Gellan Gum – O,O'-Bis(2-aminopropyl)-polyethylene glycol hydrogel for controlled fertilizer release

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Abstract

Gellan gum-jeffamine superabsorbent hydrogel obtained with different crosslink density by using different amounts of (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (EDC) and N-Hydroxysuccinimide (NHS). FTIR and Thermal analysis can confirm crosslinking. Morphology analysis indicates denser structures for samples with higher crosslinking points. The swelling degree in high acyl gellan gum hydrogels was equivalent to 145 times their dry weight, and 77 times when low acyl gellan gum was used. Hydrogels also showed a 450 minute water retention opposed to 280 minutes for pure water, evidencing good humidity control capacity and use in arid climates. They also demonstrated maximum release of commercial fertilizer of about 400 mg per gram for MKP and about 300 mg per gram for NPK.

1 INTRODUCTION

Modern agriculture is always evolving, demanding higher standards of quality and in food production, leading to higher usage of water fertilizers, pesticides, and other related resources. With an increasing world population, high fossil fuel prices and water shortages, the optimization and improvement of the agricultural production systems is essential[1,2].

In order to improve productivity and reduce losses, the use of hydrogels may become an alternative. Hydrogels can be prepared from hydrophilic polymers or macromolecules, where the polymer chains crosslinking (chemically or physically)

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maintain their three-dimensional structure, making it possible to swell large amounts of water without dissolution[3,4]. A wide variety of polymers (natural or synthetic) can be used to prepare hydrogels, depending on the desired application. The structural integrity (provided by crosslinks), high water content and soft consistency (similar to natural tissue)[5] qualify them to be used as a scaffold for tissue engineering[6,7] and wound healing[3]. Their network structure can be engineered to optimize[8] their use as a substrate for cell growth[9] and as controlled release systems for chemicals[10,11]. Recent studies are using hydrogels as a support for enzymes[12] and proteins immobilization[13].

Controlled release of chemicals was first studied for pharmaceuticals[14,15], but the same principles can be applied in fields such as agriculture regarding the release of fertilizers, nutrients and herbicides. When dry polymer chains are compressed, they retain the molecules of interest. When in contact with water, the hydration process causes polymer chains to expand, releasing the controlled molecules to the enviroment[16]. Therefore, these systems can be used for gradual and controlled release of chemicals, increasing the presence of the latter in the soil and avoiding saturation[17]. In addition, the water retention capacity of hydrogels can assist in the gradual release of water, allowing for the control of soil moisture.

Gellan Gum, commercialized by CP Kelco under trade name *Gelrite*®, is obtained through the fermentation of non-pathogenic aerobic bacteria culture *Sphingomonas paucimobilis*[18,19]. Gellan Gum features high molecular weight, desacylated anionic polysaccharide constituted of repeated units of β-1,3-D-glucose, β-1,4-D-glucoronic acid and α-1,4-L-ramnose in a 2:1:1 ratio[20]. Gellan gum can be obtained in two forms: high acyl (native) and low acyl (approximately half of glucose residues been substituted by acetate and L-glycerate)[21]. The presence of the acetate group has great influence on the characteristics of the resulting gel. While the native gum forms soft, elastic and opaque gel, the deacylated gum forms hard, tough and bright gel[22].

The (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (EDC) is one of the most popular compound for combining substances that contain amines and carboxylate groups[23]. EDC is soluble in water, and can be added directly to a solution without organic solvents[24]. Both reagent and the reject of the crosslinking reaction (isourea) can be easily removed from the medium[25].

Carbodiimides N-substituted can react with carboxylic acids to form highly reactive o-acylisourea intermediate; this type of intermediate reacts immeadiately with

nucleophiles such as amine, resulting in an amide bond[26]. The reaction of EDC with the carboxylate group, in order to form the ester intermediate (o-acylisourea), occurs slowly and can be hydrolyzed in aqueous solution. The advantage of adding N-hydroxisuccinamide (NHS) to the reaction is an increase in the solubility and stability of active intermediate[27].

Most controlled release systems used in agriculture contain superabsorbent hydrogels derived from polyacrylamide, due to its price and large capacity of water absorption.[28]. Polyacrylamide has been used as a soil conditioner and for controlling humidity since 1950[29-31], with the growth of agroecology and green chemistry, it is necessary to replace synthetic polymers for greener solutions (even though it is a more expensive solution)[32,33]. In this paper the synthesis of a new superabsorbent hydrogel is proposed, based on gellan gum and Jeffamine 130 (O,O′-Bis(2-aminopropyl)polypropylene glycol) using EDC/NHS as a crosslinker, aiming to develop its use in fertilizer controlled release[2].

2 MATERIALS AND METHODS

2.1 Materials

Gellan gum (GG) (M_w ~1,000,000 Da) high acyl (HA) and low acyl (LA) were kindly provided by CPKelco; Jeffamine (O,O′-Bis(2-aminopropyl)polypropylene glycol) (M_w ~130 Da) 99% was obtained from Fluka; N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride 98% (EDC) from Sigma-Aldrich; N-Hydroxysuccinimide 98% (NHS) from Aldrich; and 2-(N-Morpholino)ethanesulfonic acid 99,5% (MES Buffer) from Sigma. All reagents were used without further treatments.

2.2 Hydrogel preparation

The hydrogels were prepared dissolving 0,1g of GG (HA or LA) in 20 ml of MES buffer (pH = 5). After complete dissolution, 0,4 mL of Jeffamine was added to the solution. Different crosslinking densities were obtained by adding 1,2,3 and 4 mmol of EDC and NHS to the solutions, and named 1,2,3 and 4 according to the quantities of EDC/NHS added to the sample.

After mixing for 2 hours, the hydrogels were cryogelated[34] in a freezer at -20°C for approximately 6 hours and then heated to room temperature. This process was repeated 3 times. The samples were washed several times and dried at 40°C; no further treatment was used.

2.3 Swelling Degree

The swelling degree (S) was obtained by weighting swollen hydrogel. Approximately 0.1g of dry gel was submerged in water at room temperature for 24h. Then, the swollen sample was removed from the water and water excess was drained. Measuring was performed using an analytical 0.001g precision weighing scale.
The swollen degree (S) was calculated using the equation (1)[35].

$$S = \frac{W_{wet} - W_{dry}}{W_{dry}} \qquad (1)$$

where, w_{wet} is the weight of the hydrated sample and w_{dry} the weight of the dry sample.

2.4 Network parameters

The density between crosslinks (d_x) was calculated using equation 2,

$$d_x = \frac{1}{\upsilon M_c} \qquad (2)$$

where, υ is the specific volume of the polymer and M_c is the average molecular mass between crosslinks.

The average molecular mass between crosslinks have been extensively studied by Flory and represented by the Flory-Rehner equation (3)[36,37],

$$M_c = \frac{-\rho_p V_s V_r^{1/3}}{[\ln(1 - V_r) + V_r + \chi V_r^2]} \qquad (3)$$

where, V_s is the molar volume of the solvent, ρ_p the density of the polymer, ρ_s the solvent density, V_r polymer volume fraction (equation 4) and χ Flory-Hoggins parameter (equation 5), which correlates the affinity between solvent and polymer.

$$V_r = \left[1 + \frac{\rho_p}{\rho_s}\left(\frac{M_a}{M_b}\right) + \frac{\rho_p}{\rho_s}\right]^{-1} \quad (4)$$

Here, M_a is the mass of hydrated polymer and M_b is the mass of dry polymer.

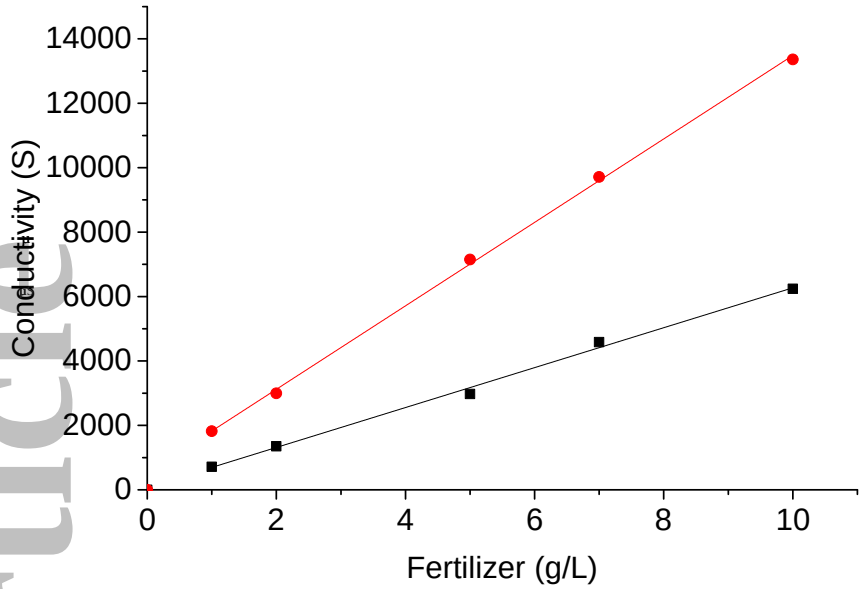
$$\chi = \left(\frac{V_s}{RT}\right)(\delta_{tpol} - \delta_{tsol})^2 \quad (5)$$

Here, V_s is molar volume of the solvent; δ_{tpol} - the solubility parameter of the polymer and δ_{tsol} - the solubility parameter of the solvent.

2.5 Fertilizer release

Commercial fertilizer monopotassium phosphate (KH_2PO_4; MKP; Yara Fertilizer Brazil Ltda.) and NPK 20-5-20 (NH_4NO_3, P_2O_5 and K_2O; Agro Brasil) were both dissolved in Millipore Milli-Q® water resulting in solutions of 1g/L, 5g/L and 10 g/L. After this process, the samples were immersed in those solutions for 24 hours; next, they were oven dried at 40 ºC until constant weight was achieved. Last, they were stored in the desiccator. For release measuring, the hydrogels with fertilizer were then immersed in 14 mL of Milli-Q® water and the conductivities were measured using conductivimeter Hanna HI 2550 every 30 min or 60 min. Since conductivity varies linearly with concentration in the range used, it is possible to determine the amount of fertilizer released through the conductivity using a calibration curve described in Figure 1.

Figure 1 - Calibration curve correlating conductivity (µS) of fertilizer solution with concentration for MKP (■) and NPK (●).



2.6 Analytical Techniques

The infrared spectroscopy (FTIR) analysis was performed using Shimadzu model IRAffinity1.

Thermogravimetric analysis (TGA) was made using TA Instruments TGA Q50 with a heating rate of 10°C/min under an N_2 flow.

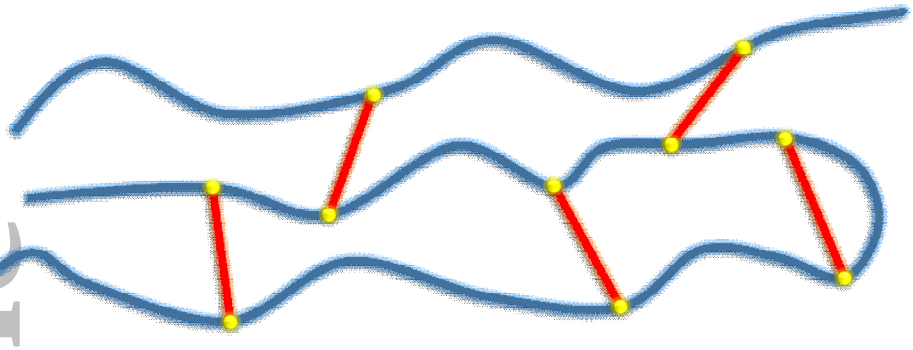
Scanning Electron Microscopy (SEM) images were obtained with ZEISS LEO 440 (Cambridge, England) operating with 20 kV electron beam and equipped with OXFORD detector (model 7060). Before the examination, the dry samples were covered with a 6 nanometer thick gold layer using Coating System BAL-TEC MED 020 (BAL-TEC, Liechtenstein) at $2x10^{-2}$ mbar pressure level, a 60 mA current and a deposition rate of 0.60 nm/s.

3 RESULTS AND DISCUSSION

EDC/NHS is a zero length crosslinker between carboxylic acids and amines, resulting in an amide bond. Both Jeffamine amine ends when crosslinked to gellan gum carboxyl groups can act as a bridge to maintain hydrogel structure. This structure can be predicted as shown in Figure 2.

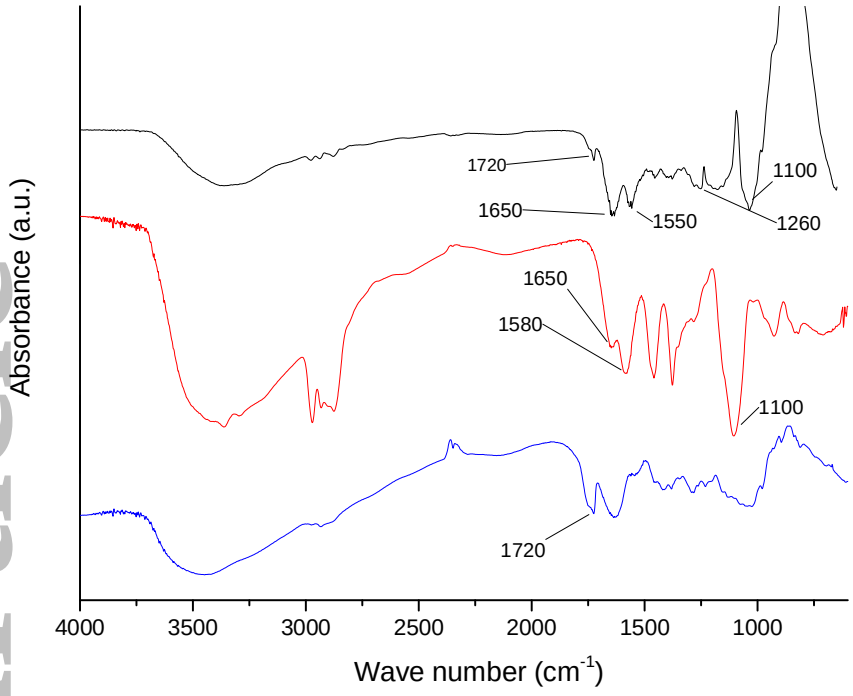
Figure 2 – gellan gum-Jeffamine hydrogel proposed structure after crosslinking, represented by gellan gum (blue line), Jeffamine (red line) and crosslinking points

(yellow dots).



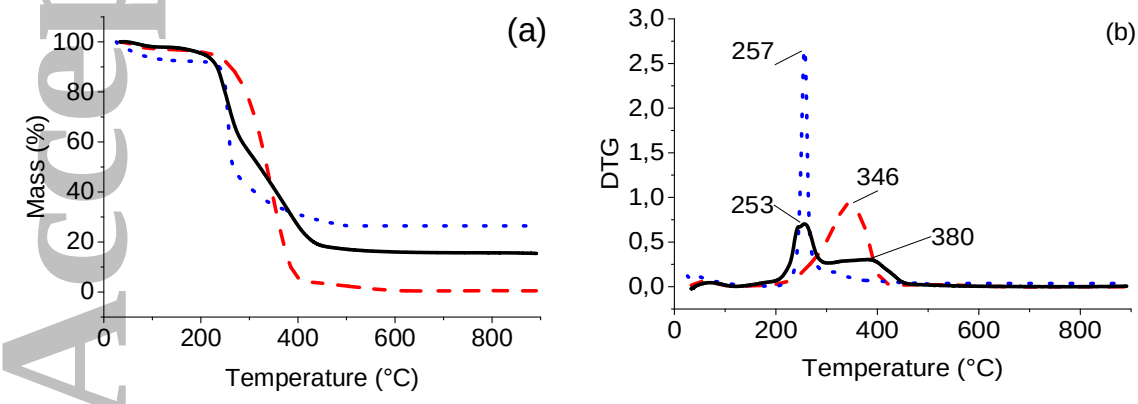
The FTIR spectra (Figure 3) serves as a means to confirm the structure presented in Figure 2: the amide bond formation can be observed at 1650 cm^{-1} band, associated with C=O stretching vibration (amide I), whereas 1550 cm^{-1} band is associated with N-H in-plane deformation coupled with C-N stretching (amide II)[38] and the 1260 cm^{-1} band with C-N stretching deformation coupled with N-H deformation (amide III). The 1720 cm^{-1} band is associated with carboxylic acid C=O deformation, showing higher intensity in gellan gum spectra. The 1100 cm^{-1} band is related to C-O-C stretching, also present in jeffamine spectra[39,40], confirming the formation of crosslinking between gellan gum and jeffamine chains.

Figure 3 – FTIR spectra for gellan gum (—) jeffamine (—) and hydrogel (—)



TGA and DTG thermograms for both reagents and the hydrogel formed are presented in figure 4.

Figure 4 – Thermograms of TGA (a) and DTG (b) for gellan gum(···), jeffamine (- -) and hydrogel (—) presenting their thermal degradation.

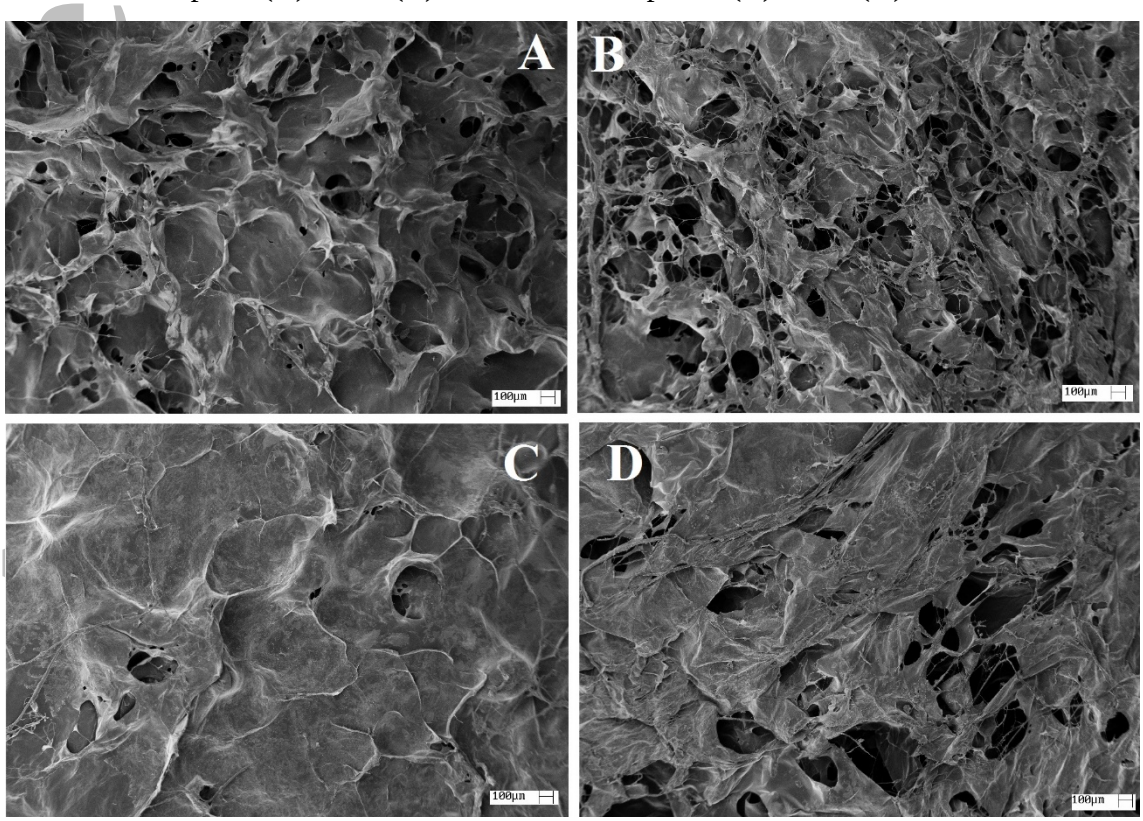


The thermogram in Figure 4 (a) revealed an initial mass loss of 8% for the GG, 2% for Jeffamine and 3% for the GG-Jeffamine hydrogel up to 150°C. This mass loss can be attributed to the water adsorbed in polymers. The DTG (Figure 4 b) presents peaks of thermal degradation confirmed for GG at 257°C and for the Jeffamine at

346°C. The hydrogel thermogram shows two peaks of degradation at 253°C and 380°C. The peaks of similar degradation indicate the presence of both polymers in the hydrogel composition, and the shift in temperature peak can be associated with polymer chains interactions[41].

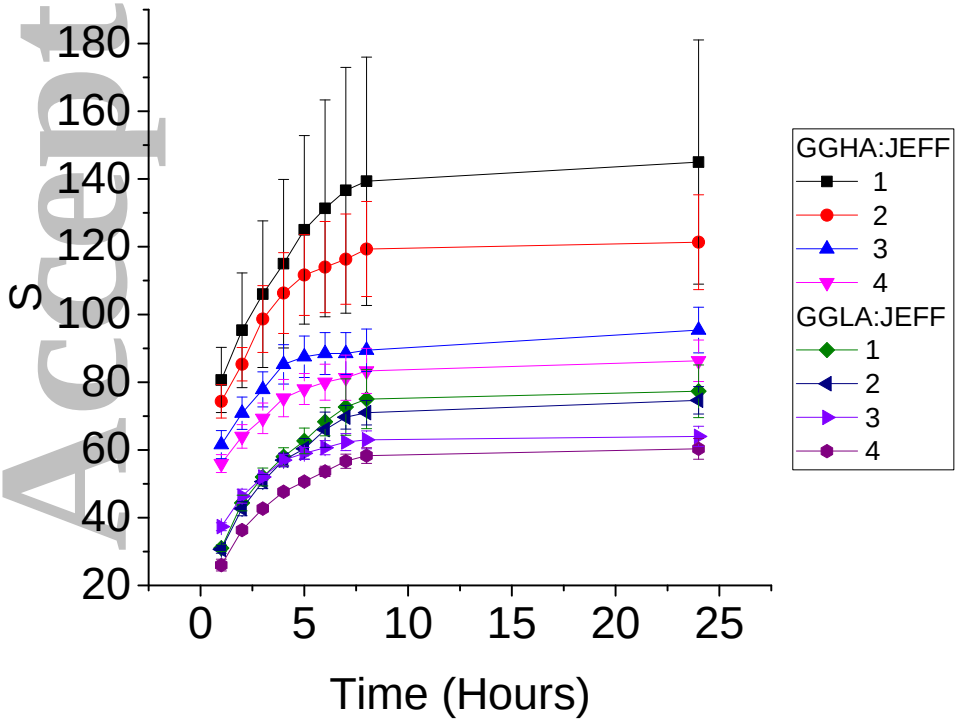
To analyze the morphology of hydrated hydrogels, the samples were lyophilized and pictures of the surfaces were taken by Scanning Electron Microscopy (SEM). Figure 5 presents samples 2 (A) and 4 (B) for GGHA and samples 2 (C) and 4 (D) for GGLA. For GGHA it is possible to observe that sample 2 (A) showed fewer empty spaces while sample 4 (B) has more empty spaces and larger pores. This observation can be associated with more crosslinking promoted by higher presence of EDC/NHS in the preparation. GGLA sample 2 (C) presented denser structure with fewer small pores, while sample 4 (D) show large pores and more empty spaces. By comparing both GGHA and GGLA it is possible to associate structure with swelling, where smaller pores (A) can swell more than larger pores (D)[42]. SEM images also present highly porous structure with irregular pores[27].

Figure 5 – Scanning Electron Microscopy images for lyophilized structure of GGHA-Jeffamine sample 2 (A) and 4 (B) and GGLA samples 2 (C) and 4 (D).



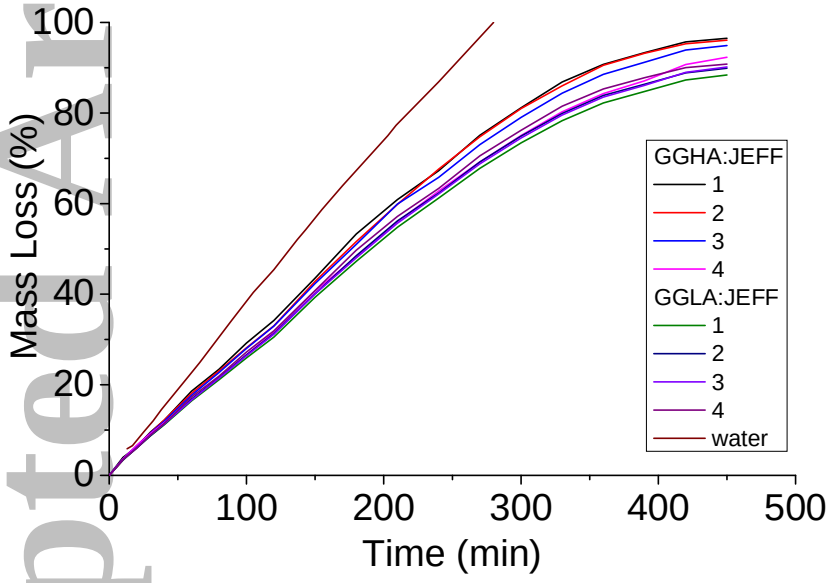
The main characteristic of a Hydrogel is the ability to hydrate in water. In most cases, it is noticeable a higher absorption through time. The hydrophilic chains of polymers absorb water until hydration forces are counterbalanced by the strength of the expansion of the polymer chain[43]. The swelling kinetics for samples prepared with GGHA is shown in Figure 6. In both cases, samples prepared with lower quantities of EDC/NHS have higher water absorption, showing values equal to 145 times the dry weight for GGHA and 77 times for GGLA. These results are expected since larger quantities of EDC/NHS in the synthesis of the hydrogel promote higher crosslink density, lowering maximum water absorption. Flory and Rehner[44] defined crosslinking point of molecules as rigid, so there is no water absorption at this point. Thus, a higher absorption reflects a smaller number of crosslinkings. These values are comparable to other natural polymer hydrogels like gellan gum-carboxymethyl chitosan[45], and presented a lower swelling degree when compared to gellan gum-chitosan prepared by our research group[42], and approximately one third of the swelling degree when compared to synthetic hydrogels[46].

Figure 6 – Swelling Degree (S) for gellan gum (HA and LA) Jeffamine hydrogels through time (1,2,3,4,5,6,7,8 and 24 hours after immersed).



In order to evaluate the humidity control of the hydrogels, the water evaporation kinetics in 1.0g of hydrated samples was performed, and the weight was measured through time at 70°C. Figure 7 displays water loss in percentage through time for the studied samples. It is possible to observe that, in this system, all pure water is lost in about 280 min, while the water absorbed in hydrogels is lost in about 450 min. This means that pure water completely evaporates in about 60% of the time when compared to the hydrogel samples. Values are comparable to polyacrylamide-methylcellulose hydrogels[47].

Figure 7 – Water evaporation kinetics (mass loss) at 70°C over time for GGHA and GGLA hydrogels.



With maximum swelling values, it is possible to calculate the network parameters using equations (2) to (5). Values of polymer volume fraction (V_r), molar mass between crosslinking (M_c) and crosslinking density (d_x) were calculated based on the maximum absorption of hydrogels at pH = 7. Considering that crosslinking points are rigid on the network structure (hydrophobic), these points do not influence the absorption of water by the polymer network. In this case, a higher density of crosslinking indicates lower absorption of the polymer chain. Those values are represented in Tables 1 and 2.

Table 1 – Values of swelling degree (S), polymer volume fraction (V_r), molar mass

between crosslinking (M_c) and crosslinking density (d_x) values for GGHA:JEFF hydrogels.

GGHA:JEFF	**S**	**V_r (10^{-3})**	**M_c (10^3)**	**d_x (10^{-4})**
1	145	7.6	8.34	2.39
2	121	10.2	5	4.0
3	95	13.6	3.43	5.82
4	85	16.5	2.83	7.05

Table 2 - Values of swelling degree (S), polymer volume fraction (V_r), molar mass between crosslinking (M_c) and crosslinking density (d_x) values for GGLA:JEFF hydrogels.

GGLA:JEFF	**S**	**V_r (10^{-3})**	**M_c (10^3)**	**d_x (10^{-4})**
1	77	16.3	2.86	6.96
2	74	19.7	2.36	8.44
3	64	21.9	2.13	9.36
4	60	24.6	1.9	10.51

Since the GGHA hydrogels presented better water adsorption, they were tested as matrixes for fertilizer controlled release. To evaluate the use of hydrogels in chemicals controlled release systems, they were tested with commercial fertilizers MKP and NPK. The dry gel was immersed in 3 different fertilizer solutions (1g/L, 5g/L and 10g/L) for 24 hours and then oven dried at 40°C. The fertilizer release was evaluated after the dry sample immersion in water and the conductivity was measured over time. Figures 8 and 9 exhibit milligrams of fertilizer released per gram of hydrogel used over time.

Figure 8 – Values of MKP release per hydrogel gram in water for GGHA:JEFF hydrogels using [MKP] 1 g/L, [MKP] 5 g/L and [MKP] 10 g/L.

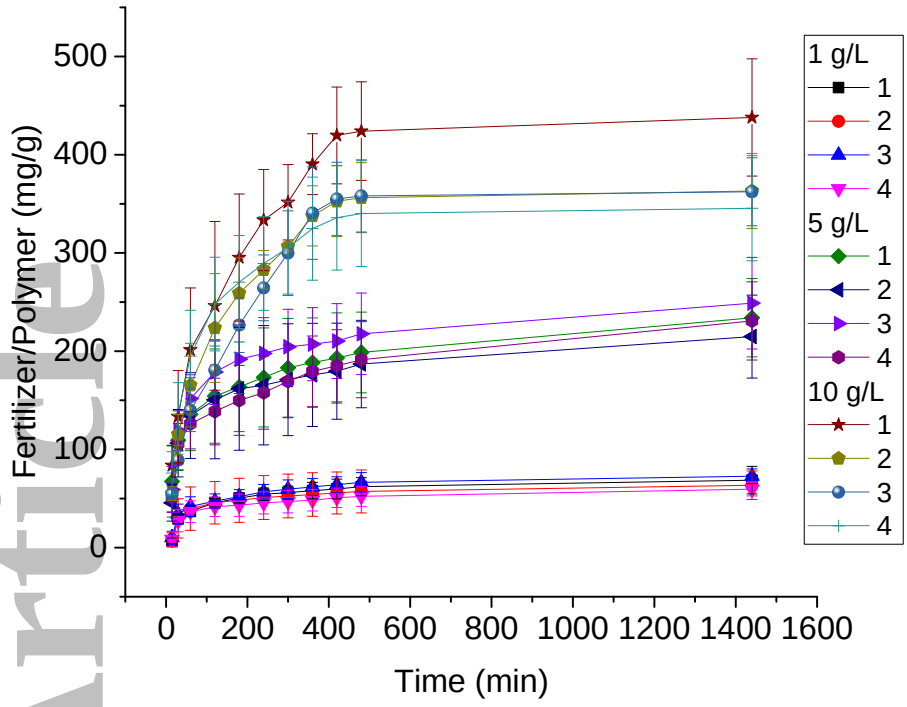
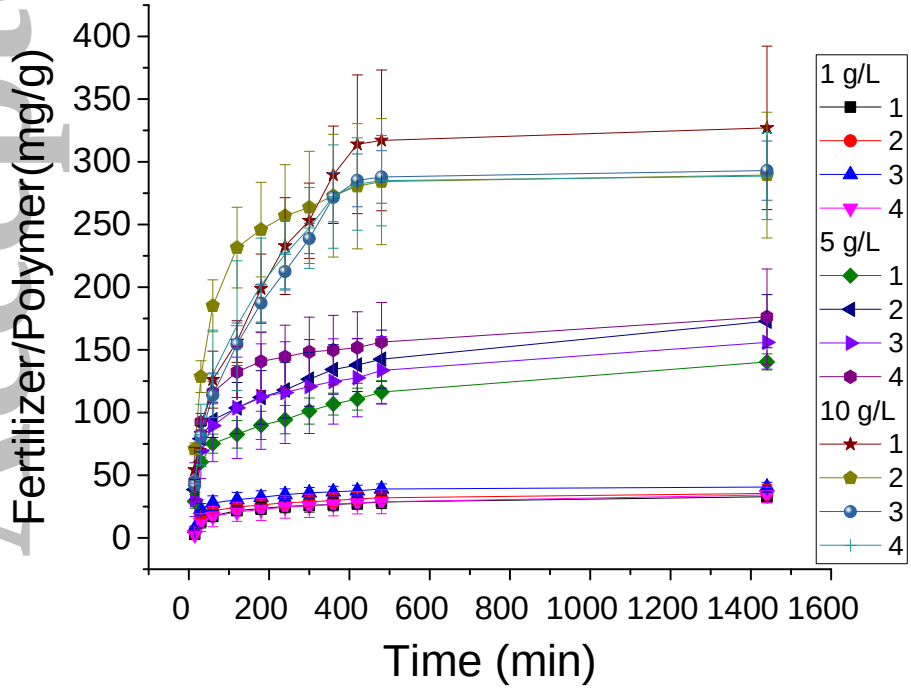


Figure 9 - Values of NPK release per hydrogel gram in water for GGHA:JEFF hydrogels using [NPK] 1 g/L, [NPK] 5 g/L and [NPK] 10 g/L.



From figures 8 and 9 it can be observed that maximum release happens at about 8 hours after the hydrogels get in contact with water, showing a higher release time than gellan gum-carboxymethyl chitosan hydrogel[45], but still lower than hydrogels based on Sodium alginate-g-Poly(acrylic acid-co-acrylamide)/Clinoptilolite[48]. After this time, the fertilizer concentration in the solution remains practically constant indicating the end of the release. The results also show a constant partial amount of fertilizer released as a function of time, and the final amount released is dependent only on fertilizer solution concentration and independent of the hydrogel swelling degree and composition[47,49,50]. The same behavior was observed in gellan gum-chitosan prepared by our research group[42]. The two analyzed fertilizers showed increased releasing per gram of polymer MKP compared to NPK, with the same concentrations used. Better efficiency is noted in the release of commercial fertilizer MKP, reaching about 400 mg of fertilizer per gram of hydrogel, while NPK releases about 300 mg per gram of dry hydrogel, which is probably due to the structure of the fertilizer and interactions with the hydrogel polymer chains[42], still more study is needed in this aspect.

4 CONCLUSION

In this paper, it is presented the synthesis and characterization of superabsorbent hydrogels based on gellan gum and Jeffamine; different amounts of crosslinking between those two polymers were obtained using different amounts of EDC/NHS. This crosslinking was confirmed by FTIR spectra and thermogravimetric analysis. Morphology was analyzed by SEM images of lyophilized samples, indicating denser structures for samples with higher crosslinking points. The synthetized hydrogels presented a swelling degree equivalent to 145 times their dry weight for GGHA, and 77 times for GGLA. Hydrogels also showed a 450 minute water retention as opposed to 280 minutes for pure water, evidencing good humidity control capacity and use in arid climates. Then, samples with higher water adsorption were tested as matrixes for fertilizer controlled release, showing complete release after 500 minutes regardless of gel formulation or fertilizer concentration. They presented maximum release of about 400 mg per gel gram for MKP and of about 300 mg per gel gram for NPK. Those results accredit gellan gum-Jeffamine hydrogels as good materials for fertilizer-controlled release and soil humidity control.

5 ACKNOWLEDGEMENTS

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