

Research Article

Polyelectrolyte Complexation versus Ionotropic Gelation for Chitosan-Based Hydrogels with Carboxymethylcellulose, Carboxymethyl Starch, and Alginic Acid

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The preparation of gels by charge interaction methods has been extensively studied, but it is not yet clear how these methods influence gel characteristics. The objective of this work was to study differences in morphology and surface charge of hydrogels prepared by ionotropic gelation, polyelectrolyte complexation, and a combination of both methods. Thus, the anionic charge was provided by carboxymethylcellulose (CMC), carboxymethylated starch (CMS), and alginic acid (AA); calcium chloride (CaCl₂) and chitosan (CS) were used for the ionotropic gelation and polyelectrolyte complexation, respectively. Those materials are commercially available, have low toxicity, and are widely used in the area. These compounds interact through physical crosslinks, which are affected by physical changes of the medium. Our results showed that these two methods produced changes in the morphology of the hydrogels. CMC gels exhibited larger pores in the presence of CaCl₂. In polyelectrolyte complexation, CMS produced an increased agglomeration of particles, while the addition of CaCl₂ to AA generated dispersed particles of size in the order of millimeters. Mixing both ionotropic gelation and polyelectrolyte complexation methods yielded gels of varied charge (568 mV for CMC, 502 mV for CMS, and 1713 mV for AA). FTIR spectra of the hydrogels showed interactions between the different polymeric compounds, being the greatest changes between 1250 and 1600 cm⁻¹, due possibly to the replacement of Na by Ca at crosslinking points. Therefore, the method of gel preparation employed had a major influence on the size and pore distribution, parameters which in turn influence encapsulation and drug delivery in these systems.

1. Introduction

Hydrogels are three-dimensional networks capable of absorbing large quantities of water or biological fluids, therefore, they attract interest in medicine [1]. Polysaccharides have been studied during the last years as raw materials in the encapsulation of drugs forming a mesh structure by charge interaction between anions and cations. Polysaccharides have peculiar properties like good solubility in aqueous environments, high stability, null toxicity, biocompatibility, biodegradability, and have the ability to encapsulate drugs [2–4]. In drug delivery applications, polysaccharides possessing hydroxyl, carboxyl, and amino groups are bioadhesives and could increase the residence time [3].

Several types of hydrogels have been prepared by noncovalent crosslinking, incorporating anionic polymers, such as carboxymethylcellulose, carboxymethylated starch or alginic acid, on cationic polymers like chitosan (polyelectrolyte complexation) [5–10]. Also, the same anionic polymers have been used with CaCl₂ (ionotropic gelation) [11, 12] or a mix of polymers with the salt [13, 14].

In ionotropic gelation, hydrogels are produced due to the ability of polyelectrolytes to crosslink with counterions, forming a meshwork structure of ionically crosslinked. The polyelectrolyte complexation technique forms hydrogels by the addition of one polyelectrolyte to another polyelectrolyte [15] having opposite charge.

There have been several proposals to generate hydrogels with a particular structure. Here, the method chosen plays an important role in the morphology. Some methods that show great promise as a tool for hydrogel manufacture are ionotropic gelation and polyelectrolyte complexation. Each method has some advantages and limitations.

This paper compares the ionotropic gelation, polyelectrolyte complexation, and the mixture of both procedures, on the morphology of gels prepared from carboxymethylcellulose, carboxymethyl starch, and alginate acid with chitosan.

2. Materials and Methods

2.1. Materials. Carboxymethylcellulose sodium salt (CMC) average Mw ~250,000 and degree of substitution 0.7 (CAS 9004-32-4), chitosan (CS) medium molecular weight (75–85% deacetylation) (CAS 9012-76-4), and alginate acid (AA) of medium viscosity (CAS 9005-38-3) are products of Sigma-Aldrich. Carboxylated starch (CMS) was purchased from DFE Pharma. Calcium chloride dihydrate (CAS 10035-04-8) was supplied by Carlo Erba.

2.2. Hydrogel Formation. Polymer solutions were prepared at such concentrations that were able to pass through the needle and formed a separate drop without problems of fluency [16].

2.2.1. Cationic Substance Concentrations. CS concentration was fixed in 0.01% w/v (dissolved in acetic acid 1% v/v) [17]. CaCl₂ was used at 1 and 4.9% w/v dissolved in water (in previous work, lower concentrations did not produce a gel).

2.2.2. Anionic Polymer Concentrations. CMC solutions were prepared at 2% w/v, CMS at 3.2% w/v, and AA at 3 and 0.5% w/v. For ionotropic gelation only, concentrations of 1.6% w/v for CMC and 5.6 for CMS were used. In order to solubilize the polymers, it was necessary to leave each solution under magnetic stirring for 24 hours. CaCl₂ was solubilized under magnetic agitation for 2 hours approximately.

2.2.3. Ionotropic Gelation. Each anionic polymer was dissolved in deionized water (to the desired concentration), and approximately 10 mL of this solution was dropped using a programmable syringe pump (New Era Pump System Inc.), passing through a needle (21 G) from a plastic syringe into a beaker containing 10 mL of the CaCl₂ solution; this system was kept under continuous stirring (600 rpm) for 10 minutes at room temperature. Then, the mix was stirred at the same conditions for 20 minutes more, and the hydrogel was kept 24 hours at room temperature to reach the equilibrium. Afterward, the sample was centrifuged at 3300 rpm for 30 minutes and the precipitate was removed from the supernatant. Finally, the gels were washed with distilled water and separated again by centrifugation.

2.2.4. Polyelectrolyte Complexation. The procedure followed a previously reported methodology [18, 19]. Briefly, a CS solution in acetic acid 1% w/v was used as a cationic agent,

instead of CaCl₂. The mix was stirred at the same conditions for 20 minutes, and later, the hydrogel was kept 24 hours at room temperature until the equilibrium was reached. The sample was then centrifuged at 3300 rpm for 30 minutes and the precipitate was removed from the supernatant. Finally, the gels were washed with distilled water and separated again by centrifugation.

A change to evaluate the effect of the aggregation order was made for the CMC polyelectrolyte complexation, dropping CS 0.016% w/v onto a CMC 0.8% w/v solution.

2.2.5. Preparation of Gels by Simultaneous Polyelectrolyte Complexation and Ionotropic Gelation. A mix of both procedures was carried on by solubilizing the CaCl₂ in the CS solution (2 hours of agitation), and then dropping the CMC on this mixture. The mix was stirred at the same conditions for 20 minutes more, and the hydrogel was kept 24 hours at room temperature to reach the equilibrium. Thus, the sample was centrifuged at 3300 rpm for 30 minutes, and the precipitate was removed from the supernatant, washed with distilled water and separated again by centrifugation.

Three gel samples were prepared for each system in order to compare results.

2.3. Morphological Characterization of the Gels. Images of the wet gels were obtained by an optical microscope (Leica DMREB, software LAS, version 7.1. Camera Leica DFC320). A drop of the sample was put on a slide with a drop of water to disperse the gel before the observation. Then, a coverslip was put upon the sample and was observed.

The surface morphology and internal structure of lyophilized gels were observed by a scanning electron microscope (SEM; HITACHI TM-1000). The samples were cooled with liquid nitrogen and freeze-dried for 24 hours, then stored and characterized.

2.4. Zeta Potential. Zeta potential of the preparations was measured after equilibrium (around 24 hours after the preparation) was established. The zeta potential was read with a Zetasizer Nano ZS90 with DS170 cuvettes, using acetic acid as a solvent for chitosan and hydrogels made by both polyelectrolyte complexation and the mix of ionotropic gelation and polyelectrolyte complexation. Water was used as a solvent for the polymers and hydrogels made by ionotropic gelation. Three readings of zeta potential values were made for each sample.

2.5. FTIR Characterization of Gels. FTIR spectra of freeze-dried samples were recorded with a PerkinElmer FTIR spectrometer, model spectrum GX with an attachment of attenuated total reflectance (ATR) and crystal diamond, taking spectra between 4000 and 600 cm⁻¹ with a resolution of 4.00 cm⁻¹ and 16 scannings.

3. Results

It has been suggested that polymers containing carboxyl groups are better than sulfated polymers for the encapsulation

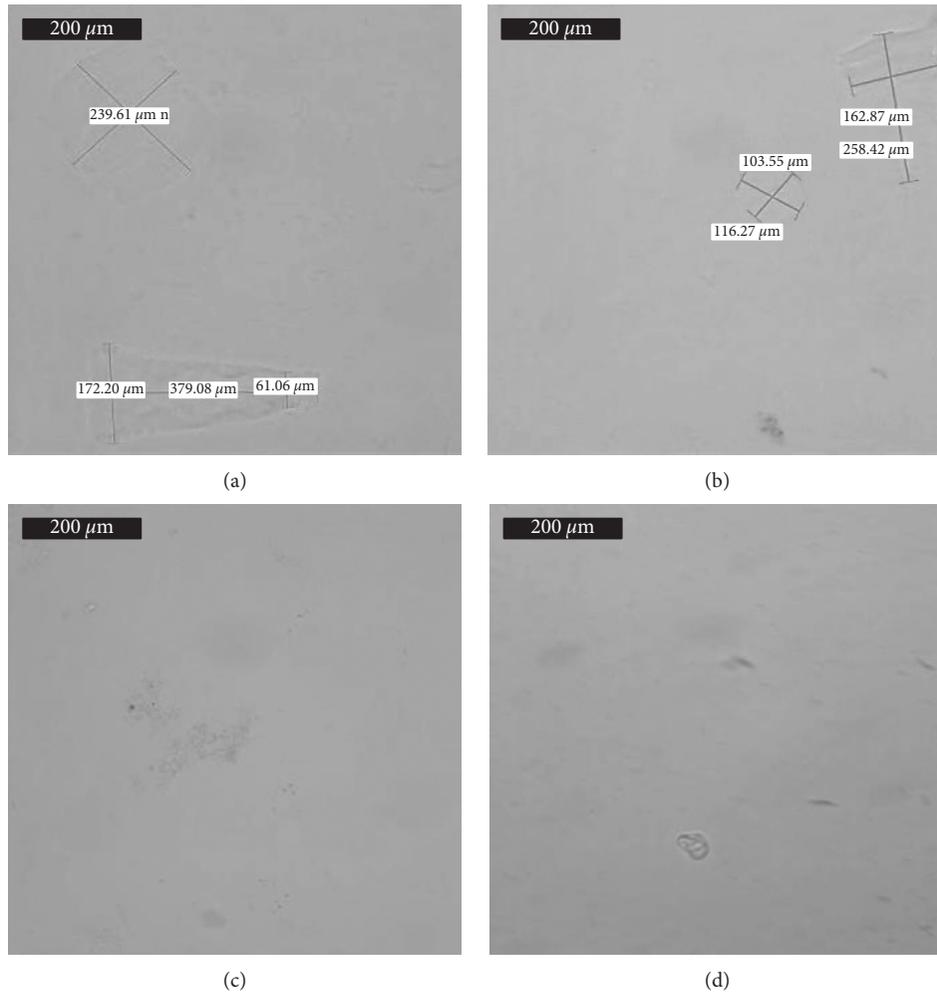


FIGURE 1: Optical micrographs of hydrogels made from CMC 2%: (a) ionotropic gelation with CaCl_2 1% w/v; (b) ionotropic gelation with CaCl_2 4.9% w/v; (c) polyelectrolyte complexation with CS 0.01% w/v; (d) combination of ionotropic gelation and polyelectrolyte complexation (CaCl_2 1% w/v). Scale (a, b) 200x and (c, d) 100x.

and controlled release of drugs [20]. All polyanions used in the investigation have carboxyl groups [20, 21]. The mixture of the different polyanions with the CS produced a hydrogel at a pH lower than 4, and the morphology of hydrogels is very sensitive to the preparation procedures [16, 22, 23]. In this study, differences which are related to the method used in each polymer gel were observed from SEM images. Gulrez et al. [21] claim that, at acidic pHs, the mechanism promotes hydrogen bonding, which induces a decrease in the solubility in water and results in the formation of an elastic hydrogel.

3.1. Carboxymethylcellulose- (CMC-) Based Hydrogels.

Optical microscopy images of CMC hydrogels showed that mixing the polymer with CaCl_2 yielded conical and spiral-like particles without any visible agglomeration. On the contrary, the polyelectrolyte complexation method generates membranes in the form of sponges of different sizes, which could be reduced by the simultaneous use of the two methods of hydrogel preparation.

Micrographs obtained with an optical microscope are reported in Figure 1 for a CMC concentration of 2%, to

compare hydrogels made of CMC by different methods. Ionotropic gelation generated a transparent hydrogel, which became cloudy when the CaCl_2 concentration was increased. Micrographs showed that CMC hydrogels appearance changed with the method used for their production. Conical and spiral shapes were observed by using ionotropic gelation, while polyelectrolyte complexation produced a sponge-like membrane. On the contrary, the mix of procedures yielded a cloudy and viscous suspension (as CaCl_2 concentration increased) with elongated particles.

Many studies about gelation with CMC and CaCl_2 are reported in the literature, but few microscope images are showed and analyzed. Dhanaraju et al. [22], studied the ionotropic gelation of Na-CMC and Na-alginate at 5% fixed concentration of CaCl_2 , finding that the mean size of hydrogel beads increased at higher concentration of both polymers. Huei et al. [24] showed, with optical images, that polyelectrolyte complexation of CMC/gelatin ionically crosslinked using aqueous ferric.

Hosny et al. [23] found that the size of CMC gel beads was affected by the polymer concentration (i.e., 3% w/v); however, more homogeneous forms were obtained at

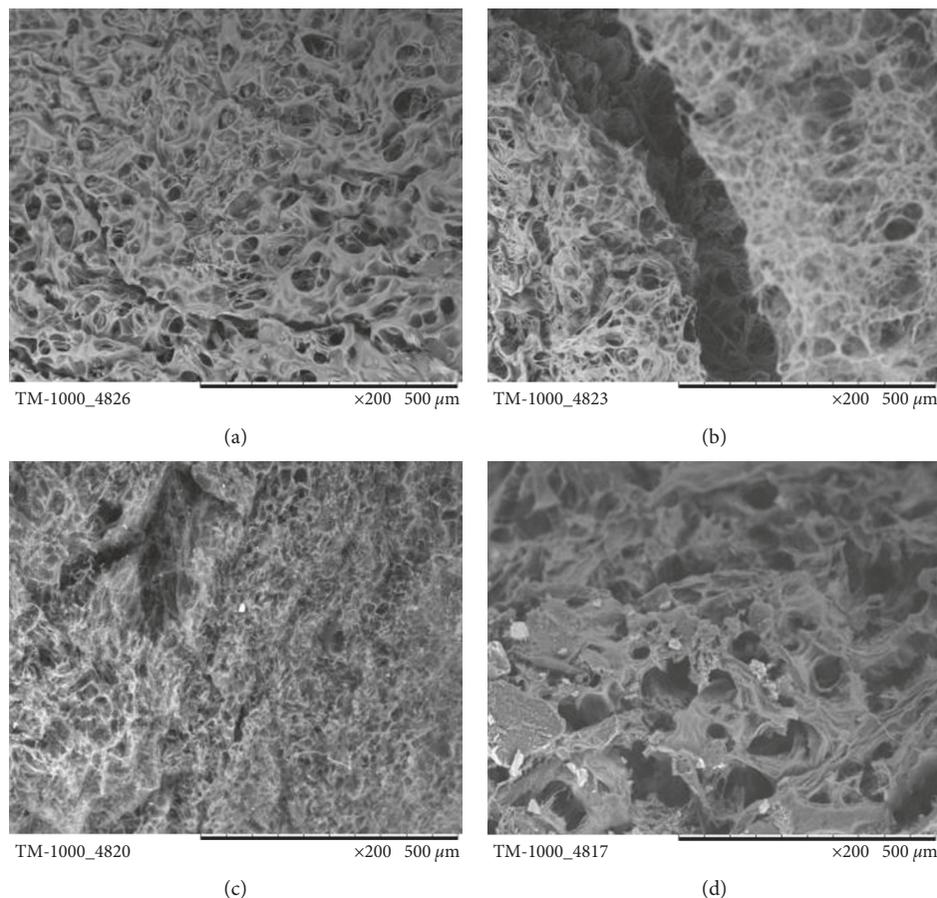


FIGURE 2: SEM images for hydrogels made from CMC 2%: (a) ionotropic gelation with CaCl_2 1% w/w; (b) ionotropic gelation with CaCl_2 4.9% w/v; (c) polyelectrolyte complexation with CS 0.01% w/v; (d) combination of ionotropic gelation and polyelectrolyte complexation (CaCl_2 1% w/w). Scale 200x.

concentrations of 2%, attributing the increased size to the increment in viscosity of the polymer solutions.

Figure 2 shows SEM images for hydrogels made from CMC 2% by different methods. All methods produced sponge-like particles with an observable macroporous structure. In the case of ionotropic gelation, the thickness of the membrane seemed to increase at lower calcium chloride concentrations. The polyelectrolyte complexation gave a membrane with smaller pores while combining the ionotropic gelation and complexation methods produced a thicker membrane with larger pores. SEM images of both ionotropic gelation and polyelectrolyte complexation showed traces apparently of calcium salt not solubilized.

From SEM images, it is evident that the combination of methods rendered a network of larger pores and a denser network, while the ionotropic gelation generated smaller pores and a less dense network. Polyelectrolyte complexation produced even smaller pores and a spongy appearance of the membrane. Dhanaraju et al. [22] only reported SEM images where the observed porous membranes are similar to the sponges obtained in this study.

In order to examine the effect of varying CaCl_2 concentration on hydrogels made by ionotropic gelation, the CMC concentration was decreased to reach 1.6%, and

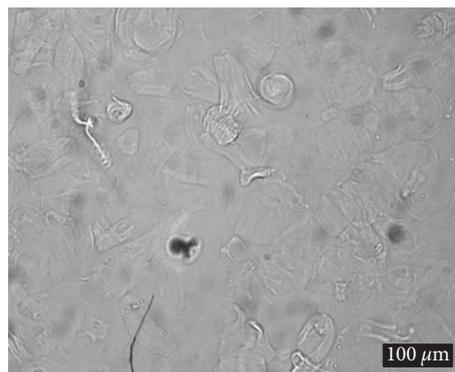


FIGURE 3: Optical micrographs of a hydrogel sample made by ionotropic gelation from CMC 1.6% and CaCl_2 4.9% w/v. Scale 50x.

different concentrations of CaCl_2 were used (4.9, 7.35 y 14.7% w/v) to guarantee the formation of gel beads (Figure 3).

All CaCl_2 concentration levels resulted in particles with truncated-conical and spiral shape in the micrometer range, remaining as a sponge when the gel was lyophilized. The amount and size of these particles increased with increased quantities of the CaCl_2 present in the solution, suggesting that the more CaCl_2 concentration, the more gel was

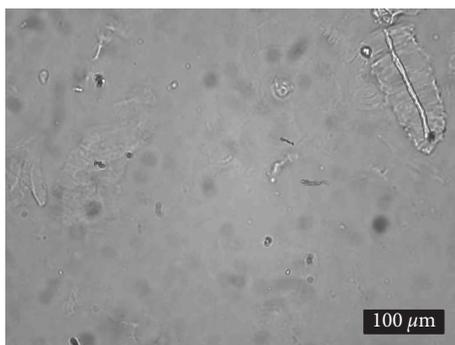


FIGURE 4: Optical micrographs of a hydrogel sample made by polyelectrolyte complexation of CS 0.016% w/v over CMC 0.8% w/v. Scale 50x.

produced. The shape noted in these hydrogels was not observed in the polyelectrolyte complexation of CMC and CS, as well as in the combination of polyelectrolyte complexation and ionotropic gelation of CMC, CS, and CaCl_2 .

Similar particles were obtained in the case of polyelectrolyte complexation, only when CS 0.016% w/v was added drop by drop on CMC 0.8% w/v (changing the sequence of polymers addition). However, the recovery of the precipitate after centrifuging was difficult, due to its smaller size (Figure 4).

3.2. Carboxymethylated Starch- (CMS-) Based Hydrogels.

Optical micrographs are shown in Figure 5 at a CMS concentration of 3.2%. The resulting particles did not appear to change noticeably with the gel-forming method employed. The ionotropic gelation generated more individualized particles, while agglomeration was detected in polyelectrolyte complexation. The agglomeration process seemed to decrease at lower calcium chloride concentrations, as well as possibly due to interaction with CS chains in the combined procedure.

The increase in the concentration of both CMS and CaCl_2 in the ionotropic gelation did not produce major differences in the gel particle structures (Figure 6).

Figure 7 shows SEM images of hydrogel samples made of CMS 3.2% by different methods. All methods studied generated particles similar to raw starch, and only in the case of the polyelectrolyte complexation, it was observed in structures that could be caused by agglomeration of particles.

It is known that CMS gels in water generating particles [25]. Ionotropic gelation and the combination of gel-forming methods also yielded these types of particles. Moreover, polyelectrolyte complexation (without added salt) produced CMS hydrogels with an increased particle agglomeration, a fact confirmed by SEM images which showed amorphous macrostructures of apparently linked particles.

The difference observed between ionotropic gelation and coacervation is the initial agglomeration of the particles of CMS. According to the literature, when the particles agglomerate, they form networks between them, generating cavities attributed to the hydrophilic groups that tend to remain on the surface of the polymer. These cavities are suitable for drug loading and release [26].

Mihaela Friciu et al. [27] indicate that the largest granules observed in the CMS hydrogel are probably due to the association of numerous small particles forming larger granules. Figures 7(c) and 7(d) containing CS show particles with rough surfaces and larger than those obtained by ionotropic gelation, due to an alteration of the structure of the starch by the association of hydrogen between hydroxyl groups that promote repulsion and leads to reorganization of the network. The complex formation shows a greater amount of ionic interactions that lead to the formation of agglomerates of small particles that cover the larger granules as happens in the complexation of CMS with lecithin [27]. The formation of these large particles could help drug storage.

3.3. Alginate Acid- (AA-) Based Hydrogels.

Optical micrographs of hydrogels made from AA (0.5%) employing different techniques are reported in Figure 8. Polyelectrolyte complexation of AA and CS produced an important increment in the particle size, growing from micrometers to a millimeter order, compared to the particles generated by the ionotropic gelation method.

By increasing the AA concentration, particles with a larger size and more defined shape were obtained by means of the polyelectrolyte complexation and the combination of methods (Figure 9).

The most significant differences observed were that with alginate acid and salt addition the mixture generates spheres in the order of millimeters (the shape of the falling drop) for both ionotropic gelation and the combination of ionotropic gelation and polyelectrolyte complexation, suggesting that the cationic substances are able to envelop the AA polyanion. The blending of AA with Ca^+ ions has been widely studied for the formation of hydrogels by crosslinking [4], and most studies about polyelectrolyte complexation involve AA and CS [23].

Hosny et al. [23] reported that spheres of the millimetric order were formed by ionotropic gelation of alginate acid with aluminum ions. Dhanaraju et al. [22] also suggested that gel particles increased in size with a higher concentration of AA because the viscosity increased the size of the falling drop. For polyelectrolyte complexation, a few small points are observed. Hosny et al. [23] indicated as well that, in polyelectrolyte complexation, higher concentrations of AA (3%) yielded less homogeneous spheres with less stability while decreasing the concentration produced spheres of different sizes. Spheres of the millimetric order could be obtained by polyelectrolyte complexation by increasing the CS concentration.

In another study, Blemur et al. [16] found that the ionotropic gelation of AA with CaCl_2 formed a porous structure, while chitosan interacted with alginate to form microspheres which exhibited porosity like alginate.

Mi et al. [28] found that chitosan and alginate beads with higher crosslink density were spherical and had a relatively gross and an even cross-section and affirm that the particles consist of an inner core and an outer layer; those results are similar to the observed in this work. When the cationic charge was increased, particles with a size in the order of mm were obtained which had an even surface. Similarly, the

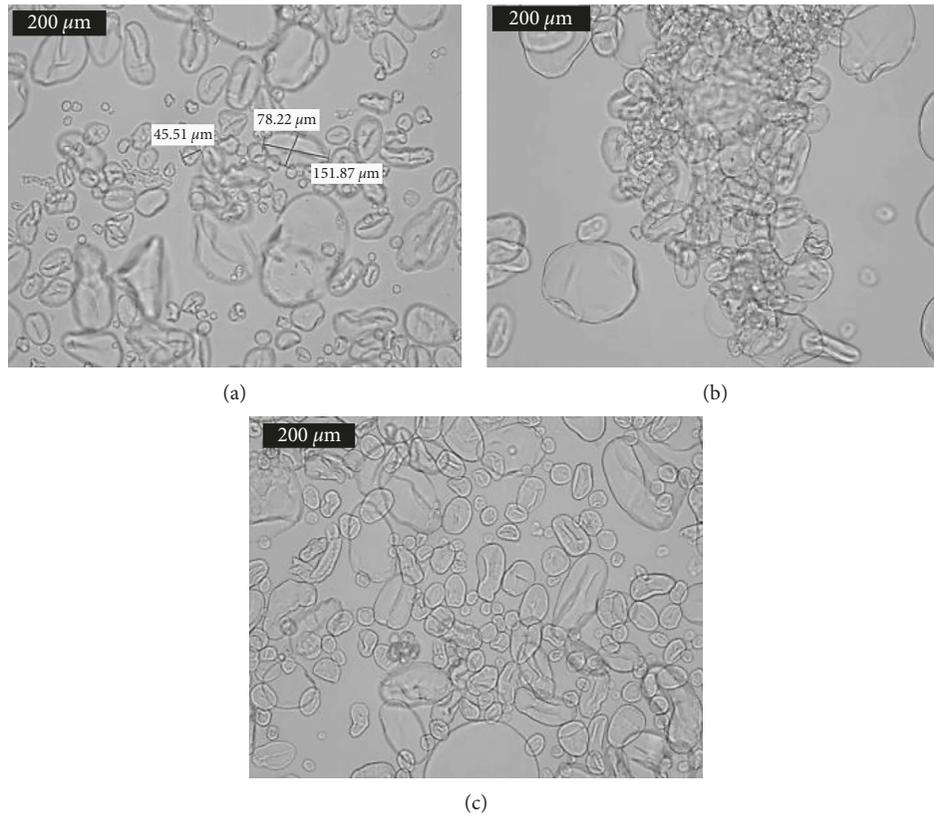


FIGURE 5: Comparison of hydrogel beads made from CMS 3.2% by (a) ionotropic gelation with CaCl_2 4.9% w/v, (b) polyelectrolyte complexation with CS 0.01% w/v, and (c) combination of ionotropic gelation and polyelectrolyte complexation (CaCl_2 1% w/w). Scale 100x.

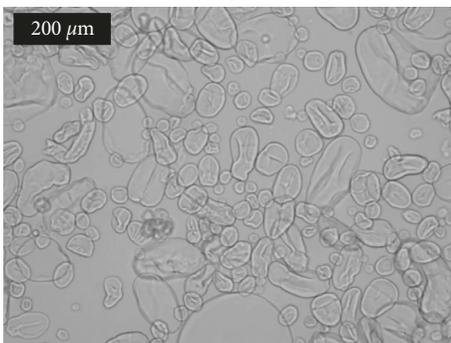


FIGURE 6: Optical micrograph of a hydrogel sample made by ionotropic gelation of CMS 5.6% and CaCl_2 4.9%. Scale 50x.

complexation of alginate with chitosan also has porous walls (data not shown), and Xu et al. [29] showed that the mixture of AA with CS forms a random network that decreases pore formation with the increase in AA.

Finally, it was concluded that each polymer combination produces gels with different pore sizes that could modify the performance of the gels in drug delivery applications. When the superficial pores are in contact with water, they fill with water and facilitate the initial diffusion of the drug, controlled by the dissolution of the solute in the water of the pores and by its diffusion [30]. Besides, when the gel is hydrophilic, a progressive swelling occurs and produces changes in the shape and size of the pores with a drug

diffusion through them, then the release will be both through the pores filled with water and through the swollen polymer.

Release decreases with pore size [31], depending on the porosity of the hydrogel, the size of the drug, and the chemical properties of each, and the drug will diffuse slowly into the gel. Diffusion is regulated by movement through the polymer matrix or by mass erosion of the hydrogel to as it decomposes. Environmentally sensitive hydrogels effectively open their pores for drug diffusion [32]. Then, it can be proposed that, for CMC- and AA-based hydrogel, the release occurs in both ways and the liberation could be slower for hydrogel obtained by polyelectrolyte complexation, and in the case of CMS-based hydrogel, the release is by swelling without dependence of the mechanisms of the formation.

3.4. Zeta Potential of Hydrogels. Table 1 shows the effect of the salt (calcium chloride) on the charge interaction of hydrogels. Calcium chloride has a smaller size than CS and can reach more sites for interaction with the anionic polymer, thus reducing the negative charge of the CMS or AA polymers.

In the study, it was also found that there was a greater equivalence of charge when the two methods of gel formation are combined. Sadeghi et al. [33] suggested that zeta potential is an indicator of charge, which is available on the gel surface, and the values reflect the charge that is not neutralized. They obtained higher negative zeta potential

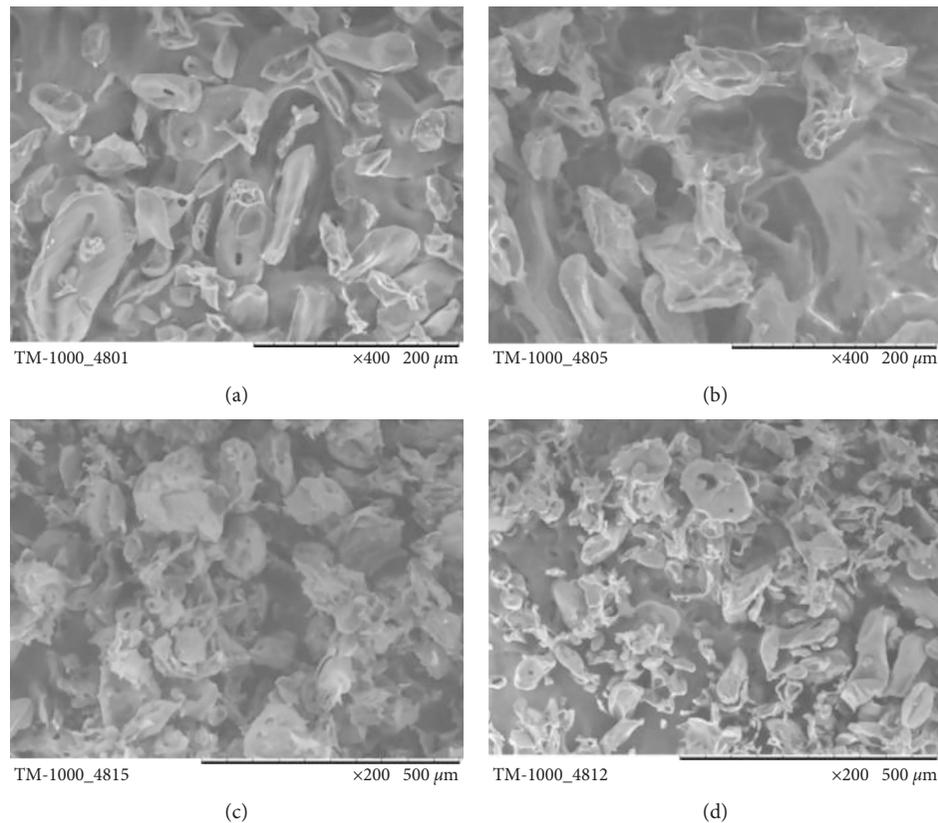


FIGURE 7: SEM micrographs of hydrogel samples made of CMS 3.2% by (a) ionotropic gelation with CaCl_2 1% w/w, (b) ionotropic gelation with CaCl_2 4.9% w/v, (c) polyelectrolyte complexation with CS 0.01% w/v, and (d) ionotropic gelation and polyelectrolyte complexation (CaCl_2 1% w/w). Scale (a, b) 400x and (c, d) 200x.

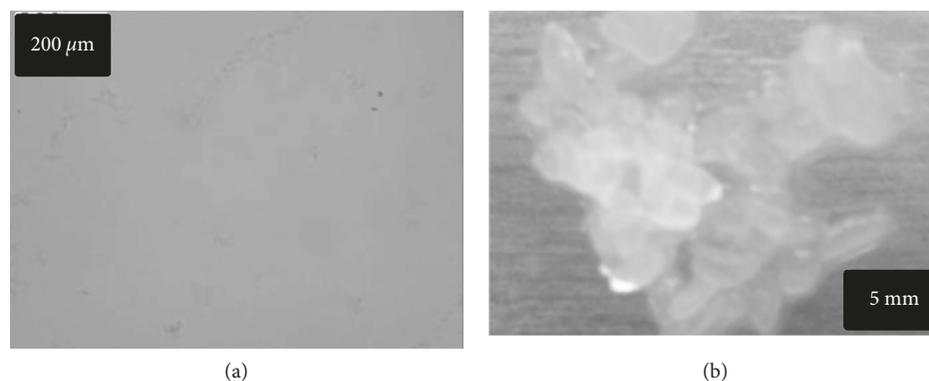


FIGURE 8: Micrographs of hydrogel samples made of AA 0.5% by (a) ionotropic gelation with CaCl_2 1% w/v and (b) polyelectrolyte complexation with CS 0.01% w/v.

values for particles made by the complex polyelectrolyte method than with ionotropic gelation because the salt (cationic electrolyte present in this system) gives more positive charge, in order to neutralize more negative charges.

Then, according to Sadeghi et al. [33], polyelectrolyte complexation reveals that not all anionic polymer charges were balanced, probably due to the low concentration of chitosan. The addition of cationic electrolyte (such as in the combination of methods) decreases the magnitude of negative

charges and suggests an increased molecular interaction for the resulting zeta potential values that are close to zero.

Particles with positive zeta potential values are more stable. According to Lamarra et al. [34], a higher negative charge gives larger and less stable particles, which was also observed in this work. On the contrary, negative zeta potential values seem to propitiate aggregation. It is known that positive zeta potential keeps a thicker double-layer that appears to prevent aggregation [35].

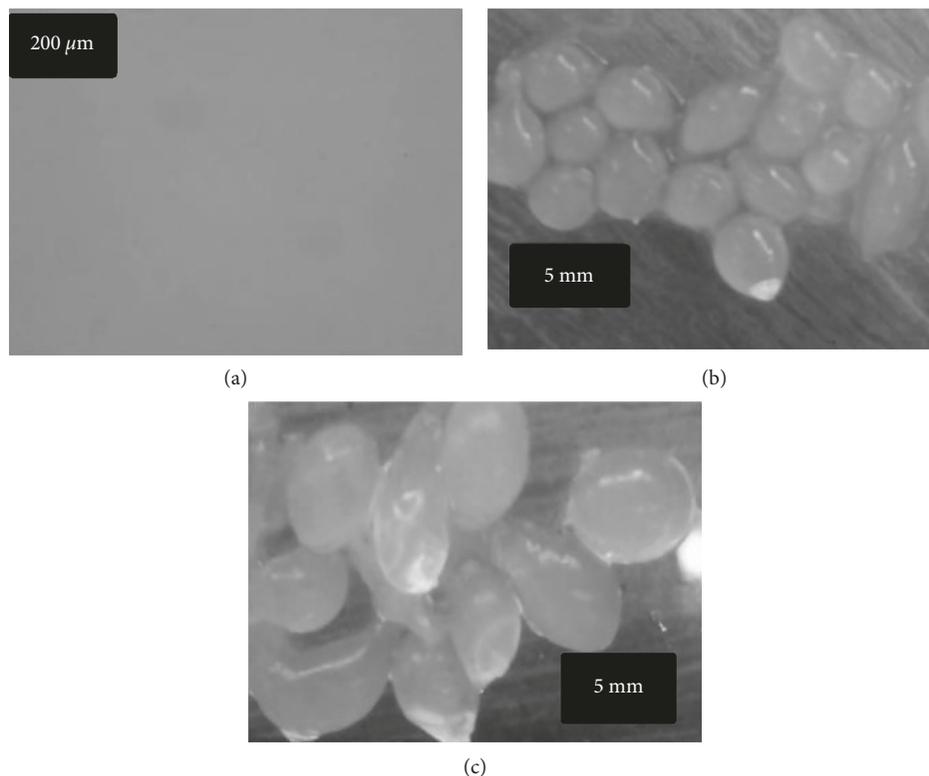


FIGURE 9: Optical microscope images of hydrogel samples made of AA 3% by (a) ionotropic gelation with CaCl_2 1% w/v, (b) polyelectrolyte complexation with CS 0.01% w/v, and (c) combination of ionotropic gelation and polyelectrolyte complexation (CaCl_2 1% w/v).

TABLE 1: Zeta potential for samples made by polyelectrolyte complexation and ionotropic gelation-polyelectrolyte complexation.

Anionic polymer/CS ratio	Zeta potential (mV) ionotropic gelation + polyelectrolyte complexation	Zeta potential (mV) polyelectrolyte complexation
CMC/CS, 200	-394	-962
CMS/CS, 320	-134	-636
AA/CS, 50	330	-786
AA/CS, 300	-37	-1750

3.5. FTIR of Hydrogels. FTIR spectra showed interactions between the cationic and anionic polymers in the polyelectrolyte complexation. The same was noted between the anionic polymers and calcium chloride for the ionotropic gelation and between the three components for the combination of ionotropic gelation and polyelectrolyte complexation methods.

Figures 10 and 11 show the IR spectra of hydrogel samples prepared from CMC and CMS by ionotropic gelation, polyelectrolyte complexation, and a combination of both. Both samples showed membrane-like structures in the micrometer order.

The characteristic absorption bands of chitosan -OH bond are at 3352 cm^{-1} . At 2918 cm^{-1} there is the signal of $-\text{CH}_2$ groups. Also, the characteristics bands at 1640 , 1570 , and 1460 cm^{-1} , belonging to vibration of carbonyl bonds ($\text{C}=\text{O}$) of the amide group and protonated amine group (NH_3^+) can be seen. Signals of C-H bonds are identified at 1420 and 1380 cm^{-1} . The bands at 1300 and 1250 cm^{-1} correspond to C-N stretch, while the bands at 1150 , 1040 , and 1030 cm^{-1}

belong to C-O group (COH , COC , and CH_2OH). The band at 1150 cm^{-1} is attributed to asymmetric vibrations of CO resulting from the deacetylation of chitosan. The band at 890 cm^{-1} is related to the glycosidic bonds [36].

Figure 10 shows bands for CMC at 1570 cm^{-1} , 1412 cm^{-1} , and 1315 cm^{-1} , which are assigned to asymmetrical COO^- stretching, symmetrical stretching, and C-H bending, respectively, indicating the presence of the carboxymethyl ether group. The band at 3315 cm^{-1} corresponds to -OH stretch. Also, the band at 2918 cm^{-1} can be assigned to CH_2 stretching of the $-\text{CH}_2$ groups. Bands at 1080 , 1040 , and 1030 cm^{-1} belong to C-O-C and C-O characteristic of polysaccharides. The band at 890 cm^{-1} is attributed to the glycosidic bond and the saccharide structure [37].

The CMC/CS complex has the characteristic bands of both chitosan and carboxymethylcellulose, namely, glycosidic bonds, OH, $\text{C}=\text{O}$, CH_2 , and amide (Figure 10), which is evidence of the formation of the complex. The CMC/ CaCl_2 gel has the characteristic bands of both carboxymethylcellulose and CaCl_2 , and finally, the CMC/CS/ CaCl_2 spectrum

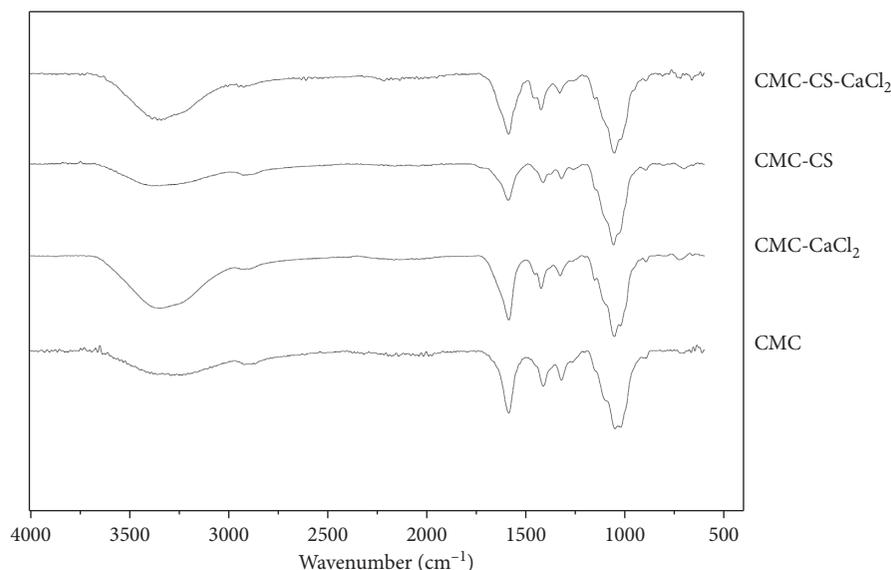


FIGURE 10: FTIR Spectra of hydrogels made from CMC (a) 2% by (b) ionotropic gelation with CaCl_2 1% w/v, (c) polyelectrolyte complexation with CS 0.01% w/v, and (d) combination of ionotropic gelation and polyelectrolyte complexation mixing.

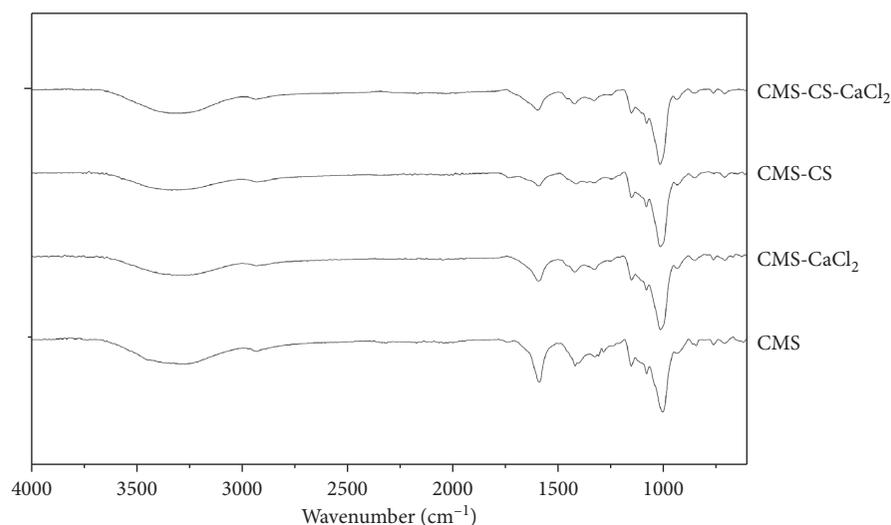


FIGURE 11: FTIR Spectra from hydrogel samples made of CMS (a) 3.2% by (b) ionotropic gelation with CaCl_2 1% w/v, (c) polyelectrolyte complexation with CS 0.01% w/v, and (d) combination of ionotropic gelation and polyelectrolyte complexation.

shows characteristic of all compounds, indicating that all groups of interest are present.

The CMC/CS gel has all the characteristic bands of CMC, and also a new band in 1725 cm^{-1} due to C=O stretch; carbonyl groups are very sensitive to atoms and groups nearby, so the shift of this signal is due to the different chemical environment of the carbonyl group in the complex. The spectrum shows characteristic bands of chitosan 1640 , 1380 , and 1150 cm^{-1} . The signal at 1040 cm^{-1} is more attenuated than the band at 1030 cm^{-1} probably due to the same changes in the environment.

Interaction of CS polymer chains in complexation with CMS brings major differences in 3300 cm^{-1} , which suggests a rearrangement of hydrogen bonds when forming the complex, and also between 1700 and 1250 cm^{-1} bands which probably results from the new environment of the C=O. For

ionotropic CMC gels, respective to the CMC alone spectra, the presence of calcium chloride increases the 3300 cm^{-1} band, makes a new band at 1490 cm^{-1} appear, decreases the 1300 cm^{-1} band, makes appear a peak in 1200 cm^{-1} , and reduces the 1100 cm^{-1} band (Figure 10). By comparison to the CMC spectrum, CS in the polyelectrolyte complexation reduces the 1620 cm^{-1} band, makes the peak at 1300 cm^{-1} more defined due to the increased concentration of C=O bands contributed by the carboxymethylated starch; also CS causes the band at 1150 cm^{-1} to disappear and also attenuates the band at 1050 cm^{-1} .

The FTIR spectrum of CMS shows peaks similar to those of CMC. Additionally, it has a weak absorption at 1730 cm^{-1} , probably due to the C=O stretch and asymmetrical COO-stretching of carboxylic acids groups present in carboxylated

starch molecules [38]. Wang et al. [39] report that CMS has a strong peak around 1625 cm^{-1} belonging to carboxylic groups and also another around 1421 cm^{-1} of OH groups, as well as another at 1300 cm^{-1} due to CH.

For CMS/CS in polyelectrolyte complexation gels, there is a noticeable band at 1700 cm^{-1} , which can be due to C=O bonds in a different chemical environment. The addition of calcium chloride and its interaction with CS gives a band at 1450 cm^{-1} ; both hydrogels have bands at 1300 and 1320 cm^{-1} characteristic of CMS (Figure 11).

The CMS/CS complex polyelectrolyte has more characteristic bands of carboxymethylated starch than those of chitosan (Figure 11). The spectrum shows a decrease in the signal at 3400 cm^{-1} attributed to the -OH bonds; also the band at 1730 cm^{-1} was shifted to 1700 cm^{-1} . A new band begins to be noticed at 1650 cm^{-1} attributed to N-H bending, and the band at 1590 cm^{-1} related to COO- groups of CMS is smaller. Bands at 1420 and 1315 cm^{-1} from the CMS are also observed, which overlapped the amine bands of CS. Besides, there is an additional band at 1250 cm^{-1} , probably belonging to C-N stretching of chitosan. All these signals and their changes are evidence of the interaction between CMA and CS, strongly related to the new chemical environment of the polymeric chains and their functional groups in the formation of the complexes.

Regarding the interactions between the polymers and calcium chloride in the different gel preparations, the major differences were observed at 3300 cm^{-1} and between 1700 and 1250 cm^{-1} . The OH, C=O, and C-N bonds are the functional groups mostly affected by the interactions of the polymer chains in the system. For CMC gels in relation to the CMC spectra, the formation of a salt increased the 3300 cm^{-1} band, made the 1490 cm^{-1} band appear, decreased the 1300 cm^{-1} band, made a peak in 1200 cm^{-1} appear, and reduced the signal at 1100 cm^{-1} (Figure 11). In the case of CMS, differences were noticeable in the appearance of a peak at 1450 cm^{-1} and the overlapping of bands between 1320 and 1300 cm^{-1} . Cho et al. [40] reported that stretching vibrations of O-H bonds from alginate, calcium chloride, resveratrol, and alginate microspheres that appeared in the range of $3207.59\text{--}3486\text{ cm}^{-1}$ were changed after forming resveratrol-loaded cyclodextrin nanospheres complexes. The O-H stretching peak of alginate shifted to lower wavenumbers when resveratrol was encapsulated into alginate microspheres, suggesting that a molecular interaction between alginate and the drug was formed by hydrogen bonding. In the present work, changes between 1600 and 1250 cm^{-1} bands in spectra obtained of gels made by ionotropic gelation and a combination of ionotropic gelation and polyelectrolyte complexation can be assigned to the replacement of Na^+ by Ca^{++} , which may indicate points of crosslinking due to the interaction of the components [40].

4. Conclusions

This contribution provides a more specific knowledge regarding the influence of three gel-forming methods, namely, ionotropic gelation, polyelectrolyte complexation, and a combination of both, on the morphology, size, and potential zeta of the hydrogels formed.

Charge interaction between one of the anionic polymer (CMC, AA, or CMS) and CS, CaCl_2 , or a mixture of both, produce hydrogels with different morphological characteristics dependent on the method used. Regarding the size of hydrogel particles, in case of the ionotropic gelation, the addition of a salt, such as calcium chloride, yields definite individualized particles with AA generating particles in the range of millimeters being smaller when CaCl_2 is using without CS. The small size of calcium ions allows a more efficient diffusion to neutralize anionic charges along the anionic polymer chains. The polyelectrolyte complexation produces particles that tend to agglomerate. This is due probably to the difficulty of cationic and anionic polymeric chains to establish proper electrostatic interactions. The combination of ionotropic gelation and polyelectrolyte complexation techniques yields zeta potential values that approach to a neutralization of charges. The presence of calcium chloride guarantees a more balanced interaction between the negative and positive charge of polymers.

FTIR characteristic bands of the individual polymers employed in the different gel systems were reported. Shifting of these bands, mostly to higher wavenumbers, can be regarded as evidence of the molecular interactions taken place between their polymeric chains when forming the gel complexes.

Features like shape and variation of gel pores may influence negatively or positively the process of encapsulation and drug delivery, being crucial when choosing the type of hydrogel to encapsulate active substances.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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