Journal of Applied Polymer Science

Gellan gum–*O,O′*-bis(2-aminopropyl)-polyethylene glycol hydrogel for controlled fertilizer release

R. C. Sabadini,[1] **M. M. Silva**,[2] **A. Pawlicka**,[1] **J. Kanicki**[3]

[1]Instituto de Química de São Carlos, Universidade de São Paulo, 13566-590 São Carlos SP, Brazil
[2]Centro de Química, Universidade do Minho, Gualtar, 4710-057 Braga, Portugal
[3]Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI 48109
Correspondence to: R. C. Sabadini (E-mail: sabadini@usp.br)

ABSTRACT: A gellan gum–Jeffamine superabsorbent hydrogel was obtained with different crosslink densities using different amounts of (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) and *N*-hydroxysuccinimide. Infrared spectroscopy and thermal analysis confirm the crosslinking. A 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 min water retention, as opposed to 280 min for pure water, evidencing good humidity control, suitable for use in arid climates. They also demonstrated a maximum release of commercial fertilizer of about 400 mg per gram for KH_2PO_4 and about 300 mg per gram for NPK 20-5-20. © 2017 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2018**, *135*, 45636.

KEYWORDS: crosslinking; gels; swelling

Received 14 February 2017; accepted 25 July 2017
DOI: 10.1002/app.45636

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 agricultural production systems are 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 crosslinking polymer chains (chemically or physically) maintain their three-dimensional structure, making it possible to swell with 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 scaffolds 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 protein 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 the 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 the trade name Gelrite, is obtained through the fermentation of the nonpathogenic aerobic bacteria culture of *Sphingomonas paucimobilis*.[18,19] Gellan gum features a high-molecular-weight deacylated 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 being substituted by acetate and L-glycerate).[21] The presence of the acetate group has a great influence on the characteristics of the resulting gel. While the native gum forms a soft, elastic, and opaque gel, the deacylated gum forms a hard, tough, and bright gel.[22]

The compound [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] (EDC) is one of the most popular

compounds 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 the reagent and the reject of the crosslinking reaction (isourea) can be easily removed from the medium.[25]

N-substituted carbodiimides can react with carboxylic acids to form a highly reactive *o*-acylisourea intermediate; this type of intermediate reacts immediately 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*-hydroxysuccinamide (NHS) to the reaction is an increase in the solubility and stability of the active intermediate.[27]

Most controlled-release systems used in agriculture contain superabsorbent hydrogels derived from polyacrylamide, due to its price and large capacity for water absorption.[28] Polyacrylamide has been used as a soil conditioner and for controlling humidity since 1950,[29–31] but 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 the controlled release of fertilizer.[2]

EXPERIMENTAL

Materials

Gellan gum (GG; M_w ~1,000,000 Da) high acyl (HA) and low acyl (LA) were kindly provided by CP Kelco (Atlanta, GA). Jeffamine [*O,O′*-bis(2-aminopropyl)polypropylene glycol; M_w ~130 Da; 99%] was obtained from Fluka (Morris Plains, NJ). *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride 98% (EDC), *N*-hydroxysuccinimide 98% (NHS), and 2-(*N*-morpholino)ethanesulfonic acid 99.5% (MES buffer) were obtained from Sigma-Aldrich (St. Louis, MO). All reagents were used without further treatment.

Hydrogel Preparation

The hydrogels were prepared by dissolving 0.1 g 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 the samples were named 1, 2, 3, and 4 according to the quantities of EDC/NHS added to the sample.

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

Swelling Degree

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

The swelling degree (*S*) was calculated using eq. (1)[35]:

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

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

Network Parameters

The density between crosslinks (d_x) was calculated using eq. (2):

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

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 has been extensively studied by Flory and is represented by the Flory–Rehner eq. (3)[36,37]:

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

where V_s is the molar volume of the solvent, ρ_p the density of the polymer, ρ_s the solvent density, V_r the polymer volume fraction [eq. (4)], and χ the Flory–Huggins parameter [eq. (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.

$$\chi = \left(\frac{V_s}{RT}\right)(\delta_{t\ pol} - \delta_{t\ sol})^2 \quad (5)$$

Here, V_s is the molar volume of the solvent, $\delta_{t\ pol}$ is the solubility parameter of the polymer, and $\delta_{t\ sol}$ is the solubility parameter of the solvent.

Fertilizer Release

The commercial fertilizers monopotassium phosphate (KH_2PO_4; MKP; Yara Brasil (Porto Alegre, Brazil)) and NPK 20-5-20 (NH_4NO_3, P_2O_5, and K_2O; Agro Brazil (São Paulo, Brazil)) were both dissolved in Millipore (Billerica, MA) Milli-Q water, resulting in solutions of 1 g/L, 5 g/L, and 10 g/L. After this process, the samples were immersed in those solutions for 24 h; next, they were oven-dried at 40 °C until a constant weight was achieved. Last, they were stored in a desiccator. To measure release, the hydrogels with fertilizer were then immersed in 14 mL of Milli-Q water, and the conductivities were measured using the conductivity meter Hanna Instruments (Woonsocket, RI) 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 based on the conductivity using a calibration curve as shown in Figure 1.

Analytical Techniques

The infrared spectroscopy (FTIR) analysis was performed using a Shimadzu (Kyoto, Japan) model IRAffinity1.

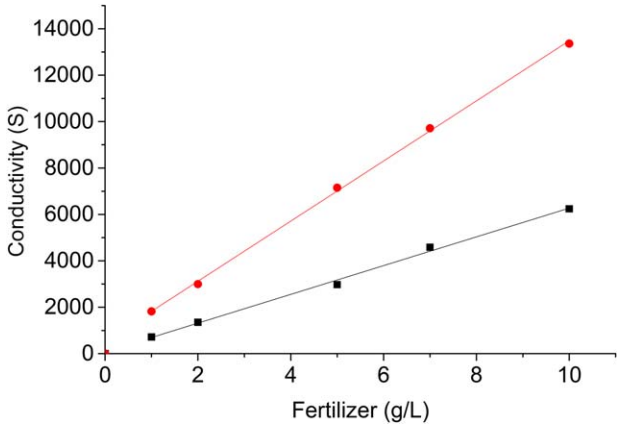

Figure 1. Calibration curve correlating conductivity (μS) of fertilizer solution with concentration for MKP (black squares) and NPK (red circles). [Color figure can be viewed at wileyonlinelibrary.com]

Thermogravimetric analysis (TGA) was done using a TA Instruments (New Castle, DE) TGA Q50 with a heating rate of 10 °C/min under a N_2 flow.

Scanning electron microscopy (SEM) images were obtained with a Zeiss LEO 440 (Cambridge, England) operating with a 20 kV electron beam and equipped with an Oxford detector (model 7060). Before the examination, the dry samples were covered with a 6 nm thick gold layer using Coating System BAL-TEC MED 020 (BAL-TEC, Liechtenstein) at 2×10^{-2} mbar pressure level, a 60 mA current, and a deposition rate of 0.60 nm/s.

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 the hydrogel structure. This structure can be predicted as shown in Figure 2.

The FTIR spectra (Figure 3) serve as a means of confirming the structure presented in Figure 2: the amide bond formation can be observed at the 1650 cm^{-1} band, associated with the C═O stretching vibration (amide I), whereas the 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 is attributed to the C—N stretching deformation coupled with N—H deformation (amide III). The 1720 cm^{-1} band is associated with

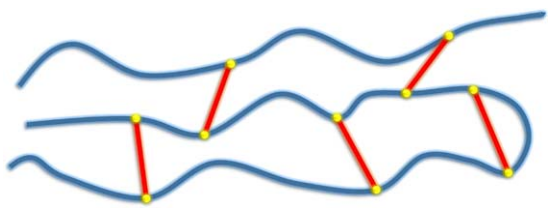

Figure 2. Proposed structure of gellan gum–Jeffamine hydrogel after crosslinking, represented by gellan gum (blue lines), Jeffamine (red lines), and crosslinking points (yellow dots). [Color figure can be viewed at wileyonlinelibrary.com]

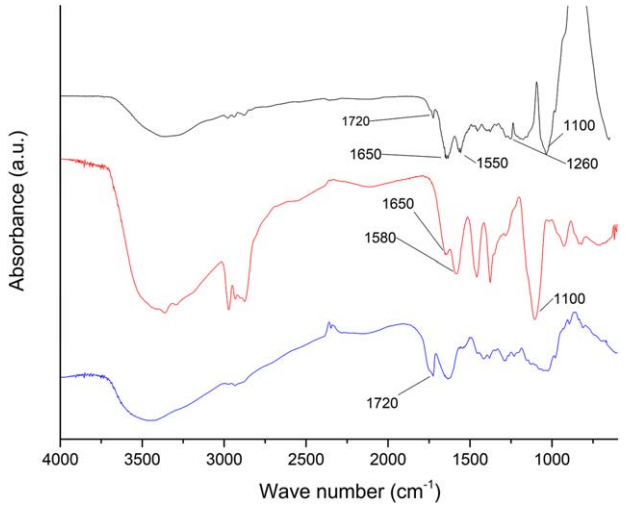

Figure 3. FTIR spectra for gellan gum (blue, bottom) Jeffamine (red, center), and hydrogel (black, top). [Color figure can be viewed at wileyonlinelibrary.com]

carboxylic acid C═O deformation, showing higher intensity in the gellan gum spectra. The 1100 cm^{-1} band is related to C—O—C stretching, also present in the Jeffamine spectra,[39,40] confirming the formation of crosslinking between gellan gum and Jeffamine chains.

TGA and DTG thermograms for both reagents and the hydrogel formed are presented in Figure 4. The thermogram in Figure 4(a) reveals 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 the 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 the temperature peak can be associated with polymer chain interactions.[41]

To analyze the morphology of hydrated hydrogels, the samples were lyophilized, and pictures of the surfaces were taken by SEM. Figure 5 presents samples 2 (A) and 4 (B) for high-acyl gellan gum (GGHA) and samples 2 (C) and 4 (D) for low-acyl gellan gum (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 being promoted by the large quantity of EDC/NHS in the preparation. GGLA sample 2 (C) presents a denser structure with fewer small pores, while sample 4 (D) shows large pores and more empty spaces. By comparing 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 structures with irregular pores.[27]

The main characteristic of a hydrogel is its ability to hydrate in water. In most cases, a higher absorption through time is noticeable. The hydrophilic chains of polymers absorb water

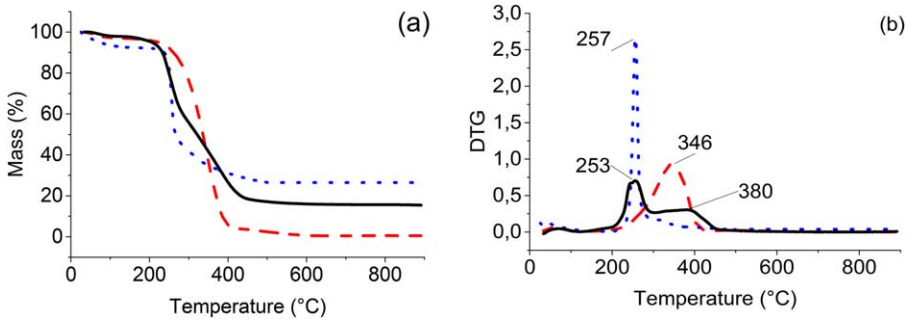

Figure 4. Thermograms of (a) TGA and (b) DTG for gellan gum (blue dotted line), Jeffamine (red dashed line), and hydrogel (solid line), presenting their thermal degradation. [Color figure can be viewed at wileyonlinelibrary.com]

until the hydration forces are counterbalanced by the strength of the expansion of the polymer chain.[43] The swelling kinetics for samples prepared with GGHA are 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, which lowers maximum water absorption. Flory and Rehner[44] defined the crosslinking point of molecules as rigid, so there is no water absorption at this point. Thus, a higher absorption reflects a smaller number of crosslinks. 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]

In order to evaluate the humidity control of the hydrogels, the water evaporation kinetics in 1.0 g of hydrated samples was performed, and the weight was measured through time at 70 °C. Figure 7 displays the 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 the 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. The values are comparable to that of polyacrylamide–methylcellulose hydrogels.[47]

With maximum swelling values, it is possible to calculate the network parameters using eqs. (2-5). The values for 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 I and II.

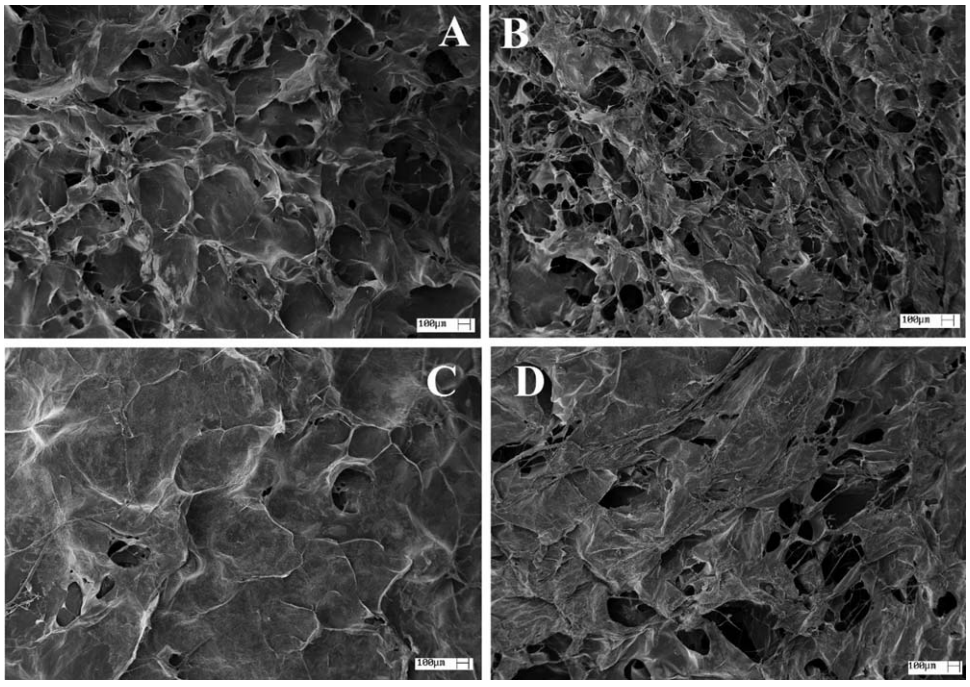

Figure 5. SEM images for lyophilized structure of GGHA–Jeffamine samples 2 (A) and 4 (B) and GGLA samples 2 (C) and 4 (D).

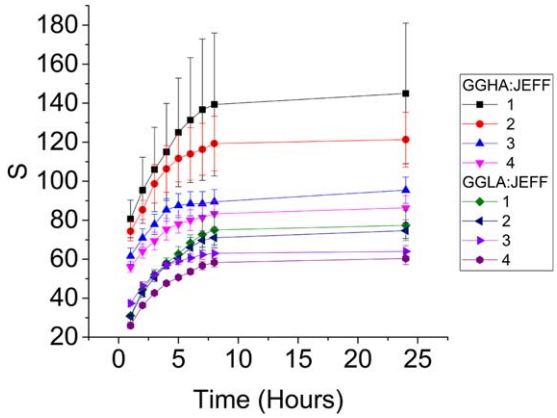

Figure 6. Swelling degree (S) for gellan gum (HA and LA)–Jeffamine hydrogels through time (1, 2, 3, 4, 5, 6, 7, 8, and 24 h after immersion). [Color figure can be viewed at wileyonlinelibrary.com]

Since the GGHA hydrogels presented better water adsorption, they were tested as matrixes for controlled release of fertilizer. To evaluate the use of hydrogels in chemical controlled-release systems, they were tested with commercial fertilizers MKP and NPK. The dry gel was immersed in three different fertilizer solutions (1 g/L, 5 g/L, and 10 g/L) for 24 h and then oven-dried at 40 °C. The fertilizer release was evaluated after the dry sample was immersed 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.

From Figures 8 and 9 it can be observed that the maximum release happens at about 8 h after the hydrogels come 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 the fertilizer solution concentration and independent of the hydrogel swelling degree and

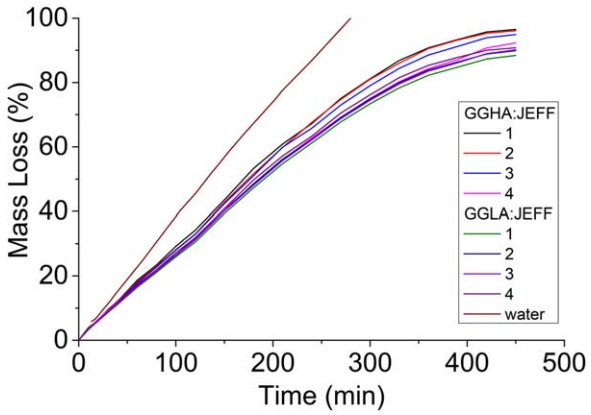

Figure 7. Water evaporation kinetics (mass loss) at 70 °C over time for GGHA and GGLA hydrogels. [Color figure can be viewed at wileyonlinelibrary.com]

Table I. Swelling Degree, Polymer Volume Fraction, Molar Mass Between Crosslinking, and Crosslinking Density Values for GGHA–Jeffamine Hydrogels

Sample	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 II. Swelling Degree, Polymer Volume Fraction, Molar Mass Between Crosslinking, and Crosslinking Density Values for GGLA–Jeffamine Hydrogels

Sample	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

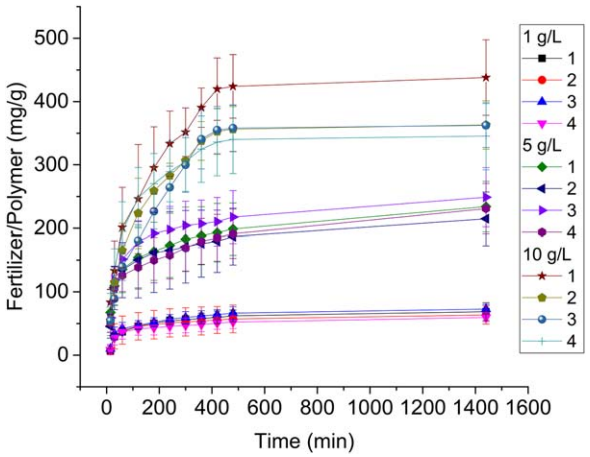

Figure 8. Values of MKP release per hydrogel gram in water for GGHA–Jeffamine hydrogels using [MKP] 1 g/L, [MKP] 5 g/L, and [MKP] 10 g/L. [Color figure can be viewed at wileyonlinelibrary.com]

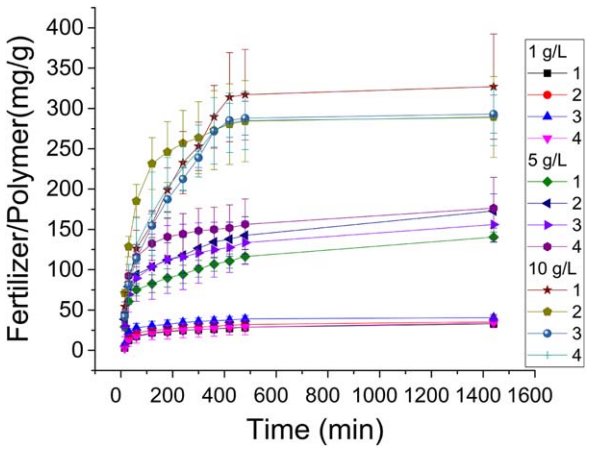

Figure 9. Values of NPK release per hydrogel gram in water for GGHA–Jeffamine hydrogels using [NPK] 1 g/L, [NPK] 5 g/L, and [NPK] 10 g/L. [Color figure can be viewed at wileyonlinelibrary.com]

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 release 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] although more study is needed on this aspect.

CONCLUSIONS

In this paper, we present 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. The morphology was analyzed by SEM images of lyophilized samples, indicating denser structures for samples with higher crosslinking points. The synthesized hydrogels presented a swelling degree equivalent to 145 times their dry weight for GGHA, and 77 times for GGLA. Hydrogels also showed a 450 min water retention, as opposed to 280 min for pure water, evidencing good humidity control, suitable for use in arid climates. Then, samples with higher water adsorption were tested as matrixes for controlled release of fertilizer, showing complete release after 500 min regardless of gel formulation or fertilizer concentration. They presented a maximum release of about 400 mg per gram of gel for MKP and about 300 mg per gram of gel for NPK. These results confirm gellan gum–Jeffamine hydrogels as good materials for controlled release of fertilizer and soil humidity control.

ACKNOWLEDGMENTS

We would like to thank FAPESP, CNPq, and CAPES for their financial support. R. C. Sabadini acknowledges the support provided by CNPq (process 152252/2016-9), and M. M. Silva acknowledges the mobility grant provided by CNPq (PVE grant 406617/2013-9) for the success of this research. The authors would also like to thank Fausto and Maria for the translation.

REFERENCES

1. Davidson, D.; Gu, F. X. *J. Agr. Food Chem.* **2012**, *60*, 870.
2. Ni, B. L.; Liu, M. Z.; Lu, S. Y.; Xie, L. H.; Wang, Y. F. *J. Agr. Food Chem.* **2011**, *59*, 10169.
3. Fan, L. H.; Tan, C.; Wang, L. B.; Pan, X. R.; Cao, M.; Wen, F.; Xie, W. G.; Nie, M. *J. Appl. Polym. Sci.* **2013**, *128*, 2789.
4. Pal, K.; Banthia, A. K.; Majumdar, D. K. *Des. Monomers Polym.* **2009**, *12*, 197.
5. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27.
6. Drury, J. L.; Mooney, D. *J. Biomater.* **2003**, *24*, 4337.
7. Jeong, B.; Kim, S. W.; Bae, Y. H. *Adv. Drug Deliv. Rev.* **2012**, *Supplement 154*, 64.
8. Lin, C.-C.; Metters, A. T. *Adv. Drug Deliv. Rev.* **2006**, *58*, 1379.
9. Baysal, K.; Aroguz, A. Z.; Adiguzel, Z.; Baysal, B. M. *Int. J. Biol. Macromol.* **2013**, *59*, 342.
10. Tian, K.; Xie, C. S.; Xia, X. P. *Colloids Surf. B* **2013**, *109*, 82.
11. Wu, L.; Liu, M. Z. *Carbohydr. Polym.* **2008**, *72*, 240.
12. dos Santos, J. C. S.; Bonazza, H. L.; de Matos, L. J. B. L.; Carneiro, E. A.; Barbosa, O.; Fernandez-Lafuente, R.; Gonçalves, L. R. B.; de Sant'Ana, H. B.; Santiago-Aguiar, R. S. *Biotechnol. Rep.* **2017**, *14*, 16.
13. dos Santos, J. C. S.; Rueda, N.; Torres, R.; Barbosa, O.; Gonçalves, L. R. B.; Fernandez-Lafuente, R. *Process Biochem.* **2015**, *50*, 918.
14. Ferris, C. J.; Panhuis, M. I. H. *Soft Matter* **2009**, *5*, 3430.
15. Guzman-Villanueva, D.; Smyth, H. D. C.; Herrera-Ruiz, D.; El-Sherbiny, I. M. *J. Nanomater.* **2011**, *2011*, DOI: 10.1155/2011/507508.
16. Colombo, P. *Adv. Drug Deliv. Rev.* **1993**, *11*, 37.
17. Torelli-Souza, R. R.; Bastos, L. A. C.; Nunes, H. G. L.; Camara, C. A.; Amorim, R. V. S. *J. Appl. Polym. Sci.* **2012**, *126*, E408.
18. Jansson, P. E.; Lindberg, B.; Sandford, P. A. *Carbohydr. Res.* **1983**, *124*, 135.
19. Shah, J. N.; Jani, G. K.; Parikh, J. R. *Pharmacol. Rev.* **2007**, *5*, http://journaldatabase.info/articles/gellan_gum_its_applications_review.html
20. de Souza, C. F.; Riegel-Vidotti, I. C.; Cardoso, M. B.; Ono, L.; Lucyszyn, N.; Lubambo, A. F.; Sens, C. V.; Grein-Iankovski, A.; Sierakowski, M. R. *Carbohydr. Polym.* **2014**, *114*, 48.
21. Giavasis, I.; Harvey, L. M.; McNeil, B. *Crit. Rev. Biotechnol.* **2000**, *20*, 177.
22. Quinn, F. X.; Hatakeyama, T.; Yoshida, H.; Takahashi, M.; Hatakeyama, H. *Polym. Gels Networks* **1993**, *1*, 93.
23. Wang, C.; Yan, Q.; Liu, H. B.; Zhou, X. H.; Xiao, S. *J. Langmuir* **2011**, *27*, 12058.
24. D'Este, M.; Eglin, D.; Alini, M. *Carbohydr. Polym.* **2014**, *108*, 239.
25. Hermanson, G. T. In Bioconjugate Techniques, 2nd ed.; Hermanson, G. T., Ed.; Academic Press: New York, **2008**.
26. D'Este, M.; Eglin, D.; Alini, M. *Carbohydr. Polym.* **2014**, *108*, 239.
27. Cao, H.; Xu, S. Y. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 567.
28. Cao, L. F.; Yang, H. L.; Zhou, Y.; Zhao, F.; Xu, P. F.; Yao, Q. Q.; Yu, N.; Hu, Z. W.; Peng, Z. Q. *Energy Build.* **2013**, *62*, 590.
29. Seybold, C. A. *Commun. Soil Sci. Plant Anal.* **1994**, *25*, 2171.
30. Agnihotri, S. A.; Aminabhavi, T. M. *Int. J. Pharm.* **2006**, *324*, 103.
31. Zhan, F. L.; Liu, M. Z.; Guo, M. Y.; Wu, L. *J. Appl. Polym. Sci.* **2004**, *92*, 3417.

32. Pretty, J. *Philos. Trans. R. Soc. B* **2008**, *363*, 447.
33. Hazell, P.; Wood, S. *Philos. Trans. R. Soc. B* **2008**, *363*, 495.
34. Giannouli, P.; Morris, E. R. *Food Hydrocolloids* **2003**, *17*, 495.
35. Coutinho, D. F.; Sant, S. V.; Shin, H.; Oliveira, J. T.; Gomes, M. E.; Neves, N. M.; Khademhosseini, A.; Reis, R. L. *Biomaterials* **2010**, *31*, 7494.
36. Hatakeyema, T.; Yamauchi, A.; Hatakeyema, H. *Eur. Polym. J.* **1984**, *20*, 61.
37. Barikani, M. H. C. *Iranian J. Polym. Sci. Technol.* **1992**, *1*, 1.
38. Pasqui, D.; De Cagna, M.; Barbucci, R. *Polymers* **2012**, *4*, 1517.
39. Roeges, N. P. G. A Guide to the Complete Interpretation of Infrared Spectral of Organic Structures; Wiley: Hoboken, NJ, **1994**.
40. Sam, S.; Touahir, L.; Andresa, J. S.; Allongue, P.; Chazalviel, J. N.; Gouget-Laemmel, A. C.; de Villeneuve, C. H.; Moraillon, A.; Ozanam, F.; Gabouze, N.; Djebbar, S. *Langmuir* **2010**, *26*, 809.
41. Ostrowska-Czubenko, J.; Gierszewska-Drużyńska, M. *Carbohydr. Polym.* **2009**, *77*, 590.
42. Sabadini, R. C.; Martins, V. C. A.; Pawlicka, A. *Cellulose* **2015**, *22*, 2045.
43. Omidian, H.; Zohuriaan-Mehr, M. *J. Polymer* **2002**, *43*, 269.
44. Flory, P. J.; Rehner, J. *J. Chem. Phys.* **1943**, *11*, 521.
45. Tang, Y.; Sun, J.; Fan, H.; Zhang, X. *Carbohydr. Polym.* **2012**, *88*, 46.
46. Kabiri, K.; Zohuriaan-Mehr, M. *J. Macromol. Mater. Eng.* **2004**, *289*, 653.
47. Bortolin, A.; Aouada, F. A.; de Moura, M. R.; Ribeiro, C.; Longo, E.; Mattoso, L. H. C. *J. Appl. Polym. Sci.* **2012**, *123*, 2291.
48. Rashidzadeh, A.; Olad, A.; Salari, D.; Reyhanitabar, A. *J. Polym. Res.* **2014**, *21*, 344.
49. Pourjavadi, A.; Doulabi, M.; Soleyman, R.; Sharif, S.; Eghtesadi, S. A. *React. Funct. Polym.* **2012**, *72*, 667.
50. Watkins, K. A.; Chen, R. *Int. J. Pharm.* **2015**, *478*, 496.