FULL PAPER

Efficiency of Zinc and Calcium Ion Crosslinking in Alginate-coated Nitrogen Fertilizer

Claudia Dyana Santos Peretiatko*, Ediane Andreza Hupalo, José Ricardo da Rocha Campos, Cristiane Regina Budziak Parabocz

Programa de Pós-Graduação em Tecnologia de Processos Químicos e Bioquímicos - Universidade Tecnológica Federal do Paraná – UTFPR- campus Pato Branco, Via do Conhecimento, KM 01, s/n - Fraron, Pato Branco - PR, 85503-390.

Abstract:

The work evaluated the difference of nitrogen retention in sodium alginate hydrogel microspheres with 50% of nitrogen, to allow the production of controlled release fertilizers. The crosslinking with the calcium and zinc ions occurs in a differentiated way, and it is emphasized that zinc binds more slowly and covalently. After the spheres were elaborated, a leaching test was performed to simulate the release of nitrogen by soil moisture. In this case, the zinc nitrate-reticulated beads showed a lower loss of the nitrogen content compared to calcium, with values of 6.7129% of nitrogen for zinc nitrate, corresponding to about 50% of the initial value, and 43.6345 % of nitrogen for calcium chloride corresponding to about 90% of the initial value. The characterization of the samples was performed by fourier transformed infrared (FTIR) analysis, and characteristic calcium and zinc alginate compounds were observed, in the same way as the connections caused by nitrogen. It can be defined that the zinc-crosslinked alginate obtained a lower swelling capacity and higher retention of nitrogen than with calcium chloride, being an alternative for the preparation of slow-release fertilizer.

Keywords: alginate; hydrogel; microspheres; nitrogen fertilizer; calcium; zinc; crosslink

1. Introduction

Nitrogen (N) is a limiting and more required nutrient in grass crops, such as corn, wheat, oats. In the corn plant, for example, the adsorption of N occurs by the adsorption of ammonium which is incorporated into the organic compounds in the roots and the nitrate which is readily mobile in the xylem and can be accumulated in the vacuole of the roots, aerial parts and storage organs. When the plant has nitrogen deficiency some symptoms are visible, such as the bleaching of the new leaves and the occurrence of chlorosis in the old leaves that become yellowish, and that will entail in a plant more susceptible to diseases and losses in productivity.

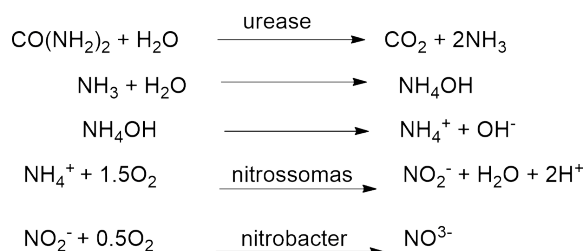In Brazil, the main source of nitrogen fertilizer is urea, due to its high nitrogen content and relatively low price. Commercial urea has about 46% Nitrogen, but only about 30 to 50% of nitrogen applied through the urea is actually used by the plant, the rest is lost. Nitrogen fertilizers are mostly soluble in water, and they are easily converted into easily leachable or volatilized molecules, and this leads to the loss of efficiency of these fertilizers [10]. In addition to leaching and volatilization losses, the micro-organisms are still hydrolyzed by urea.

Applied to the soil urea presents two reaction paths, first the dissolution in the soil and direct adsorption by the roots and according to the hydrolysis by the urease of microorganisms and vegetal remains as well as the reactions below (Eq. 1), in the case of hydrolysis by urease.

After fertilization, it is expected that the two forms of nitrogen adsorbed by the plant are ammonium (NH_4^+) and nitrate (NO_3^-), which is the

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predominant form of adsorption by plants [19]. When the soil is well aerated and poorly compacted, hydrolysis of ammonium nitrate occurs easily, but nitrate is more susceptible to leaching or denitrification or to soil removal by microbial immobilization. In contrast, NH_4^+ is much less subject to losses due to leaching or denitrification than NO_3^- therefore, fertilization with ammonium can be more efficient than only with the use of nitrates [3].

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

$$NH_3 + H_2O \longrightarrow NH_4OH$$

$$NH_4OH \longrightarrow NH_4^+ + OH^-$$

$$NH_4^+ + 1.5O_2 \xrightarrow{\text{nitrossomas}} NO_2^- + H_2O + 2H^+$$

$$NO_2^- + 0.5O_2 \xrightarrow{\text{nitrobacter}} NO^{3-}$$

Eq. 1

Urea itself presents instability in the soil due to its high solubility in water. Therefore, it is important to look for alternatives that can, at the same time, effectively nourish the plant and present less N loss in the system, thereby causing less environmental impact [13]. This justifies the search by means of immobilization of urea for later use as slow-release or controlled fertilizer (FLC) to avoid losses of urea by leaching increasing the useful life of the fertilizer.

One way to avoid the loss of nitrogen is to coat the urea grain with a protective barrier that releases the nutrient in the soil, increasing the nitrogen retention time in the fertilizer, that is, supplying the nutrient slowly [20]. Nutrient immobilization is a biotechnology technique and in addition to fertilizer research, it is used in a range of fields, such as pharmaceuticals, environmental protection and food production. For the immobilization of elements protects them from the environment, it creates the possibility of reuse, besides being able to be used for adsorption of cells on the conveyor surface [16].

The aim of this research was to evaluate the retention of nitrogen from urea coated on hydrogel spheres formed from sodium alginate and urea, crosslinked with the calcium (Ca^{+2}) and zinc (Zn^{+2}) divalent cations separately, for comparison between which of the two cations is better to carry out alginate cross-linking and to immobilize the urea. The analyzes performed for this verification were the nitrogen analysis by the micro-kejadhal

method, and to determine the properties of the spheres the Fourier Transform Infrared (FTIR) analysis was performed [16].

Sodium alginate was chosen as the coating polymer, since it is a biodegradable polymer, derived from brown algae. Alginate is considered a linear biopolymer containing β-D-mannuronate (M) and α-L-guluronate (G) compounds bound linearly by 1,4-glycosidic bonds (Figure 1) [14].
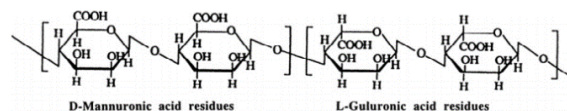
Figure 1. Chemical structure of alginate, β-D-mannuronate (M) and α-L-guluronate compounds (G). Source: reference [4].

Alginate in the presence of divalent cations such as Ca^{+2}, Ba^{+2} form gels, and crosslinking occurs through the attachment of these cations to the negative charges of the carboxy groups of the alginate chain. This connection forms a structure similarly compared to an "egg box", according to Figure 1 [16].

This binding characteristic determines the mechanics of the hydrogel, such as swelling and diffusion capacity. In addition, because it does not require high temperatures for its handling, the alginate stands out to perform several immobilizations including enzymes and cells, besides the use in controlled release fertilizers [15].

Alginate gels may be formed by divalent cations such as Ba^{+2}, Pb^{+2}, Sr^{+2} or Cu^{+2}, but these cations can not be used to immobilize cells due to their toxicity [16]. However, the Ca^{+2} and Zn^{+2} cations are described by CERCIELLO (2017) in the use of drug immobilization, with emphasis on zinc (Zn^{+2}) for prolonging the release of the drug. As regards thermal stability and flame retardant properties, hydrogels crosslinked with Ca^{+2} and Zn^{+2} are shown to be improved, with flame retardation in alginate fibers and decreased release of products by gas pyrolysis [8].

Gelling and alginate cross-linking occurs by ion exchange between the sodium ions of the guluronic acids and the divalent cation of the gelling solution, forming the egg carton structure as shown in Figure 2. The polymer bond involves

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about 20 monomers, and the chain can still dimerize to form bonds with other chains forming the gel networks in the form of precipitates [4].
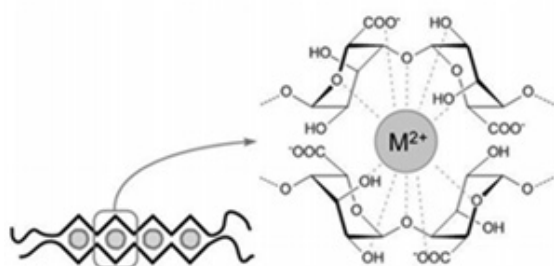
Interaction of a alginate with divalent ion

Figure 2. Chemical structure of the β-D-mannuronic acid alginate, α-L-guluronic acid alginate monomers and interaction with divalent cations forming an egg box structure model. Source: Adapted from reference [12].

By bonding the material to the alginate prior to gelation, a "block" gel is formed, because upon gelation the formed bead already has the material to be incorporated or retained. For example, when forming alginate fibers, they are immersed in a bath of divalent ions, which may be Ca^{+2} or Zn^{+2}, forming calcium alginate fibers [4]

2. Material and Methods

Reagents

All reagents used were of analytical grade. Sodium alginate pH 5.5-7.7 (próquimios) and calcium chloride 96% purity (Vetec) were used calcium chloride and zinc nitrate at 98% purity, urea used with a maximum of impurity of 0.0235% (próquimios).

Obtaining alginate and urea spheres

The methodology used to obtain the alginate beads was an adaptation of LIU (2016) and CERCIELLO (2017). 3 g of sodium alginate was dissolved in 100 mL of distilled water to form a polymer solution of 3% w/v. This solution was heated in a water bath, 60°C, for 1 hour until complete dissolution and stabilization of the solution. After stabilization, the solution was cooled to 50°C and 3.27 g of urea corresponding to 50% nitrogen w/w was added. We waited 30 min to stabilize the solution and start dripping.

The dripping occurred with the use of a Tecnal peristaltic pump, model TE-BP-01-MINI. The main hose used was 3mm thick internal silicone, and a silicone hose was also attached to the connector with a 2mm internal thickness, where the solution sucked. Through the pump rotation system, the solution was brought to the other end of the hose and dripped at a rate of 90 drops per minute.

Two solutions of sodium alginate and urea were prepared, each of which was independently dripped into respective solutions of calcium chloride ($1 \, molL^{-1}$) and zinc nitrate ($1 \, molL^{-1}$). Both gelifications of the solution were at low temperature, 1.2 and 1.3°C respectively. The beads remained in the gelling solution under stirring for 1 hour.

After 1 hour the beads were removed and washed with 100mL of distilled water in order to remove some excess of ions under the beads. After washing, they were placed in glass petry dishes and routed for oven drying at 42°C for 72 hours. After drying they were removed from the petry dishes and stored in desiccator for analysis.

Leaching Test

For the leaching test, the system of BORGES (2013) was adapted, which in its thesis carried out the leaching test for phosphorus and nitrogen. A beaker was weighed 1g of sample and the volume of 100 mL was filled with distilled water of pH 7.72 and the system rested for 72h at 25°C. After this time the beads were removed and dried again in an oven at 42°C for 72 hours. The spheres after leaching were sent for analysis of nitrogen content by the micro-kejadahl method, in the same way the spheres that did not go through the leaching process were sent. The samples that were followed for the analyzes were named:

• AS50CASL - alginate sphere with 50% nitrogen w/w, crosslinked with calcium without leaching (SL);

• AS50CAU - alginate sphere with 50% nitrogen w/w, crosslinked with calcium after leaching;

• AS50ZNSL - alginate sphere with 50% nitrogen w/w, crosslinked with zinc without leaching (SL);

• AS50ZNU - alginate sphere with 50% nitrogen w/w, crosslinked with zinc after leaching.

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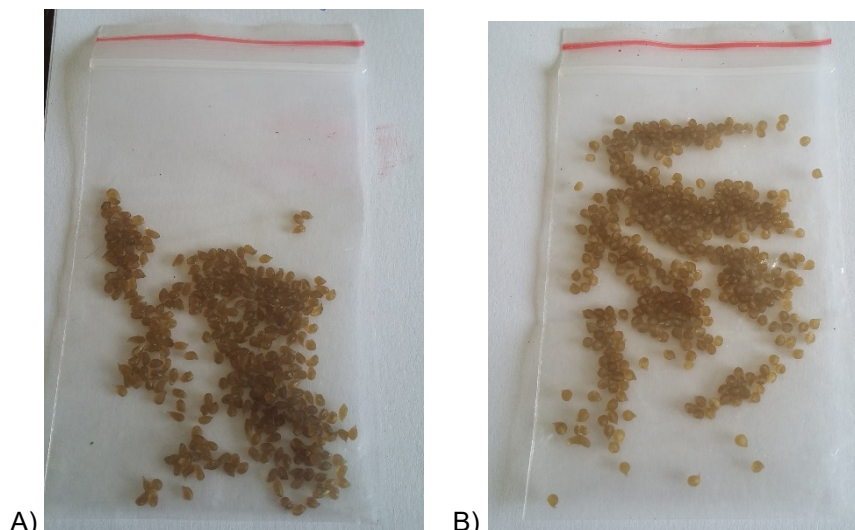
Figure 3. A) Alginate beads with 50% nitrogen gelled with Calcium Chloride. B) Alginate beads with 50% nitrogen gelled with Zinc Chloride. Source: Own author.

Nitrogen Analysis

Nitrogen content analysis consists of the classical kejadahl method, adapted from Tedesco et al. [18], using a Tecnal model 040/25 digester block, a Tecnal model temperature controller TE-007MP, and a nitrogen distiller Tecnal model TE-0363. This analysis aims to determine the percentage of nitrogen present in the samples.

Digestion of the sample

The preparation of the sample consists of its digestion with sulfuric acid and catalytic mixture (100 g of sodium sulfate, 10 g of copper sulphate and 1 g of metal selenium) in order to break down the bonds and decomposition of the organic matter to obtain as product only the inorganic material, as described in the Eq. 2 [17]:

$$\text{Organic matter} + H_2SO_4 \longrightarrow \text{Inorganic Matter} + SO_2 + CO_2 + H_2O$$
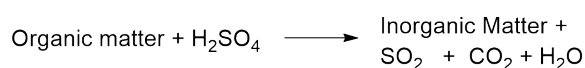
Eq. 2

Weigh out 0.2 g of sample, 0.7 g of catalytic mixture in the digestion tube and finally in the hood and next to the digester block 5 mL of concentrated H_2SO_4 is added in the digestion tubes and then the digester block. After reaching the temperature of 375 °C the sample remains for 1h. After this period the digestion tubes are removed, and the cooling of the tubes is awaited, and the samples are swollen to 50 mL. And the nitrogen determination is carried out.

Determination of Nitrogen

During digestion the sulfuric acid transforms all the nitrogen forms of the sample into the ammoniacal form (NH_4^+), thus forming the ammonium sulfate. The distillation in strongly basic medium causes the release of the ammonia in the hydroxide form. The solution containing boric acid with indicator acquires the greenish color as the ammonium borate is formed. By means of titration with H_2SO_4 of known concentration, it is possible to quantify the nitrogen content of the sample [17]. After titration, the nitrogen content (% N) is calculated by means of the following equation:

$$\%N = \frac{(mL\ H^+ - mLPB)\ x700x5x5xfc}{1000}$$

mL H^+ = volume spent in the titration; mLPB = volume spent on standard titration; fc = correction factor (0.959)

Infrared Spectrometry with Transmittance Fourier Transform (FTIR)

For the accomplishment of the analysis of infrared spectroscopy the Perkin Elmer spectrometer, Frontier model, in ATR was used.

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For the reading of the spectra the following condition was used 64 scan scans, resolution 2 cm^{-1}, in the region of 4000 to 400 cm^{-1}, all by transmittance. The aim of the analyzes was to provide structural information on the alginate / urea sphere.

3. Results and Discussion

Obtaining the Spheres

The solution of alginate and urea presented a viscous characteristic, with a slightly yellowish and translucent coloration. The still wet beads were light yellow in color, with a large volume, after drying, their lotion decreased and they had a dark yellow coloration (Figure 3). The hydrogels were solid after drying with a shape nearer to beads, with a dark yellow coloration.

Leaching and Nitrogen Analysis Test

The leaching analysis occurred following a model proposed by BORGES (2013). In different beakers were added 100 mL of distilled water and 1.0 g of calcium alginate hydrogel beads and zinc. During the resting period one can observe the swelling of the spheres, as observed in Figures 4 and 5 after the removal of the spheres from the water. This occurred according to the alginate property of moisture absorption and swelling capacity, as in the studies of Cerciello et. al. [2], where it was clearly observed the expansion of the granules due to the penetration of the H_3O^+ ion, since the assay took place in acidic environment for drug retention. In addition, alginate beads also showed swelling ability and were soluble in other substances such as DMEM (Dulbecco's Modified Eagle Medium) as the release medium used in the work of Malagurski et. al. [9], which verified the release of mineralized zinc in alginate beads. After the leaching test, the already dried samples were taken to perform the nitrogen content analysis, Micro-Kejadahl method and provided the following results described in the Table 1.

The values of the final percentage of nitrogen after leaching show that the alginate was not effective to immobilize the urea nitrogen. Even during the processing of the beads, the only sample remaining with an approximate theoretical value was the sample AS50CASL, which

remained with approximately 47% of nitrogen. However, when the leaching loss ratio is observed, it is observed that the zinc nitrate crosslinked beads obtained a smaller difference between the leaching and non-leaching samples. The percentage difference in this case was only 3.3564% N.

Table 1. Final percentage of nitrogen in the calcium and zinc crosslinked samples, without leaching (SL) and after leaching (U).

Sample	Nitrogen content (%N)
AS50CASL	46,9910
AS50CAU	3,3565
AS50ZNSL	11,7477
AS50ZNU	8,3912

An important factor, already cited, is the alginate swelling ability and crosslinking of the same by the divalent ions. These factors were investigated by Sharma et al. [15], who verified that the swelling capacity varied with the percentage of the alginate solution used. Ranging from 0.5 to 5.0 g of alginate, he observed in his work that the swelling reduces from 1.0 g of alginate in solution and can be explained by the formation of a more compact polymer network. In addition, with increasing concentration of the crosslinking agent it was also observed that the amount of water absorbed by the nanoparticles decreased with increasing concentration of the crosslinking agent, which in this case was used calcium chloride. The calcium chloride in higher concentration implies in the formation of a larger quantity of pores of smaller size that provide less water absorption, consequently less swelling capacity. However, the release of water by nanoparticles occurs differently, in the study presented, observed that greater amount of alginate resulted in a greater loss of water in a shorter time, ie, the rate of water loss increases gradually with increasing alginate content. An example of this, and the difference in the release of water in solution of 0.5 g of alginate and of 3.0 g of alginate, to 0.5 g of alginate, 50% of water was lost between 15 days and 3.0 g alginate was lost almost 90% of water in the same period.

These data described by Sharma et al. [15], help to explain the fact that the spheres produced in this work have released urea quickly. Because

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it is soluble in water and the formulation used in the production of spheres (3.0g alginate content and calcium chloride solution and 1.0 mol L^{-1} zinc nitrate solution) provides less water absorption, but its release quickly, the urea was lost more quickly. Analyzing each sample, calcium chloride showed greater alginate crosslinking capacity than zinc nitrate, retaining a higher amount of nitrogen during the preparation of the beads, but at the release, calcium chloride was the sample that lost most nitrogen in aqueous solution. In contrast, zinc nitrate, although not as efficient in retention during spheres development, proportionally retained a greater amount of nitrogen, this can be due to the fact that the alginate and zinc bonds occur more slowly, in covalent bonds and difficult to break, providing better crosslinking with alginate and less loss of water.

In the use of drugs, according to the literature, also the difference in the crosslinking and consequently the release of substances in alginate films, crosslinked with calcium and zinc, is also observed. In the use of chitosan hydroxyethyl acrylate (HC) together with alginate for slow release of paracetamol, it was observed that calcium chloride had greater swelling power compared to zinc crosslinking (Zn^{+2}). In simulation of the stability in simulated digestive medium and in distilled water, films crosslinked with zinc ions, were more stable than calcium, however the stability of these hydrogels were influenced by the HC indexes and not by the crosslinking conditions. In the release of the drug the hydrogels did not show significant differences, suggesting that this combination could be used for delayed drug release [18].

In the use of alginate with mineralized zinc, slower drug release also occurred, maintaining the integrity of the microspheres after 10 days of incubation in a physiological environment using DMEM again. Occurrence of Zn (II) zinc but still remaining part of the alginate microspheres, showing that the alginate hydrogel can act not only as deposits of minerals but can release them to the environment [9]. This was also observed in the research samples, since they also retained a certain amount of urea, which was later released in aqueous solution of the calcium and zinc-cross-linked samples.

Infrared Spectroscopy with Transmittance Fourier Transform (FTIR)

Alginate FTIR spectra are shown in Figures 4 and 5. Figure 4 shows alginate beads with 50% nitrogen w/w crosslinked with calcium chloride, and Figure 5 represents the bead with 50% crosslinked nitrogen w/w with zinc nitrate.
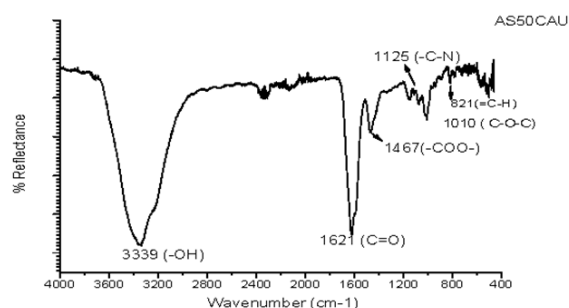
Figure 4. Fourier Transformed Infrared (FTIR) spectrum of the alginate and urea spheres (50% nitrogen w/w) cross-linked with Calcium Chloride.
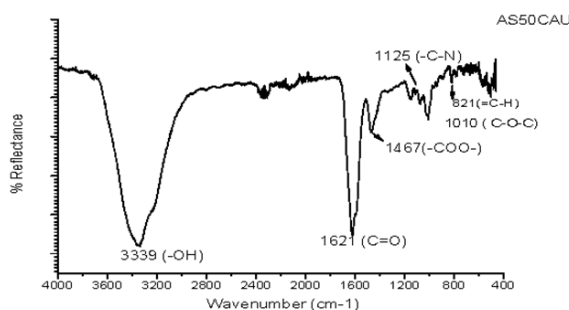
Figure 5. Fourier transform infrared (FTIR) spectrum of the alginate and urea spheres (50% nitrogen w/w) crosslinked with zinc nitrate.

In both spectra, biopolymer characteristic bands appear clearly at 3339 and 3482 cm^{-1} relating to OH bonds [6], bands 1621, 1645 to a lesser extent for crosslinking with zinc ion and 1467cm^{-1} refers to the symmetrical stretching carboxylate (R-COO) [18] which can be salified carboxylic group having a (Ca^{+2}, Zn^{+2}, etc.) revealing that binding occurs between the alginate, calcium ion and zinc ion. The band observed in Figure 5 in 1788 is characteristic to free carboxyl anions present in the formation of zinc alginate, as was also observed by Campañone et al. [21]. The bands observed in the 1010 and 1024 cm^{-1} spectra are characteristic of C-O-C bonds [6].

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The lower intensity bands such as 821 and 834 cm^{-1} correspond to the hydrocarbon bonds (-C-H) and the bands 1125 and 1111 cm^{-1} to the -C-N-type bonds. Since the last two bands mentioned are in a region close to that described by Saxena et. al. [14] identified as C-N, for a cross-linked sample with calcium ion. The 1010 and 1024 cm^{-1} bands are symmetric C-O-C axial deformation characteristics of the alginate (1033 cm^{-1}), indicating that the polymer chain has not been altered.

4. Conclusions

During the research, it was observed that both calcium and zinc divalent ions are able to crosslink alginate beads with urea, but the efficiency of crosslinking was different for each ion. The calcium ion during the processing of obtaining the spheres allowed greater retention of the nitrogen in the spheres but was not able to avoid the fast release of this nutrient. The swelling capacity observed was higher in the sample cross-linked with the calcium ion, since the zinc makes covalent bonds and therefore, the pores of the spheres with zinc can present of smaller size compared to the balls with calcium, according to the literature. The swelling capacity was shown to be essential for nitrogen retention in the sample. Because urea is highly soluble in water, the greater the capacity to absorb water and to lose water, the greater the inability to retain the urea in the sphere, consequently losing the nitrogen content in the samples.

Despite having the lowest nitrogen content after the spheres were made, those that were crosslinked with zinc nitrate obtained a lower nitrogen loss, demonstrating that the zinc ion is actually able to decrease pore size and form more stable bonds with alginate. It can be a study instrument for the elaboration of nitrogen fertilizers with a certain zinc content. The characterization of the samples by infra-red showed that both had similar characteristics. However, certain displacements and decreases in band intensities occur. As in the case of the bands 3339 cm^{-1} for the calcium ion and 3482 cm^{-1} for the zinc ion that considerably decreases the intensity of the band. It was also observed in both samples the bands 1621 cm-1, 1645 cm-1 in less intensity for the cross-linking with zinc ion, and 1467 cm^{-1} that besides representing the symmetrical stretch of carboxylate indicate the bonds with the divalent ions. As observed in the spectrum in this region zinc has a lower band, which may justify a lower retention of nitrogen during the confection of the spheres due to the smaller amount of zinc and alginate bonds. This could perhaps be remedied by increasing the concentration of the zinc nitrate solution used in gelling by increasing the degree of crosslinking or by increasing the residence time of the beads in the solution.

The studies show that for the preparation of a fertilizer slow release of nitrogen, it is necessary that the samples lose more slowly the nutrient which was not observed in a satisfactory. The use of calcium crosslinked alginate was not as effective as zinc crosslinking in nitrogen retention. However, in both cases, for better quality of the material it is necessary to release the nitrogen more slowly, so new research on other polymer sources or combinations of polymers sources is important in the search for greater retention of nitrogen in the material. Thus, other tests can and should be carried out in the search for slow release fertilizers with biopolymers and in the search for new ways of using the zinc ion for crosslinking.

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