.47	72.67 ± 3.17	–	–	–	–	–	–	–
11H1-3	0.809 ± 0.032	844.84 ± 51.48	0.16	79.43	88.78 ± 2.16	0.144	1.731	0.996	2.447	0.924	3.488	0.994
41L0-3	0.733 ± 0.040	777.44 ± 34.05	0.11	92.76	–	–	–	–	–	–	–	–
41L0.5-3	0.751 ± 0.043	779.02 ± 61.49	0.20	67.20	84.89 ± 4.69	–	–	–	–	–	–	–
41L1-3	0.757 ± 0.043	817.25 ± 52.01	0.16	90.18	76.44 ± 4.38	–	–	–	–	–	–	–
41H0-3	0.791 ± 0.034	821.13 ± 20.61	0.06	73.89	–	1.374	1.109	0.976	–	–	–	–
41H0.5-3	0.814 ± 0.025	799.92 ± 49.21	0.16	88.30	74.27 ± 3.60	–	–	–	–	–	–	–
41H1-3	0.830 ± 0.025	803.37 ± 48.85	0.16	75.24	72.93 ± 3.15	0.098	1.866	0.992	1.534	0.909	3.830	1.000
11H1-5	0.819 ± 0.022	924.56 ± 31.64	0.09	76.83	84.54 ± 2.89	0.143	1.765	0.989	1.035	0.902	4.409	0.949
41H1-5	0.849 ± 0.035	887.22 ± 70.77	0.20	69.55	77.89 ± 1.22	0.215	1.669	0.983	1.465	0.933	0.589	0.968

and so larger particles were built (Kulkarni, Mangond, Mutalik, & Sa, 2011; Martins, Sarmento, Souto, & Ferreira, 2007).

The increase of particles size was caused by increasing of drug concentration from 0.5% to 1.0% (excepted sample 41H1-3) which is consistent because when more drug is available to fill the interstitial spaces of polymer network, the structure is expanded and the inward shrinkage is hindered, resulting in larger particles (Kaity, Isaac, & Ghosh, 2013; Kulkarni, Mangond, Mutalik, & Sa, 2011).

The increase of crosslinker concentration from 3% to 5% promoted an increasing in particle size for both polymeric proportions ($p < 0.05$). This interesting behavior can be related to the formation of a more branched structure in higher cross-linking degrees, enlarging the bead size. There was no consistent relation between particle size and polymer ratio.

The Span index ranged between 0.06 and 0.20 (Table 1), indicating the homogeneity of particle size distribution (Gaumet, Vargas, Gurny, & Delie, 2008).

The high circularity values (0.730–0.849) and the narrow variation among them demonstrates some circularity and homogeneity of shape. The increase of polymer concentration led to more circular beads ($p < 0.05$) (Table 1), except for the sample 11H0-3. Besides, the presence of drug favored the circularity of beads while no correlation was established between beads shape and polymer ratio.

The highest circularity value exhibited by sample 41H1-5 ($p < 0.05$) indicates that in this higher crosslinking degree, a stronger structure should be built which was more resistant against the impact with the crosslinker solution, so that the droplet shape was preserved.

3.4. Percent yield values

The percent yield values presented in Table 1 (59.91–92.76%), with mean value among all batches of 79.37%, indicate that the ionotropic gelation technique employed in this work was efficient for obtaining beads.

3.5. Drug entrapment efficiency

EE% ranged from 52.22% to 88.78% (Table 1), with sample 11L0.5-3 showing the lowest value ($p < 0.05$). These values were higher than those reported by Agnihotri, Jawalkar, and Aminabhavi (2006) in a study with gellan gum microspheres crosslinked with Ca^{2+} and Zn^{2+} for cephalexin controlled release (69.24%).

For sample 1:1, the increasing of polymer and drug concentrations improved the encapsulation process (Table 1). This behavior can be attributed to some factors as the larger size of beads built in these conditions, the increased amount of sites available to crosslink with Al^{3+} and the high viscosity of the polymeric dispersion at 4% polymer concentration, these last been those that impaired the drug diffusion to the crosslinking solution during the preparation process (Nayak, Das, & Maji, 2012). These variables did not influence the drug entrapment of 4:1 samples ($p > 0.05$), which showed no significant difference among their EE% ($p > 0.05$).

No straight correlation was observed between gellan gum:pectin ratio and EE% or between cross-linker concentration and EE% ($p > 0.05$).

3.6. Evaluation of drug–polymer interactions

In gellan gum spectra (Fig. 2) a broad band was identified between 3650 and 3100 cm^{-1} that is assigned to hydroxyl groups present in the glucopyranose ring. The band at 2925 cm^{-1} is due to stretching vibration of —CH_2 groups, and bands at 1609 and 1418 cm^{-1} are due to presence of carboxylate anions (COO^-). A band at 1157 cm^{-1} and that one near to 1050 cm^{-1} can be assigned to ethereal and hydroxylic C—O stretching, respectively (Agnihotri, Jawalkar, & Aminabhavi, 2006; Lee, Tsai, Wen, & Huang, 2012).

The pectin spectra (Fig. 2) exhibited a broad band between 3600 and 3100 cm^{-1} due to hydroxyl stretching vibration, which can form inter- and intramolecular hydrogen bonds (Gnanasambandam & Proctor, 2000). Bands assigned to C—H

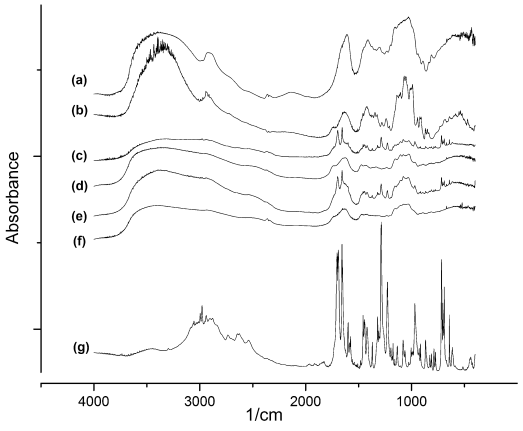

Fig. 2. FTIR spectra of (a) gellan gum; (b) pectin; (c) sample 11L0.5-3; (d) sample 11L0-3; (e) sample 41L0.5-3; (f) sample 41L0-3 and (g) ketoprofen.

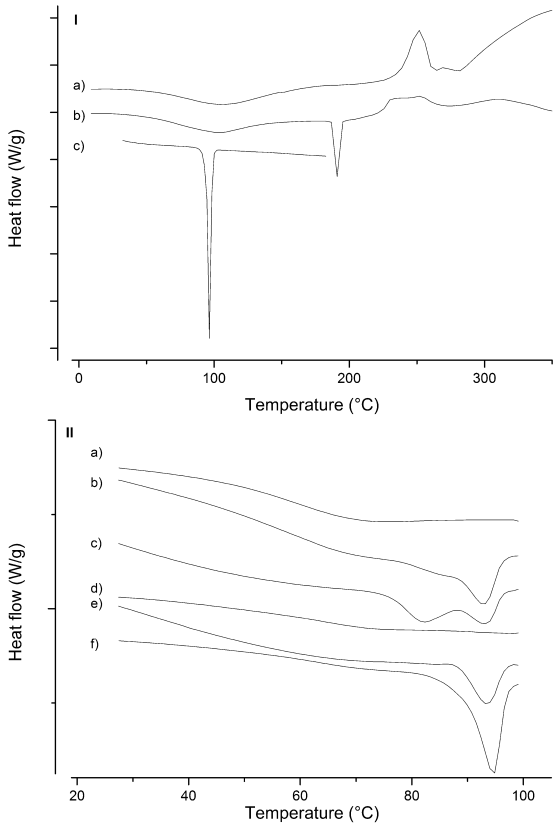

Fig. 3. (I) DSC curves of (a) gellan gum; (b) pectin and (c) ketoprofen and (II) DSC curves of gellan gum/pectin beads (a) 11H0-3; (b) 11H0.5-3; (c) 11H1-3; (d) 41H0-3; (e) 41H0.5-3 and (f) 41H1-3.

symmetrical and asymmetrical stretching occurred between 3000 and 2800 cm^{-1}. A characteristic peak appeared at 1745 cm^{-1} and corresponds to stretching vibration of ester carbonyl groups (C=O), although discreet, because it is low methoxylation pectin. Another characteristic band appears at 1617 cm^{-1} due to C=O stretching of carboxylate anion (COO^-) (Gnanasambandam and Proctor, 2000; Sriamornsak et al., 2008).

Strong and characteristics peaks of ketoprofen (Fig. 2) were registered at 1698 and 1655 cm^{-1} related to carboxyl and ketonic carbonyl, respectively, and one at 1228 cm^{-1} due to C—OH in-plane bending. Peaks at 1598, 1575 and 1445 cm^{-1} are due to stretching of C=C bonds of the aromatic ring (Liu & Gao, 2012; Yadav, Kumar, Singh, Bhat, & Mazumder, 2013).

Drug loaded beads (11L0.5-3 and 41L0.5-3) (Fig. 2) showed strong peaks at 1698 and 1655 cm^{-1}, which are ketoprofen characteristic peaks, while these were absent in the spectra of beads without drug (11L0-3 and 41L0-3), indicating the absence of chemical interactions between drug, polymer, and counterions after beads production, and showing that the drug remained inside the particles without molecular changes, physically trapped within the polymer chains.

3.7. DSC

Ketoprofen, gellan gum and pectin DSC curves are presented in Fig. 3I.

Ketoprofen DSC curve (Fig. 3 I) showed a single sharp endothermic peak at 96.5 °C (T_{onset} = 91.0 °C) that can be attributed to the melting of the drug (Mura, Faucci, Parrini, Furlanetto, & Pinzauti, 1999; Tiţa, Fuliaş, Bandur, Marian, & Tiţa, 2011).

A broad endothermic event between 70 and 140 °C observed in gellan gum DSC curve (Fig. 3I) was due to evaporation of bound water while the narrow exothermic peak between 230 and 260 °C is related to the polymer degradation (Verma, Ramesh, Sharma, & Pandit, 2012).

Pectin also presented a broad endothermic event between 55 and 140 °C (Fig. 3I) corresponding to loss of bound water. However, pectin presented an additional narrow endothermic peak at 191 °C that can be attributed to depolymerization of pectin chains (Soares, Castro, Cury, & Evangelista, 2013), and at 220 °C an exothermic peak occurred due to polymer degradation (Einhorn-Stoll, Kunzek, & Dongowski, 2007; Soares, Castro, Cury, & Evangelista, 2013).

DSC curves of beads containing ketoprofen (Fig. 3II) exhibited a melting peak of drug near to 95 °C, however, this peak was wider due to a partial overlap with the large endothermic event corresponding to polymer dehydration (Mura, Manderioli, Bramanti, Furlanetto, & Pinzauti, 1995). This peak was absent in beads without the drug (Fig. 3II).

3.8. TG/DTG

TG curve of ketoprofen (Fig. 4) presented a single step of mass loss between 210 and 310 °C, related to thermal decomposition of the drug (Tiţa, Fuliaş, Bandur, Marian & Tiţa, 2011). The DTG curve peak occurs in 303 °C and at the end of the process there is a mass loss of 98.8%, value very close to that related by Bannach et al. (2010).

TG profiles of beads (Fig. 4) exhibited a first event of discrete mass loss, of about 2–7.5% related to moisture evaporation. This is also represented by a small and broad peak in DTG curves between 50 and 100 °C (Soares, Castro, Cury, & Evangelista, 2013).

For beads containing drug, a thermal decomposition in two steps can be seen between 125 and 300 °C. The main narrow peak between 125 and 200 °C can be attributed to the degradation of both polymers, and appears as one single event due to crosslinking reaction (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007). The second and smaller peak between 200 and 300 °C is related to ketoprofen degradation and it was absent in beads without drug.

The set of thermal analysis data demonstrated that the mild experimental conditions of the ionotropic gelation technique avoided the degradation of both polymers and drug.

3.9. Swelling and erosion evaluation

The swelling data evidenced the pH-dependent behavior of samples, in which the swelling in pH 7.4 was up to 5.5 times higher than that in pH 1.2.

Both gellan gum and pectin are anionic polyelectrolytes and the change in media pH affects chains conformation (Narkar, Sher, & Pawar, 2010). In acidic media, there is a predominance of H^+ ions that leads to protonation of carboxyl groups, reducing electrostatic repulsion, thus polymer chains can become tighter, making the penetration of liquid in the system more difficult (Bajpai, Shukla, Bhanu, & Kankane, 2008; Oliveira, Ferrari, Carvalho, & Evangelista, 2010; Souto-Maior, Reis, Pedreiro, & Cavalcanti, 2010). In higher pH values, carboxyl groups remain in the ionized form, what increases the hydrophilicity and promotes network expansion due to electrostatic repulsion, both factors favoring the penetration of liquid in the system. In this way, the polymers show greater swelling ability in pH 7.4 (Maiti, Ranjit, Mondol, Ray, & Sa, 2011).

The sample 41H1-5 prepared with higher crosslinker concentration exhibited a decreased swelling ability ($p < 0.05$), indicating that a more rigid and packed polymer network was built, disfavoring the water entrance (Angadi, Manjeshwar, & Aminabhavi, 2013; Banerjee et al., 2012). On the other hand, the reduced ability in absorbing water by this sample in relation to 11H1-5 can be due to the lower content of pectin in its structure, once higher pectin content may favor the system hydrophilicity. Similar behavior

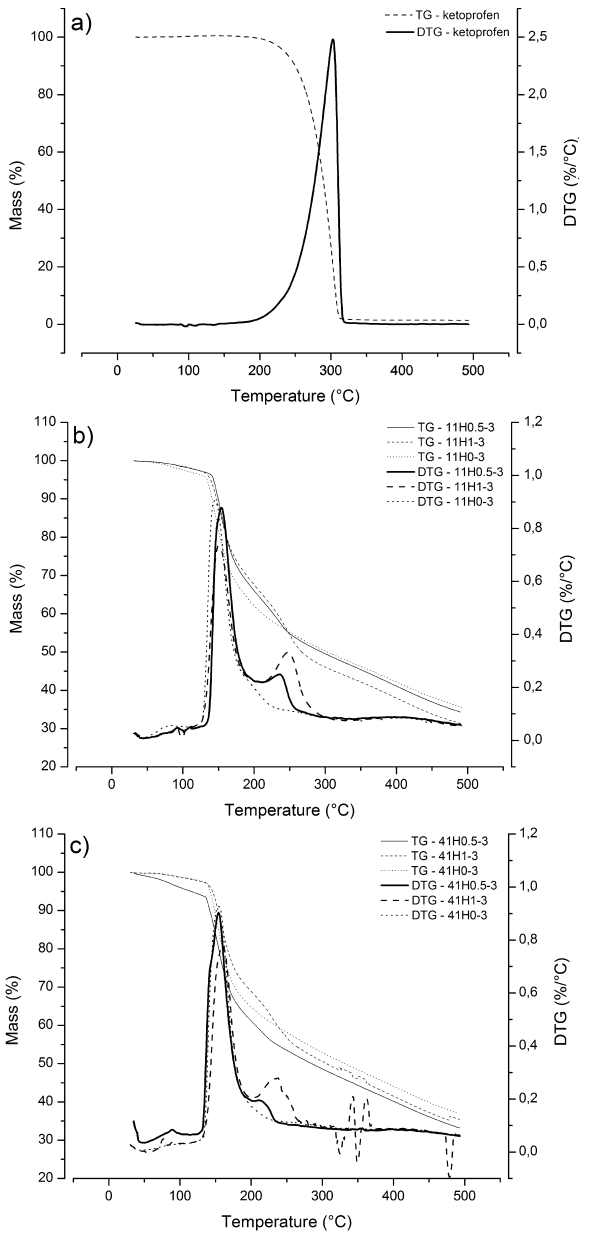

Fig. 4. Thermogravimetric analysis (TG/DTG) of (a) ketoprofen; (b) beads of gellan gum:pectin (1:1) crosslinked with Al^{3+} (3%) and (c) beads of gellan gum:pectin (4:1) crosslinked with Al^{3+} (3%).

was observed by Angadi, Manjeshwar, and Aminabhavi (2013) in a study with microparticles of chitosan and guar gum crosslinked with glutaraldehyde in which the high hydrophilicity of guar gum determined the swelling properties of the system.

Beads were highly eroded in acidic media, reaching values 4 times higher than in pH 7.4 ($p < 0.05$) and the sample 41H1-5 was the one that showed the highest mass loss (about 45%) ($p < 0.05$) (Fig. 5). This behavior suggest that when the liquid penetrates in the more rigid and tight network built at higher crosslinking degrees, an elevated hydrostatic pressure is exerted on the polymer chains, promoting the rupture of them or even the breaking of the crosslinking junctions.

3.10. In vitro mucoadhesion test

Mucoadhesion in multiparticulate systems is an important property because may avoid their rapid elimination caused by

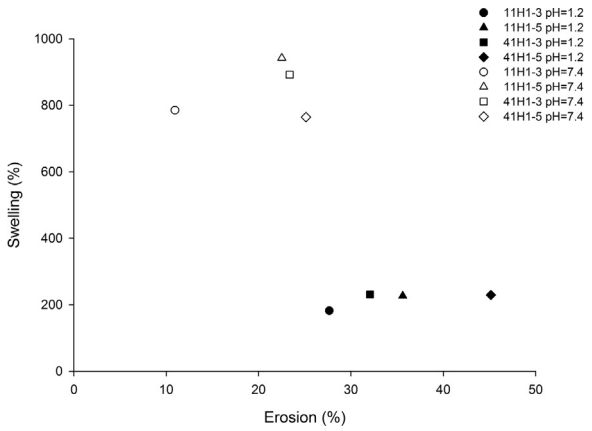

Fig. 5. Swelling and erosion behavior of beads in pH 1.2 and 7.4.

peristalsis, besides allowing longer contact with the target site (Gamboa & Leong, 2013).

For each concentration of mucin solution (Fig. 6) the amount of adsorbed mucin was not influenced by polymer proportion, crosslinking concentration and presence of drug ($p > 0.05$).

However, the amount of adsorbed mucin increased according to the mucin concentration was raised (Fig. 6a). The same behavior was observed by He, Davis, and Illum (1998) with chitosan microspheres and by Sonia and Sharma (2011) with modified and unmodified chitosan microparticles prepared by ionotropic gelation. This feature, as well as the high values of adsorbed mucin (68–83%), which were superior (about 4×) to those reported by Dhawan, Singla, and Sinha (2004) in a study with chitosan

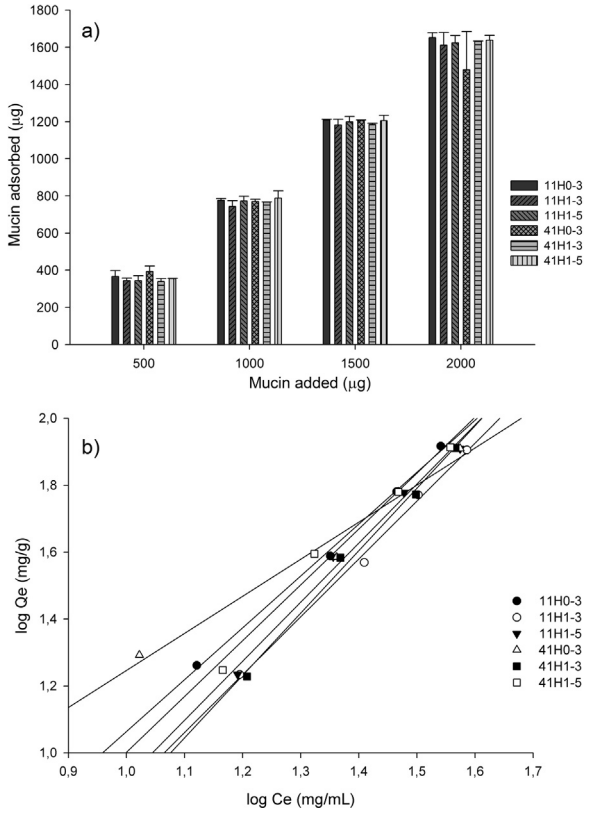

Fig. 6. In vitro mucoadhesive test: (a) relation between the amount of mucin added and the mucin adsorbed on different gellan gum/pectin beads and (b) Freundlich isotherms for adsorption of mucin in beads of gellan gum/pectin.

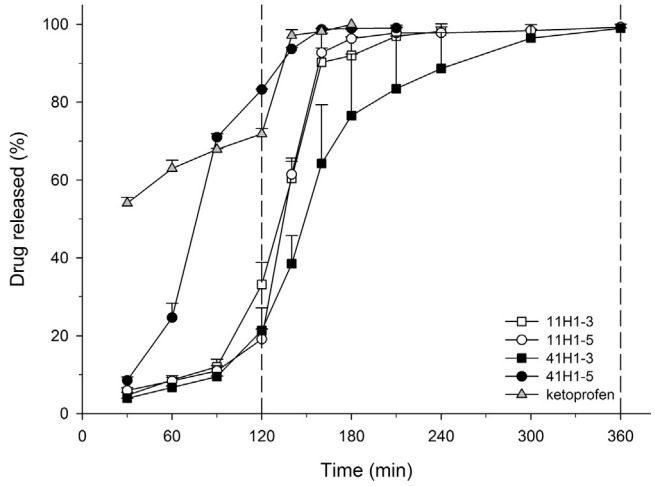

Fig. 7. *In vitro* release profile of ketoprofen free and from gellan gum/pectin beads in simulated gastric and enteric media.

microspheres, evidenced the high mucoadhesiveness of gellan gum/pectin beads.

The Freundlich model is suitable for a single-solute adsorption and heterogeneous surfaces. It is based on a multilayer distribution of this solute in the solid phase and assumes a heterogeneous distribution of adsorption heat (Freundlich & Hatfield, 1926; Guo & Gemeinhart, 2008; Ng, Losso, Marshall, & Rao, 2002; Snoeyink & Summers, 1990).

Freundlich parameters (Table 1) were obtained from a linear regression from the log C_e *vs* log Q_e plot (Fig. 6b), according to Eq. (7):

$$\log\ Q_e = \log k + \frac{1}{n}\log C_e \quad (7)$$

Values of $1/n < 1$ indicate that adsorption is based on strong interactions, and the smaller it gets, the more independent of C_e is the adsorption (constant values of k and C_e). Values of $1/n > 1$ indicate a weak interaction during adsorption and that the amount of solute adsorbed to the solid phase (Q_e) changes significantly even with small variations in C_e (Snoeyink & Summers, 1990).

The high values of $1/n$ (Table 1) corroborates that the adsorption behavior was dependent on mucin concentration. The pH of mucin solutions (4.79) was above the pKa of gellan gum (pKa 3.5), pectin (pKa 3–4) and mucin (pKa 2.6), so these structures must be negatively charged, due to ionization of polymers carboxyl groups and mucin sialic acid, so that hydrophobic interactions must be responsible for interaction during adsorption process (Sriamornsak, Wattanakorn, & Takeuchi, 2010).

3.11. Ex vivo mucoadhesion test

The concentration of free mucin in the mucous scraped off the tissue was 143 μg/ml, while no mucin was quantified in the washing solutions, demonstrating that the mucous layer of the porcine colon is persistent and was not damage by handling and washing.

In the end of the test, all beads remained strongly attached to the mucosa, evidencing the high mucoadhesive ability of these particles. The results corroborate with the *in vitro* mucoadhesion results, evidencing the high ability to remain adhered to the mucous layer.

3.12. In vitro drug release studies

The drug release profiles of ketoprofen from gellan gum/pectin beads (Fig. 7) show that in the first 120 min of dissolution in acid media (pH 1.2), samples 11H1-3, 11H1-5 and 41H1-3 released only 34%, 20% and 22% of ketoprofen, respectively, highlighting the reduced release rates in comparison to phosphate buffer (pH 7.4).

Sample 41H1-5 exhibited a marked increase in release rates, particularly after 60 min and 84% of the drug was released at 120 min. This particular behavior can be attributed to the high erosion that this sample suffers in acid media, once this process must accelerate the drug release rates.

In phosphate buffer (pH 7.4), samples 11H1-3, 11H1-5 and 41H1-3 showed higher release rates and a burst effect was observed during the first 40 min. Drug release was completed after 180 min for sample 41H1-5, while for the sample 11H1-3 this was reached after 240 min. Samples 11H1-5 and 41H1-3 were able of releasing drug up to 360 min.

The burst effect can be explained by the higher polymer swelling in pH 7.4, since the relaxation of polymer chains after swelling originates a looser structure with new channels throughout the matrix, facilitating diffusion of both media and drug, accelerating drug release rates (Mundargi, Patil, Agnihotri, & Aminabhavi, 2007; Mundargi, Patil, & Aminabhavi, 2007; Rokhade et al., 2006). Besides, the drug solubility is increased in this higher pH and the rate of the interface between dissolved and dispersed drug increases as well, so the diffusivity through the polymer matrix is improved, leading to a faster drug release (Narasimhan & Langer, 1997; Pezzini & Ferraz, 2009).

The lowest release rate was exhibited by sample 41H1-3 ($p < 0.05$). The lowest Al^{3+} concentration might have allowed a lower crosslink degree, so that even after the high liquid uptake, the swollen polymer network preserves its conformation, having a better control over drug release. These data agree with the lower erosion in pH 1.2 and higher swelling in pH 7.4 presented by this sample.

3.13. Kinetics analysis of drug release

The ketoprofen release data correlated better with Korsmeyer–Peppas, for both media (Table 1). This model is based on the Power Law, which relates exponentially the drug release with time (Eq. (8)):

$$M_t/M_\infty = k \times t^n \quad (8)$$

where M_t = amount of drug released at time t; M_∞ = amount of drug released at infinite time, i.e., the initial mass; n = release exponent and k = kinetic constant (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983).

The release coefficient n characterizes the drug release mechanism from the polymer matrices. For spherical systems, values of $n = 0.43$ indicate that the release mechanism follows Fickian diffusion, i.e., drug release is controlled by diffusion of drug molecules driven by a chemical potential (Type I transport). Values of n between 0.43 and 0.85 correspond to anomalous transport or non-Fickian diffusion, in which diffusion and swelling phenomena have similar importance in controlling drug release. For $n = 0.85$, case-II transport takes place and the swelling of the polymer drives the drug release. If $n > 0.85$, a super case-II is involved in drug release and its release rates are accelerated according to mobility of polymer chains is increased or the matrix is eroded (Kaity, Isaac, & Ghosh, 2013; Korsmeyer, Gurny, Doelker, Buri & Peppas, 1983; Lee, 1985; Maderuelo, Zarzuelo, & Lanao, 2011).

In acid media, the release exponent values (n) (Table 1) indicated that ketoprofen release from beads followed super case-II transport, which must be related to the high erosion underwent by the beads in this pH (Fig. 5).

In phosphate buffer, the release followed the same transport mechanism (super case-II); except for sample 41H1-5 that followed anomalous transport.

Both mechanisms described by mathematical model are in agreement with the findings discussed in the drug release studies.

The higher erosion of this latter sample (45.15%) in acid media in relation to other beads (27.65–35.59%) ($p < 0.05$) allowed the highest drug release rates, according to a super case-II mechanism, and, once this sample was moved to phosphate buffer, the little drug that remained was released following anomalous transport, in accordance with the greater swelling ability exhibited in this pH (Fig. 5).

Drug release kinetic from hydrophilic polymer matrix, as gellan gum/pectin beads, involve complex processes, such as polymer swelling and dissolution with subsequent drug diffusion, along with the contribution of the erosion that some systems exhibit (Mundargi et al., 2007a). It was possible to observe the influence of crosslinking degree, polymer structure, swelling, erosion and pH on controlling drug release, where the lower crosslinking and the higher amount of gellan gum in sample 41H1-3 were the conditions that allowed better control over ketoprofen release.

4. Conclusions

In this study, novel beads of gellan gum/pectin blends were successfully prepared by ionotropic gelation with a trivalent cation (Al^{3+}), avoiding organic solvent and drastic reaction conditions. The influence of concentration of polymer, drug and crosslinker as well as gellan gum:pectin ratio on beads preparation and properties were discussed in detail.

The matrix structure of beads was demonstrated by MEV while FTIR evidenced the absence of chemical bonds between drug and polymers. All beads presented high mucoadhesiveness and their swelling and erosion behavior were strongly dependent on pH. In the same manner, the drug release behavior was very sensible to pH values, so that reduced amounts of drug were released in acid media while in pH 7.4 the release rates were significantly increased and sustained up to 360 min. This sensibility to pH variation demonstrates the applicability of the beads on designing systems that allow the drug release at controlled rates throughout the GIT.

Further studies about additional strategies as dual crosslinking to reduce the erosion and the drug release in acid medium, improving the targeting of the drug to specific regions of intestine will be performed in order to modulate the drug release features according to specific therapeutic needs, widening the application of these systems in pharmaceutical and biomedical areas.

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