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RESEARCH ARTICLE

# Gellan gum microspheres crosslinked with trivalent ion: effect of polymer and crosslinker concentrations on drug release and mucoadhesive properties

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**ABSTRACT**

Gellan gum microspheres were obtained by ionotropic gelation technique, using the trivalent ion $Al^{3+}$. The percentage of entrapment efficiency ranged from 48.76 to 87.52% and $2^2$ randomized full factorial design demonstrated that both the increase of polymer concentration and the decrease of crosslinker concentration presented a positive effect in the amount of encapsulated drug. Microspheres size and circularity ranged from 700.17 to 938.32 μm and from 0.641 to 0.796 μm, respectively. The increase of polymer concentration (1–2%) and crosslinker concentration (3–5%) led to the enlargement of particle size and circularity. However, the association of increased crosslinker concentration and reduced polymer content made the particles more irregular. *In vitro* and *ex vivo* tests evidenced the high mucoadhesiveness of microspheres. The high liquid uptake ability of the microspheres was demonstrated and the pH variation did not affect this parameter. Drug release was pH dependent, with low release rates in acid pH (42.40% and 44.93%) and a burst effect in phosphate buffer pH (7.4). The Weibull model had the best correlation with the drug release data, demonstrating that the release process was driven by a complex mechanism involving the erosion and swelling of the matrix or by non-Fickian diffusion.

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Colonic drug delivery; dissolution test; ionotropic gelation; mucoadhesion; multiparticulate system

## Introduction

Oral dosage forms exploit the absorptive capacity of the gastrointestinal tract (GIT) and their wide use in the therapeutics is due to patient convenience, dose flexibility, safety, and low cost compared to other routes of administration[1,2]. After the administration of a conventional dosage form, the drug is released relatively quickly and several side effects and/or adverse reactions may appear[3,4].

The targeting of drugs to a specific organ or tissue is a great challenge in research and development of new oral controlled drug delivery systems. To achieve the site of action or absorption, the drug has to transpose many biological barriers such as others organs, rough environments, tissues, or intracellular compartments, where it may be degraded or even affect sites that are not involved in the pathological process[5].

Among the several organs along the GIT, the colon arouses particular interest for the treatment of local pathologies, such as inflammatory diseases and colonic cancer, as well as systemic pathologies, since it presents an extended transit time, reduced proteolytic activity and pH near to neutrality[6]. By these characteristics, the colon becomes a promising site for drug release, mainly those with permeability and/or stability issues in upper portions of the GIT[7,8].

A wide variety of synthetic, semi-synthetic, or natural polymers find application in the development of controlled drug release systems[9–11]. The increasing interest in the development of drug carrier systems based on natural hydrophilic polymers is justified because they are low cost, stable, biocompatible, nontoxic, have good gelling properties, and present a variety of structures able to be chemically and/or physically modified, conferring several properties that can be modulated according to specific needs[12–14].

Microencapsulation technology allows reaching different goals such as protect the drug from hostile conditions and incompatibilities, mask unpleasant taste and control the drug release rates in order to maximize therapeutic effects and minimize systemic side effects[15,16]. Microcapsules and microspheres can be obtained using many simple and low-cost materials and techniques, and their size ranges from 1 to 1000 μm[17,18].

In fact, these multiparticulate systems have several advantages over single-unit dosage forms, such as gastric emptying time more predictable and less dependent of the nutritional state; more uniform distribution of the drug on the GIT surface with reduced risk of local irritation[19,20]. For obtaining microspheres, various methods are proposed, like emulsification-solvent removal, coacervation, ionotropic gelation, polymerization, spray drying, and supercritical fluid atomization. To select the most appropriate method, it must be considered the type of the drug that will be encapsulated, the release mechanism, and the desired application[18,21].

In recent years, the development of hydrogel microspheres from polysaccharides by ionotropic gelation has been focus of important researches, because changes in polymer concentration and crosslinking degree can lead to the production of systems with different drug release profiles for specific goals[22,23].

Gellan gum (GG) is a hydrophilic and anionic exopolysaccharide obtained aerobically from the bacteria *Sphingomonas elodea* and composed by repeated units of glucose, glucuronic acid, and rhamnose in 2:1:1 molecular ratio, and two acetyl substituents,

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acetate, and glycerate, linked on glucose residue adjacent of the glucuronic acid[24,25].

GG is widely used as an additive in food industry; at low concentrations, it forms gels and it is biocompatible and biodegradable. In aqueous solution, the gelation of GG is accompanied by the chains conformational transition from random coils to double-helical conformation and then the double-helical arrangement form ordered junction zones, resulting in a three-dimensional network[11,26]. In the presence of cations, the ionotropic gelation occurs, in which polymer negative groups interact with a divalent or multivalent counter ion, building a stronger and dense hydrogel network[27,28].

These characteristics make GG an interesting material for pharmaceutical applications[22,29]. Several drug delivery systems based on GG, such as *in situ* momethasone furoate nasal gel[30], *in situ* gelling terbinafine hydrochloride ophtalmic nanoemulsion[31], and GG tablets with metronidazole[32] were developed. The development of hydrogel microspheres based on GG crosslinked with mono and divalent cations, mainly $Ca^{2+}$, has been exploited to obtain different drug delivery systems[22,24].

Trivalent cation ($Al^{3+}$) have been evaluated for microspheres production because the crosslinking with trivalent ions can be advantageous by enabling a faster crosslinking reaction due to their extra positive charge, when compared, for example, with calcium ions. Each molecule of aluminum is able to conjugate with a higher number of sites of the polysaccharides. Therefore, the use of a low concentration of crosslinking solution may allow the faster formation of a more rigid hydrogel, reducing the reaction time and consequently reducing the risk of drug solubilization or degradation in the crosslinking solution, providing a more efficient drug encapsulation and drug release control[33–35].

Maiti and coworkers reported the preparation of GG microspheres crosslinked with aluminum ions and glutaraldehyde for prolonged release of glipizide, studying the influence of the dual crosslinking (ionic and covalent) in microspheres characteristics and drug release profiles[23]. Prezotti and coworkers (2014) developed microspheres of GG and pectin blends, crosslinked with $Al^{3+}$, demonstrating the effective reduction of release rates of ketoprofen (KP) in simulated gastric media[36].

Traditional methods of performing experiments involve material and time spending, especially when new formulations are developed. The factorial design technique is an efficient method to indicate significant effects of variables and their interactions. The use of this technique helps optimizing the samples preparation and analysis of the process, minimizing the amount of time and materials spent during the development[37,38].

In this paper, GG microspheres containing KP as model drug were prepared by ionotropic gelation using aluminum chloride as crosslinker. The physicochemical characterization included analyses of size, shape, surface morphology and internal structure, swelling ability, and entrapment efficiency (EE%). The influence of the variables polymer and crosslinker concentrations on particle size, shape, and EE% was evaluated by a $2^2$ randomized full factorial design. The mucoadhesive ability of microspheres was assessed through *in vitro* and *ex vivo* analysis, and the drug release profile was evaluated in media that simulate the pH variation of the GIT.

## Materials and methods

### Materials

Low acyl GG (200–300 KDa) was kindly provide by CP Kelco (Kelcogel® CG-LA); KP (batch # 09072223) was obtained from Zhejiang Jiuhzou Pharmaceutical Co. (Taizhou, China); aluminum chloride from Vetec; Mucin type II and Total Protein Kit Micro Lowry, Peterson's Modification were purchased from Sigma Aldrich® (St. Louis, MO). All other materials used were of analytical grade and obtained from commercial suppliers.

Table 1. Composition of the beads formulations and codes for the $2^2$ randomized factorial design employed.

| Sample | GG concentration (%) ($X_1$) | $Al^{3+}$ concentration (%) ($X_2$) | Drug concentration (%) |
|---|---|---|---|
| G1Al3 | 1.0 (−1) | 3.0 (−1) | 1.0 |
| G1Al5 | 1.0 (−1) | 5.0 (+1) | 1.0 |
| G2Al3 | 2.0 (+1) | 3.0 (−1) | 1.0 |
| G2Al5 | 2.0 (−1) | 5.0 (+1) | 1.0 |
| G2Al3A-C | 2.0 | 3.0 | 0.0 |
| G2Al5A-C | 2.0 | 5.0 | 0.0 |

### Factorial design

A $2^2$ randomized full factorial design was used in this study to evaluate the effects of the variables GG concentration (X1) and crosslinker concentration (X2) on microspheres size, shape and EE%. The independent variables levels and respective values of the experimental design are given in Table 1.

### Preparation of microspheres

Microspheres were prepared by ionotropic gelation method. Aqueous dispersions of GG (1.0% or 2.0% w/v) at pH 4.0 were prepared under magnetic stirring at 60 °C. KP (1% w/v) was added under constant stirring, until complete homogenization. Microspheres without drug were prepared as control.

Polymer dispersions were dripped into the cooled (4 °C) crosslinking solution containing aluminum chloride 3% or 5%, using syringe and flat-tipped needles (23G), under constant magnetic stirring. The microspheres were kept under constant stirring for 20 min to complete the crosslinking reaction. The microspheres were separated by filtration, washed with distilled water, and dried at room temperature until constant weight.

Samples were labeled according to the GG concentration (G1 = 1% and G2 = 2%) and crosslinking solution concentration (Al3 = 3% and Al5 = 5% of AlCl3). Control microspheres without drug were labeled without suffix C (Table 1).

### Evaluation and characterization of microspheres

#### Particles size and shape analysis

Microspheres were analyzed on a stereoscope (Leica MZ APO®), and images were captured using Motic Images Advance 2.0 program. Microspheres size and shape were measured using an image analysis program (Motic Advance Images 3.2). The average diameter and circularity of 100 microspheres of each formulation were calculated. To evaluate samples polydispersity, the Span index was determined based on size distribution data, following Equation (1):

$$\text{Span} = (\text{D90} - \text{D10})/\text{D50} \quad (1)$$

where D90, D10, and D50 are the diameters (μm) determined for 90th, 10th, and 50th percentile, respectively.

#### Scanning electron microscopy

The microspheres surface and internal structure of microspheres were analyzed by field emission gun scanning electron microscopy

(FEG-SEM; JEOL JSM-7500F, Japan). To evaluate the internal structure, microspheres were frozen with liquid nitrogen and after fractured. Samples were attached to the sample holder with a double-side adhesive tape, and photomicrographs at different magnifications were taken.

### Entrapment efficiency

To determine the EE%, a known mass of dried microspheres was allowed to swell in 0.1 ml of phosphate buffer (pH 7.4) during 5 min and then crushed inside the centrifuge tube. The microspheres were added by absolute ethanol (10 ml) to dissolve the drug and kept under stirring for 1.5 h, at room temperature. Tubes were well sealed to avoid solvent evaporation. After, samples were centrifuged at 2000 rpm (5 min) to precipitate polymeric debris, and the amount of drug in the supernatant was quantified on a UV–Vis spectrophotometer (Hewlett Packard-Kayak XA), at 254 nm. Tests were performed in triplicate, and the EE% was calculated according Equation (2):

$$\%\mathrm{EE} = (\mathrm{AA}/\mathrm{TA}) * 100 \quad (2)$$

where TA was the total amount of drug added, and AA was the quantified amount of drug.

### Liquid uptake study

Swelling study was performed on an Enslin device with different media to simulate the variation throughout the GIT: simulated gastric fluid (0.1N HCl, pH 2.0), simulated intestinal fluid (pH 7.4), and simulated colonic fluid (pH 6.0), all of them without enzymes. The volume of media uptake by the sample was measured at predetermined time intervals (1, 2, 5, 10, 15, 30, 60, and 90 min). The tests were performed in triplicate and results were expressed as percentage of swelling (%S), according to Equation (3):

$$\%S = V/m \times 100 \quad (3)$$

where $m$ is the initial mass of microspheres (g); $V$ = volume (mL) of media absorbed; $\%S$ = percentage of swelling (%).

### In vitro analysis of the mucoadhesive properties

The mucoadhesiveness was analyzed by studying the adsorption of mucin on the microspheres[3]. Mucin solutions were prepared at different concentrations (50, 100, 150, and 200 μg/ml), and 20 mg of microspheres were dispersed in these solutions for 1 h at 37 °C. Then, the dispersions were centrifuged at 3000 rpm (2 min), and the supernatant was used to quantify the free mucin content by colorimetric method, using a Lowry protein assay modified by Peterson[39,40]. For the determination of free mucin in the supernatant, an UV–Vis spectrophotometer at 749 nm and reagents from Total Protein Kit, Micro Lowry, Peterson's Modification (Sigma-Aldrich®, St. Louis, MO) were used[36]. For the mucoadhesive property analysis, samples containing high amount of GG (2%) were selected, since they presented higher circularity and EE%.

### Ex vivo mucoadhesion evaluation

The *ex vivo* mucoadhesion test was performed as described by Prezotti et al. using porcine intestinal tissue. The method was based on the procedure purposed by Rao and Bur[41]. Briefly, pieces of fresh tissue (4 cm × 4 cm) were opened longitudinally and washed using saline solution (0.9%) and attached to the inclined plastic support (30 °C) of the device. The microspheres ($n = 30$) were placed on the tissue surface, allowing a 20-min contact time with the mucous layer. After this, phosphate buffer pH 6.0 was used to rinse the tissue for 5 min at a rate of 30 ml/min. The percentage of mucoadhesion was calculated from the difference between the number of microspheres attached to the biological surface at the beginning and at the end of the test.

### Dissolution test and analysis of drug release mechanism

This analysis was performed on a Hanson Dissolution Test Station SR8-Plus (Chastworth, CA) equipped with USP apparatus 1 (basket) at 50 rpm. The experiment was conducted using media with different pH values at 37 °C: simulated gastric media (900 mL of 0.1N HCl pH 1.2 with sodium lauryl sulfate 0.75%) during 120 min and enteric media (900 mL of phosphate buffer pH 7.4). At predetermined time intervals, aliquots of 3 ml were withdrawn and immediately replaced with fresh dissolution media. The amount of drug released was quantified using an UV–Vis spectrophotometer at 258 nm and 260 nm, for gastric and enteric pH, respectively. The experiment was conducted with raw KP and samples G2Al3 and G2Al5, once they presented the highest values of EE%. The tests were performed in triplicate, with a mass of microspheres containing 100 mg of KP.

Drug release data were fitted with different mathematical models (Korsmeyer–Peppas, Higuchi, First-order, Hixson–Crowell and Baker–Lonsdale) to determine the mechanism of drug release.

### Statistical analysis

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

## Results

### Evaluation and characterization of microspheres

#### Particles size and shape analysis

The diameter of the microspheres ranged from 700.17 to 938.32 μm (Table 2) with sample G1Al3 presenting the smallest mean diameter (700.17 μm) ($p < 0.05$). GG microspheres presented circularity values between 0.641 and 0.796 (Table 2). Samples showed a monodispersed and unimodal size distribution, with Span index between 0.08 and 0.18 (Table 2).

#### Scanning electron microscopy

The microspheres SEM photomicrographs showed very irregular surfaces and KP crystals in the internal structure (Figure 1).

#### Entrapment efficiency

The microspheres EE% ranged from 48.76 to 87.52% (Table 2).

#### Liquid uptake study

The results demonstrated the high liquid uptake ability of the samples, with values ranging from 216.2% to 303.3% in media with pH 1.2; from 248.3% to 255.2% in media with pH 6.0, and from 195.5% to 245.8% in media with pH 7.4 (Table 2).

Table 2. Values of circularity, size (μm), span index, EE%, and swelling (%S) of GG beads obtained through ionotropic gelation and Weibull coefficients from drug release studies (mean ± SD).

| | | | | | | | | Weibull coefficients | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | %S | | | pH 1.2 | | pH 7.4 | |
| Sample | Circularity | Diameter (μm) | Span | %EE | pH 1.2 | pH 6.0 | pH 7.4 | $r^2$ | *b* | $r^2$ | *b* |
| G1Al3 | 0.739 ± 0.059 | 700.17 ± 94,86 | 0.13 | 68.79 ± 3.36 | 222.03 ± 23.14 | 249.58 ± 16.95 | 195.49 ± 15.37 | – | – | – | – |
| G1Al5 | 0.641 ± 0.119 | 812.46 ± 103.59 | 0.18 | 48.76 ± 2.56 | 216.19 ± 36.01 | 248.32 ± 9.63 | 242.93 ± 5.82 | – | – | – | – |
| G2Al3 | 0.679 ± 0.079 | 866.52 ± 55.67 | 0.08 | 87.52 ± 8.31 | 303.03 ± 44.37 | 255.22 ± 15.30 | 245.85 ± 5.47 | 0.9898 | 1.22 | 0.9948 | 0.8819 |
| G2Al5 | 0.796 ± 0.034 | 938.32 ± 43.79 | 0.14 | 82.34 ± 2.90 | 261.31 ± 56.85 | 253.86 ± 8.29 | 204.18 ± 21.99 | 0.9546 | 1.943 | 0.9967 | 2.220 |

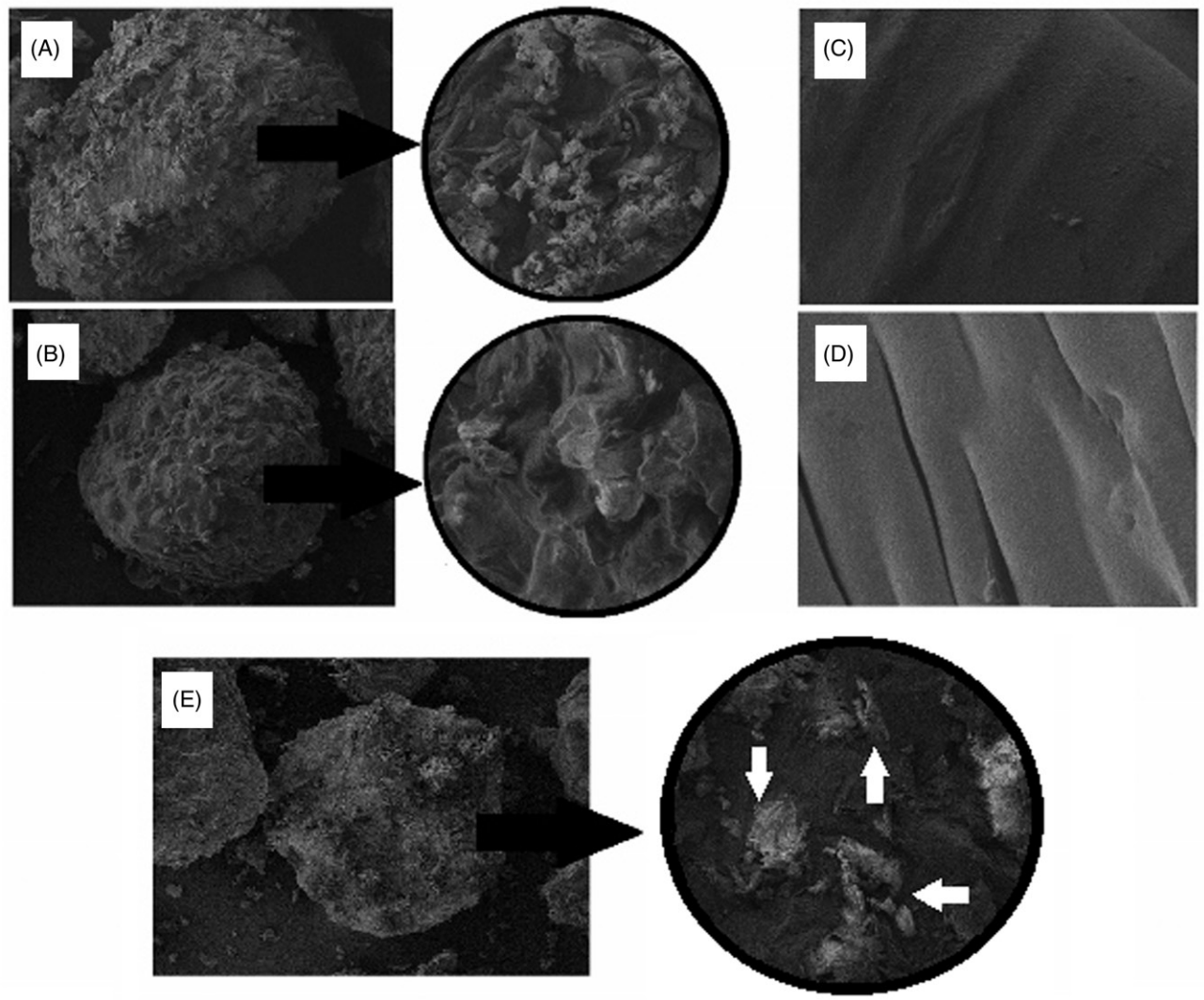


Figure 1. Photomicrographs of a) G2Al3; (b) G2Al5 evidencing the irregular surface (100× and 1000×); (c) G2Al3-C; (d) G2Al5-C evidencing the smooth surface (1000×); (e) G1Al3 evidencing the internal structure (50× and 1000×).

### In vitro *analysis of the mucoadhesive properties*

At the *in vitro* evaluation, the increase in mucin concentration increased the amount of adsorbed mucin (Figure 2). For G2Al3, the mucin adsorbed ranged from 408.97 to 1778.84 μg, and for G2Al5 from 340.45 to 1687.67 μg (Figure 2).

### Ex vivo *mucoadhesion evaluation*

The microspheres presented 100% of mucoadhesive capacity at the end of the *ex vivo* test.

### *Dissolution test and analysis of drug release mechanism*

The dissolution rates of free KP and from the microspheres are presented in Figure 3.

After 120 min in acid pH, about 72% of free KP was dissolved, and the dissolution was completed in pH 7.4 after 180 min (Figure 3). In the first 120 min in acid pH, G2Al3 and G2Al5 released 42.40% and 44.93% of the drug, respectively. The dissolution rate in acid media was lower than the free KP (about 1.64 times), demonstrating the ability of the system in controlling the drug release rates. In media with pH 7.4, a burst effect was observed, so that 87.23% and 90.75%

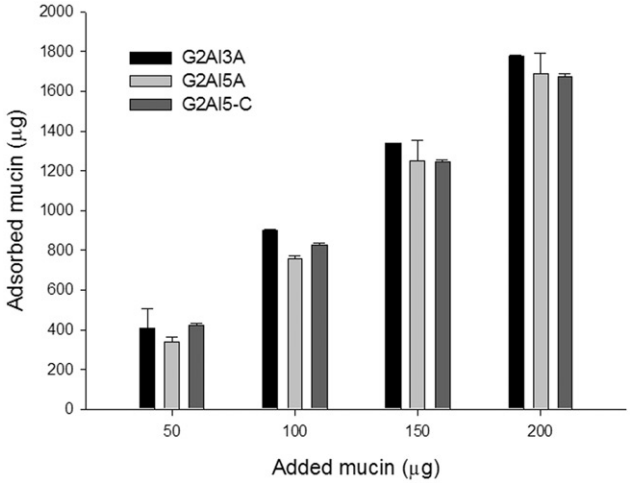


Figure 2. Relation between the amount of mucin added and the mucin adsorbed on different GG beads.

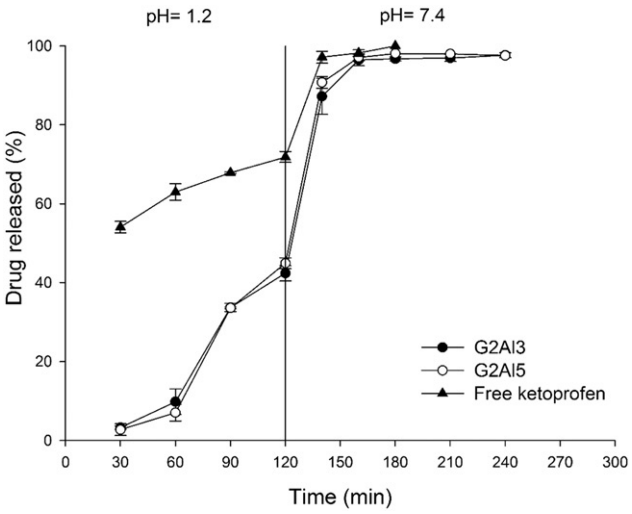


Figure 3. Drug release profile from GG beads.

of the drug was released in 140 min, and 100% of the drug was released after 240 min for G2Al3 and G2Al5 samples.

The release profiles were analyzed using mathematical models in order to describe the mechanism of drug release from the microspheres. According to the correlation coefficient ($r^2$), the model that better describes the release is the Weibull model (Table 1).

## Discussion

### Preparation of microspheres

GG microspheres were prepared by ionotropic gelation in which negatively charged GG interacted with aluminum ions. It was observed that concentrations of GG below 1% did not allow the formation of spherical particles. The low viscosity of the polymer dispersion and the superficial tension could not counteract the impact and drag forces that droplets suffer when enter in contact with the crosslinking solution, so the droplets did not penetrate correctly on the solution and nonspherical particles were formed[42,43]. When the polymer concentration was increased (2%), the viscosity of the dispersion was improved, so that the droplets were more resistant against the drag forces and spherical microspheres were built.

### Characterization of microspheres

Hydrogel droplets are formed during the dripping using a syringe and a flat-tipped needle, and these droplets should be as regular as possible and overcome the impact and drag forces with the surface of the crosslinking solution in order to maintain the spherical shape after the crosslinking reaction. The higher viscosity of the dispersions with higher polymer concentration allowed a greater resistance against deformation during the dripping process, and when the number of $Al^{3+}$ ions was increased in the gelling bath, a faster crosslinking occurred, originating a more rigid polymer network that kept the spherical shape[42–44].

From the factorial design, it can be concluded that both polymer and crosslinker concentration had significant effects on particles size ($p < 0.05$). The increase in polymer concentration from 1 to 2% led to an increase in particle size. The high polymer concentration forms a more viscous dispersion and thus bigger droplets during the dripping are formed, originating microspheres of increased diameter[36,45]. The increase of crosslinker concentration from 3 to 5% also promoted an increase in particle size ($p < 0.05$). In the presence of a large number of $Al^{3+}$ ions, a more branched network can be built, enlarging the particle size[36].

Factorial design results also showed that polymer concentration had a significant effect on particles circularity as well as a significant interaction between polymer and crosslinker concentration ($p < 0.05$). The low polymer content (1%) associated with the high crosslinker concentration (5%) decreased the particles circularity and the sample G1Al5 presented the lowest circular degree (0.641). On the other hand, the high polymer content (2%) associated with the high crosslinker concentration (5%) improved the microspheres circularity, and the sample G2Al5 showed the highest circularity (0.796; $p < 0.05$).

From SEM photomicrographs, it was possible to observe that the increase of GG and crosslinking concentrations, as well as the presence of drug, increased the surface irregularity. Control samples without drug presented a smooth and homogeneous surface, and the increase of crosslinker concentration caused more indentation. The photomicrographs from the internal structure showed that KP crystals distributed inside the polymer matrix.

Microspheres presented high percentages of drug encapsulation. From the factorial design, it can be concluded that both polymer and crosslinker concentration presented significant effects on EE% ($p < 0.05$). Polymer concentration presented a positive effect in the amount of encapsulated drug, so that at high GG content (2%), the EE% was increased. In the presence of higher amount of polymer chains, the network produced by the crosslinking with $Al^{3+}$ ions is more able to entrap the drug inside the matrix[46]. However, crosslinker concentration showed a negative effect on EE% that decreased when aluminum chloride concentration was higher (5%). There was a significant interaction effect between the two selected variables, and the condition that allowed the best EE% (87.52%) was the presence of high polymer and low crosslinker concentrations.

### Liquid uptake study

The swelling process of a polymeric material occurs at molecular level and involves the diffusion of molecules from a liquid into the polymer network. This diffusion is possible due to the mobility of local segments of the polymer chains, which accommodate the liquid molecules. However, it is known that the ionic crosslinking

process immobilizes the polymer chains, and consequently the swelling ability of the polymeric system can be reduced[47,48].

The liquid uptake ability is an important feature of drug delivery systems since the water absorption is the first step for the drug release process, for further matrix swelling and/or diffusion of the drug. However, the drug dissolution and the matrix erosion can also impel the drug release process[49,50]. This ability is also important because it can ensure the access of bacterial enzymes that act in polymer matrix degradation and the liquid uptake can assist in the mucoadhesion process, as the absorption of liquid provides polymer chains relaxation and makes them available to interact with mucus layer[51–53].

All microspheres presented a non pH sensitive liquid uptake behavior, since there were no significant differences in the percentage of swelling (%S) between the different media for all samples ($p > 0.05$).

### Mucoadhesion tests

Mucoadhesion is an important property for multiparticulate systems because it may prolong the residence time in the absorption site and promote a closer contact with the epithelial barrier, increasing drug permeability and absorption[54–56].

Colon has a low mucus turnover rate compared to the stomach and the small intestine and a slow motility; these features facilitate the contact of the polymeric system with the glycoproteins which are the structural components of mucus and primarily responsible for the adhesive interaction and enable the consolidation phase of the mucoadhesion mechanism[57,58].

The mucin adsorption values were about four times higher than those observed by Dhawan and coauthors in a study with chitosan microspheres and very similar to the results obtained by Prezotti et al. with microspheres of GG/pectin blends. The adsorption ability was not influenced by crosslinker concentration and drug presence ($p > 0.05$). Considering that the mucin solutions had pH 4.79, higher than the pKa of the GG (pKa 3.5) and mucin (pKa 2.6), both should be negatively charged due to ionization of the carboxyl groups. Thus, the electron theory does not explain the mucoadhesion process and the adsorption of mucin to the microspheres. Possibly, the electrostatic repulsion due to negative charges of the polymers made the chains farther from each other, facilitating the interaction and the bond formation with mucin glycoproteins chains (Figure 4)[59,60].

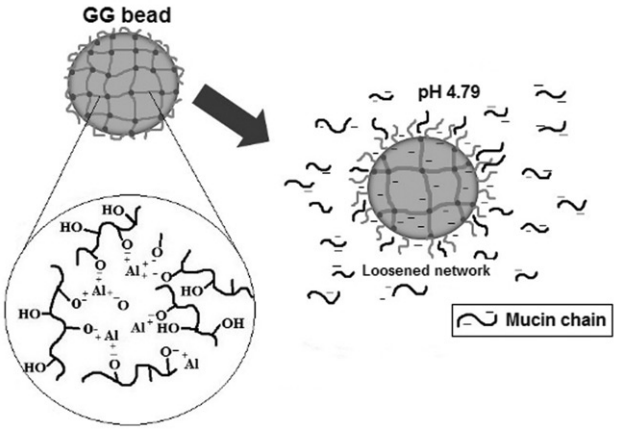


Figure 4. Schematic illustration of the mucoadhesion process by electrostatic repulsion of the polymers chains and interaction with mucin.

In the *ex vivo* mucoadhesion test, all microspheres from all samples were strongly attached to the porcine mucosa. This result corroborates with the *in vitro* mucoadhesion results, evidencing the high mucoadhesive ability of the microspheres developed in this study.

### Dissolution test and analysis of drug release mechanism

The dissolution results demonstrated that the crosslinking reaction allowed the effective control of drug release in acid media but the crosslinking degree did not affect significantly the drug release rates ($p > 0.05$). The increase in the pH should increase the dissociation of polymer carboxyl groups, resulting in a more dilated and hydrophilic polymer network, favoring the entry of liquid into the matrix and the further dissolution and release of the drug. The set of results evidenced the pH-dependent drug release behavior with reduced release rates in acid media[22,35].

The Weibull model that better described the release mechanism expresses the cumulative amount of drug at the dissolution medium at a certain time and can be adjusted to different dissolution profiles[61]. In this model, the *b* exponent value indicates the drug release mechanism through the polymer matrix. Values of $b < 0.75$ indicate Fickian diffusion, values of $0.75 < b < 1.0$ indicate a non-Fickian diffusion, and values of $b > 1.0$ indicate a complex drug release mechanism that involves diffusion, swelling, and/or erosion of the matrix[14,62]. As seen in Table 1, the *b* coefficient values were higher than 1 in both medium, except for the G2Al3, indicating that the drug release is driven by a complex mechanism involving erosion and swelling of the matrix. The G2Al3 sample showed a value of $b < 1$ at pH 7.4, indicating that the release mechanism of the drug from the matrix followed a non-Fickian diffusion, in which the drug diffusion is governed by the matrix swelling and the polymer chains relaxation. Therefore, the change in the media pH value altered the drug dissolution profile but did not change the release mechanism of the drug from the microspheres.

## Conclusions

Mucoadhesive GG microspheres were successfully prepared by ionotropic gelation with $Al^{3+}$. By applying a two-level factorial design, the effects of the polymer and crosslinker concentrations on microspheres size, shape, and EE% were demonstrated and thoroughly discussed. High polymer concentration originated more spherical and larger microspheres with higher EE%, and the water uptake of the microspheres was no sensitive to pH modifications. The high mucoadhesiveness of microspheres was demonstrated in *in vitro* and *ex vivo* assays. Microspheres were able to reduce the drug release in acid and enteric pH compared to the free drug, demonstrating that the ionic crosslinking of GG with $Al^{3+}$ allowed to produce a promising oral mucoadhesive system that controls the drug release throughout the GIT.

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## Declaration of interest

The authors report no declarations of interest.

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