# Physicochemical Properties of Superabsorbent Hydrogels Formed by Polyelectrolytic Complexation of Carboxymethylcellulose-Chitosan at Basic pH

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High water consumption during irrigation is one of the main problems in agriculture. The preparation of a material that prolongs the water retention capacity of the soil is important. This article aimed to prepare superabsorbent hydrogels by the complexation of chitosan (CHI), with high degree of deacetylation (DD), and carboxymethylcellulose (CMC), with high degree of substitution (SD). Different parameters (concentration and polymeric solutions addition order, polysaccharides' ratio, pH) were studied. Morphology, thermal stability, chemical composition, mechanical strength, and swelling kinetics of hydrogels in water were evaluated. A hydrogel formation mechanism was discussed and proposed. Results showed that most samples prepared at acid pH disintegrated during swelling test. The morphology of the hydrogels varied with the pH. It was obtained superabsorbent hydrogels (swelling > 400%) based on CMC (SD = 2.23) and CHI (DD = 92%), at basic pH, with good compression strength, and applicable as soil conditioner. The best sample was the one prepared with CMC:CHI = 1:2, polymeric solutions 1.5% m/v and pH 8.

**Keywords:** polyelectrolyte complex, polysaccharides, mechanical properties, formation mechanism, morphology

# Introduction

The industrial applications of natural polymers are increasing in areas such as packaging, pharmaceuticals, medical devices and foods, due to their attractive characteristics such as biocompatibility, biodegradability and wide availability from agricultural wastes and marine sources. Among these materials are chitin, starches (potato, corn, wheat and rice), cellulose derivatives, and gums (guar, carrageenan, alginate and pectin), besides plant proteins (gelatin, casein and collagen).<sup>1-7</sup>

The development of sustainable materials that contribute to preservation of nature is an interesting alternative, both for environmental preservation and industrial development. In this respect, biopolymers obtained from renewable sources stand out.<sup>8</sup>

Among the natural polymers that have been investigated are chitosan (CHI) and carboxymethylcellulose (CMC), which have the advantages of biodegradability, biocompatibility and better mechanical properties when

\*e-mail: ivanamello@uerj.br Editor handled this article: Fernando C. Giacomelli (Associate) complexed.<sup>9</sup> Chitosan is a cationic polymer that can form flexible and resistant films with excellent mechanical properties.<sup>1,10</sup> In turn, carboxymethylcellulose is an anionic polymer with low toxicity, wide availability in nature, high solubility in water, high sensitivity to pH variation and good ionic strength. Indeed, it is the only polyelectrolyte derived from cellulose with these characteristics. Its degree of substitution is given by the average number of carboxymethyl groups substituted *per* monomer unit, ranging from 0 to 3, with the remaining R = H (Figure 1).<sup>11,12</sup> The chemical structures of CMC are shown in Figure 1.

Polyelectrolyte complexes (PECs) are three-dimensional structures formed by a pair of ionic polymers with opposite charges in solution. The reaction to form hydrogels by polyelectrolytic complexation is an alternative to produce hydrogels with ionic crosslinks, where the main advantage of forming the PEC is the ease of preparation.<sup>13-17</sup>

Rosca *et al.*<sup>18</sup> studied the interaction of chitosan hydrochloride (acetylation degree = 20.8%) with sodium carboxymethylcellulose (substitution degree = 0.8) in aqueous solution, by potentiometric, conductometric and turbidimetric titration, as well as in solid state by Fourier transform (FTIR) spectra and thermogravimetric analysis.

# н HO NH2 NH<sub>2</sub> (a) H<sub>2</sub>COR но H-COR (b) (6) СН,ОН CICH<sub>2</sub>COOH NaOH (c)

Figure 1. Chemical structure of (a) CHI and (b) CMC, as well as (c) introduction of the carboxymethyl group in the glucopyranose ring of cellulose to obtain CMC (R = H or CH<sub>2</sub>COO<sup>-</sup>Na<sup>+</sup>).

All methods demonstrated the formation of a complex, suggesting that the interaction occurs through electrostatic forces.

Altam et al.<sup>19</sup> prepared beads composed of CMC (substitution degree = 0.7) and CHI (deacetylation degree = 95%) by interfacial complexation of these polymers via an extrusion-drop method. The results showed that the beads prepared at pH 4.5 were the strongest and could resist a force of up to 0.071 N.

Zhao et al.<sup>20</sup> prepared a PEC of CHI (deacetylation degree = 90%) and CMC (substitution degree = 0.8) by the semi-dissolution acidification sol-gel transition method. The samples exhibited good mechanical properties, with the maximum tensile strength of 0.18 MPa and elongation at break of 127.6%. The samples also had good adsorption capacity of cationic and anionic dyes, with maximum adsorption capacities of 212.83 mg g<sup>-1</sup> for sunset yellow FCF and 167.35 mg  $g^{-1}$  for methylene blue.

Shang et al.<sup>21</sup> prepared an amphoteric hydrogel film by solution blending of CHI (deacetylation degree = 90%) with CMC (substitution degree not informed) and crosslinking with glutaraldehyde. The bending of the film in an electric field was studied in different electrolyte solutions. The equilibrium bending angle of the hydrogels reached a maximum in the buffer solution at pH 5-6 with 0.2 M ionic strength. The authors suggested the use of the hydrogel in microsensor and actuator applications, especially in the biomedical field.

Chen et al.<sup>22</sup> prepared a polyelectrolyte complex based on CHI (deacetylation degree > 90%) and CMC (substitution degree = 0.7) using a dry thermo-mechanical kneading method. They also incorporated two clays, montmorillonite and sepiolite, in the formulations. The

resulting films were initially flexible, but they all became rigid with similar density after removal of excess moisture for recrystallization. The FTIR spectra were similar, suggesting that the addition of nanoclays did not change the biopolymer molecular interactions. The X-ray diffraction (XRD) results showed that the inclusion of nanoclays had no major effect.

Ferreira et al.23 studied the effects of CHI (deacetylation degree = 76.7%) and CMC (substitution degree =  $0.59 \pm 0.03$ ). with varied mixing ratios (from 1:0 to 4:0 and 0:1 to 0:4), temperatures (25, 45, 65, 85 °C), and pH levels (3.0, 3.5, 4.0, 4.5) on the PEC's properties. The authors concluded that the ratio of 1:2 and temperature of 25 °C were the best, independently of the pH value. The conditions tested led to the formation of macro and micro-structured polyelectrolyte complexes. Micro-PECs had homogeneous appearance, whereas macro-PECs presented porous network structures interspersed with heterogeneous-sized vacuoles. Also, the micro-PECs were thermally more stable than either of the biomolecules separately.

There are few systematic studies reported in the literature on the preparation of hydrogels by the polyelectrolytic complexation of CMC with a high degree of substitution and CHI with a high degree of deacetylation. Furthermore, to date, no studies have been found on the preparation of superabsorbent hydrogels based on CMC and CHI, at basic pH, for application as soil conditioners.

Sabadini et al.24 produced superabsorbent hydrogels composed of starch and gellan gum crosslinked with citric acid (CA) and incorporating bentonite (BET) and halloysite (HAL) nanoclays. It evaluated compositions from 100, 75, 25, and 0% of gellan gum to starch, all crosslinked with 10, 5, and 2% (m/m) of CA. The swelling properties in water were studied. The best swelling was observed for the sample without clay, with 100% gellan gum and 2% of CA, and it occurred in 24h. The addition of HAL to the hydrogel composition decreased this value to 296 and 8 h of immersion in water. The addition of BET clay impacted negatively on the swelling properties of the hydrogels, reaching a maximum of 205 in 48 h of immersion in water. However, the authors concluded that the hydrogels produced can be used for crop water management.

Hydrogels based on a mixture of whey proteins and alginic acid (ALG) were developed as a soil conditioner and for sustained release of the urea fertilizer.<sup>25</sup> Formulations were prepared from different proteins at a polysaccharide ratio of 1-10% m/m (with respect to protein content). The hydrogels were prepared by applying calcium chloride as the cross-linking agent. The results demonstrate how adding alginic acid promoted the possible utilization of the whey protein hydrogel in agriculture. Samples containing a higher





amount of ALG demonstrated a different response to pH in terms of swelling ratio and time, since a longer period of exposure was required to reach equilibrium. The number of swelling-drying cycles that defined the durability of the hydrogel as a soil conditioner went up in number as the concentration of ALG was raised. The water retention test performed in soil indicated a slower water evaporation ratio for the hydrogels with 5 and 10% ALG.

Hydrogels were prepared by *in situ* hydrogelation of chitosan with salicylaldehyde in the presence of urea. Scanning electron microscopy (SEM) images showed that the formulations were porous and urea crystals were embedded into the pore walls. Their ability to fertilize the soil and to hold the water were investigated. An outstanding increase of the water holding parameter, from 29 (reference soil) to 154 wt.% (for hydrogel) has been recorded. Moreover, the soil sample without hydrogels lost the absorbed water after 7 days, while those with formulations after 12 days. The authors concluded that the samples could be used as soil fertilizers improving the water-holding capacity and nitrogen percentage of the soil.<sup>26</sup>

Ghobashy *et al.*<sup>27</sup> prepared hydrogels based on a combination of irradiated natural polymer (CMC) and synthetic polymer (poly(acrylic acid), PAAc). Different sample formulations of CMC-PAAc hydrogels were prepared with different doses of CMC degradation to study their impact on the degree of swelling. Then, the sample with the best swelling was chosen to be used as a soil conditioner and for the controlled release of urea. The results showed that the sample prepared from CMC irradiated at a dose of 2 kGy was the most effective sample, presenting a degree of swelling of 155 g g<sup>-1</sup> after 300 min. The authors concluded that 1 g of this sample absorbed about 100 g of water after 90 min, and therefore this sample was proposed as the soil conditioner for agriculture.

CMC hydrogels were produced via chemical crosslink with citric acid and filled with nanocellulose (NC), montmorillonite, or vermiculite, in different contents (1, 3, 5, and 10 wt.%). The results indicated that the greater the interactions between the filler and the CMC smaller the water absorption (WA). This trend was seen mainly for high NC and vermiculite contents, with WA values of 19 and 15 g g<sup>-1</sup>, respectively. Montmorillonite resulted in high WA values, ca.  $30 g g^{-1}$ . The selected samples to incorporate NPK fertilizer were 3% NC, 1% vermiculite, and 1 and 3% montmorillonite. The efficiency of these materials was confirmed by *in vivo* tests using cucumber as a culture. The formulations showed the potential to reduce fertilizer loss during application and improve water use in agriculture.<sup>28</sup>

We aimed to prepare and characterize superabsorbent hydrogels by the polyelectrolytic complexation of

carboxymethylcellulose with high substitution degree (SD = 2.23) and chitosan with high deacetylation degree (DD = 92%), in a basic medium, without the addition of crosslinkers, to be applied as soil conditioners. For this, hydrogels need to exhibit high swelling in water and good compression strength. Thus, we evaluated the influence of different preparation conditions (concentration of solutions, ratio between polysaccharides, order of adding the polymer solutions and pH of the medium) on the morphological, thermal and mechanical properties of the PECs, along with their water absorption kinetics.

### Experimental

## Reagents

We used chitosan with average molecular weight (Mw) 190,000-310,000 Da, (CAS-No 9012-76-4), purchased from Sigma-Aldrich (Saint Louis, USA), carboxymethylcellulose Mw ca. 90,000 Da, (CAS-No 9000-11-7) provided by Denver-Especialidades Químicas (São Paulo, Brazil). Acetic acid (CAS-No 543-24-8), sodium hydroxide (CAS-No 1310-73-2), sodium chloride (CAS-No 7647-14-5) and hydrogen chloride (CAS-No 7647-01-0) were supplied by Vetec Química Fina Ltda. (Rio de Janeiro, Brazil). All the reagents were used as received.

### Instrumentation

Nuclear magnetic resonance (NMR) analysis was carried out on a Varian Mercury VX 300 spectrometer operating at 600.13 MHz (IMA/UFRJ, RJ, Brazil), Vario Macro analyzer at combustion temperature of 1,150 °C (Instituto de Química/UERJ, RJ, Brazil), Zeta potential was carried out on a Zetasizer Nano series, Malvern Instruments (IMA/UFRJ, RJ, Brazil). Thermogravimetric analysis was carried out on a TA Instruments model Q50 V6.4 Build 193 thermogravimeter (Instituto de Química/UERJ, RJ, Brazil). Fourier-transform infrared spectroscopy (FTIR) analysis was carried out on a PerkinElmer Spectrum 1000 FTIR spectrometer with an attenuated total reflectance (ATR) (Instituto de Química/UERJ, RJ, Brazil). The hydrogels samples were dried using the Lyophilizer - Terroni, model Interprise I-D (Instituto de Química/UERJ, RJ, Brazil). In determining the compressive strength, it was used Emic DL2000 universal testing machine with load cell of 500 N (Instituto de Química/UERJ, RJ, Brazil). The microscope, scanning electron microscopy (SEM) FEI Inspect 550 (Instituto de Química/UERJ, RJ, Brazil), operating at voltage of 10 KV, with a secondary electron detector was used.

The experimental method consisted of three steps: characterization of the pure polysaccharides, preparation of the hydrogels, and characterization of the hydrogels.

#### Characterization of the polysaccharides

The substitution degree (SD) of the CMC was obtained by <sup>1</sup>H NMR, through the ratio between the integrals of the methylene protons of the carboxymethyl groups and the integrals of the protons belonging to the  $\beta$ -D-glucopyranose unit linked to carbons C2, C3, C4, C5 and C6 (Figure 1c), according to equation 1.<sup>29</sup>

$$SD = (a/2)(b/6)$$
 (1)

where a: integral referring to the methylene protons of the carboxymethyl groups (3.8 ppm <  $\delta$  < 4.3 ppm); and b: integral referring to the six protons linked to the  $\beta$ -D-glucopyranose unit (2.8 ppm <  $\delta$  < 3.8 ppm).

Based on the method described by Kono *et al.*,<sup>30</sup> we performed the <sup>1</sup>H NMR analysis with approximately 30 mg of CMC, dissolved in deuterated water (D<sub>2</sub>O, purity of 99.96%), and then transferred to a probe with diameter of 5 mm, which in turn was placed in a Varian Mercury VX 300 spectrometer operating at 600.13 MHz. The number of transients was 82,000, with pulse calibrated to 90 degrees and a 2 s interval between pulses, acquisition time of 0.850 s and temperature of 25 °C.

The deacetylation degree (DD) of the chitosan was determined by elemental analysis (CHN), with application of equation  $2.^{31}$  We used a Vario Macro analyzer at combustion temperature of 1,150 °C, with time of 90 s and flow of 75 mL min<sup>-1</sup>.

$$DD(\%) = \left[1 - \frac{\frac{C}{N} - 5.145}{1.722}\right] \times 100$$
(2)

where C/N = ratio between carbon and nitrogen.

The zeta potential values were measured with a Zetasizer Nano series (Malvern Instruments). All the measurements were performed at 25 °C in triplicate.

For this purpose, we prepared an aqueous solution of CMC (0.01% m/v), with addition of  $10^{-3}$  mol L<sup>-1</sup> of an aqueous solution of sodium chloride (NaCl). For the CHI, we prepared an acid solution (2% v/v of acetic acid) at 0.01% m/v and with  $10^{-3}$  mol L<sup>-1</sup> of an aqueous solution of NaCl. These solutions were called the stock solutions and were kept under magnetic stirring for 24 h to assure complete solubilization of the polysaccharides. Aliquots of 10 mL of each stock solution were separated to adjust the

pH and for subsequent analysis of the zeta potential. The adjustment of the pH of the chitosan solution between 2 and 6 was accomplished by dropwise addition of solutions of hydrogen chloride (HCl) or sodium hydroxide (NaOH) at 2.0 mol  $L^{-1}$ . For the carboxymethylcellulose solution, the pH was adjusted in the range of 3 to 9 by adding solutions of HCl or NaOH at 0.01 mol  $L^{-1}$  each.

The mean viscometric molar masses (Mv) of the polysaccharides used in this study were determined in previous investigations.<sup>32,33</sup> The Mv values were  $4.8 \times 10^3$  (CMC) and  $1.1 \times 10^5$  (CHI).

To determine the thermal stability, we used a TA Instruments model Q50 V6.4 Build 193 thermogravimeter. About 10 mg of sample was placed in a platinum crucible and heated in a quartz oven with controlled atmosphere of ultrapure nitrogen (99.96% purity) at flow of 100 mL min<sup>-1</sup>. The temperature range analyzed was 25 to 500 °C, at a heating rate of 20 °C min<sup>-1</sup>. The degradation onset temperature (Tonset) was determined by the intersection of the tangent to the thermogravimetric (TG) curves at the zero line and the tangent at the point where the degradation speed was highest for the referred degradation stage. The temperatures at which the respective degradation speeds were maximum  $(T_{max})$ were determined from the derivate TG (DTG) curves by ascertaining the maximum point of the derivative of the mass in relation to the temperature.

The chemical composition of the polysaccharides was determined by Fourier-transform infrared spectrometry in a PerkinElmer Spectrum 1000 FTIR spectrometer with an attenuated total reflectance (ATR) accessory, since the samples were opaque. The spectral range of the analysis was 4000-400 cm<sup>-1</sup>.

## Formation of PECs based on CHI and CMC

We first prepared solutions of each polysaccharide at different concentrations (CMC-1.5 and 3.0% m/v and CHI-1.5 and 3.0% m/v). These concentration values were defined by previous investigations by our research group.<sup>34-36</sup> The aqueous solutions of CMC were prepared in beakers with 1 L capacity. After weighing, each sample was placed in a beaker followed by a corresponding volume of deionized water, and the mixture was left under magnetic stirring for 24 h at 25 °C for complete solubilization. The acid solutions of CHI were prepared in beakers with the same capacity, in which the chitosan samples were placed, followed by addition of a 2.0% v/v solution of acetic acid (CH<sub>3</sub>COOH). The mixture was submitted to magnetic stirring for 24 h at 25 °C to assure complete dissolution of the polysaccharide. After preparation, the solutions were mixed in different volumetric ratios (CMC:CHI = 2:1, 1:1 and 1:2) in beakers with capacity of 250 mL and immersed in a water bath over a hot plate to maintain temperature of 25 °C. Then, the pH of each mixture was adjusted to the range between 4 and 9 by adding a solution of sodium hydroxide (1.0 mol L<sup>-1</sup>). The final mixtures were kept under stirring for 24 h at 25 °C, after which the samples were washed with deionized water, frozen and lyophilized.

## Characterization of the PECs

To determine the swelling kinetics, approximately 0.3 g of lyophilized PEC was transferred to a 250 mL beaker, followed by addition of 100 mL of deionized water. The sample was kept immersed for different times (1, 3, 6 and 24 h). After each interval, the sample was removed from the beaker and the excess water was wiped off with paper towel. Then, the swollen sample was weighed with the analytic scale and the swelling degree (SwD) was calculated by equation 3.<sup>35</sup> All the measurements were performed in triplicate.

$$\operatorname{SwD}\left(\%\right) = \frac{M_{f} - M_{0}}{M_{0}} \times 100$$
(3)

where  $M_f$  is the mass of the swollen sample after time "t", and  $M_o$  is the initial mass of the sample before the swelling test (lyophilized sample).

To determine the compressive strength of the PEC samples, we used an Emic DL2000 universal testing machine with load cell of 500 N and test speed of 12 mm min<sup>-1</sup>. Five test specimens were prepared with dimensions of 20 mm long × 20 mm diameter. The compressive strength test was performed according to the ASTM D 1621-00<sup>37</sup> standard with adaptation. The values of maximum compressive stress ( $\sigma_{max}$ ) were determined by measuring the force (*f*) necessary to compress the samples to 50% deformation, i.e., length of 10 mm. The tests were performed in triplicate and the stress values ( $\sigma$ ) were determined by equation 4.

$$\sigma = \frac{f}{S_o} \tag{4}$$

where: f is the force exerted and  $S_0$  is the surface area of the test specimen.

SEM was used to visualize the morphology of the material in detail and to determine the size of the voids formed in each material, using the ImageJ software.<sup>38</sup> The microscope used was an FEI Inspect 550, operating at voltage of 10 KV, with a secondary electron detector. The samples were previously coated with carbon.

The thermal stability of the PECs and their chemical composition were investigated by TG and FTIR, respectively, following the same experimental procedure employed for analysis of the pure polysaccharides.

## **Results and Discussion**

The chemical composition of the polysaccharides was investigated by FTIR, where the spectra had the same characteristic absorption bands of each polysaccharide.<sup>39</sup> The values are reported in Supplementary Information (SI) section (Figure S1 and Table S1).

The SD of the CMC was determined by using equation 1, based on the data from <sup>1</sup>H NMR, and presented a value of 2.23.<sup>33,40,41</sup> When the cellulose chain is completely substituted, its substitution degree is 3.<sup>41</sup> According to the result found for the sample used in this study, we can state that the CMC had high substitution degree. The <sup>1</sup>H NMR spectrum and the corresponding attributions of the signals of the carboxymethylcellulose used in this study are reported in SI section (Figure S2 and Table S2, respectively).

The characterization of the chitosan by elemental analysis (CHN) indicated the following composition: carbon (39.19%); hydrogen (7.33%); and nitrogen (7.42%). The DD was calculated by applying equation 2, with a value of 92%.

The thermograms of the pure polysaccharides (Figure S3, SI section) revealed the presence of an initial degradation stage at temperature up to 150 °C, related to the loss of the water absorbed by the polysaccharides.<sup>13,42</sup> The temperatures of the initial degradation stages ( $T_{onsel}$ ) related to the polymer chains were 282 and 288 °C for the CMC and CHI, respectively. The temperatures where the degradation speeds were highest ( $T_{max}$ ) were 292 and 306 °C for the CMC and CHI, respectively.

The optimal pH for interaction between the polysaccharides was determined by measuring the zeta potential of the polymers individually in solution, at different pH values. The curves of variation of zeta potential in function of pH comparatively between the carboxymethylcellulose and chitosan are shown in Figure 2.

In an acid medium, the amino groups of the CHI are protonated, causing a positive potential value. As the pH increases, the protonated amino groups become progressively deprotonated. It has been previously established that chitosan is soluble in acid media up to pH of approximately 6 to 7, after which it precipitates.<sup>43</sup> Therefore, it was not possible to determine the zeta potential of the CHI at pH higher than 6. In turn, in the case of the CMC, in basic media the carboxyl groups become ionized. However, the experimental results found in this work indicated that



Figure 2. Zeta potential curves of the polysaccharides in solutions with different pH values.

as of pH 5, the zeta potential values of the CMC solutions were statistically similar. Therefore, the increase of pH promoted lesser ionization of the amino groups in the chitosan structure ( $NH_3^+$ ) and greater ionization of the carboxyl groups of the carboxymethylcellulose (COO<sup>-</sup>).

The zeta potential results shown in Figure 2 suggest that the pH range where the best electrostatic interaction between the polysaccharides occurred was from 3 to 7. It is known that during the formation of the polyelectrolyte complex, both polymers must be ionized and have opposite charges. Furthermore, the formation of this type of PEC between a weak polybase (such as CHI) and a weak polyacid (such as CMC) occurs with greater intensity in the pH range between the  $pK_a$  values of the two polymers, since more than half of the ionic groups of both will be ionized.44 In the case of the system studied here, the best formation of the CMC-CHI complex occurred at the pH range of the pK<sub>a</sub> values of the CMC  $(3.2-4.6)^{45}$  and the pK<sub>a</sub> range of the chitosan (6.2-7.5),<sup>46</sup> i.e., between 4.7 and 6.1. Therefore, the optimal pH range for interaction between the two polysaccharides, defined by the zeta potential (5-7), was in accordance with findings reported in the literature. The pH value of 3 was disregarded, because to adjust the pH to that value, the chitosan would have been positively charged, while the CMC would diminish the ionization of the carboxyl groups so that they would be neutral, causing their precipitation. Besides this, over the long run, at pH values below 4, CMC tends to undergo hydrolysis.44

CMC consists of long and rigid molecules with negative charges. Thus, its molecules in solution are stretched due to the electrostatic repulsion of the segments of the chain. Besides this, since the molecules repel each other, result in monodisperse solutions that are highly viscous and stable. A pH value below 4 suppresses the ionization of the carboxyl groups so that some of them lose their charge. Molecular association then occurs, and the viscosity increases for a time interval, but this viscosity cannot be maintained for long periods at pH values below 4 due to the occurrence of hydrolysis.<sup>44</sup>

#### PEC based on CMC and CHI

In this study, at the moment when the polysaccharide solutions were mixed to form the PEC, the pH values of the two solutions were 7.06 (CMC) and 3.03 (CHI). According to the zeta potential results (Figure 2), these values favored the formation of a polyelectrolyte complex (PEC), since the amino groups of the CHI were protonated and the carboxyl groups of the CMC were ionized.

Costa *et al.*<sup>13</sup> conducted a computational study about the formation of a PEC composed of sodium alginate and chitosan. The results showed that besides the electrostatic interaction between the ionized carboxyl groups of the alginate and the protonated amine groups of the CHI, hydrogen bonds also had a strong influence on the stabilization of the PEC, due to the high energy involved.

Since the formation of the PEC occurs by the physical mixture of the CMC and CHI solutions, and consequently by the formation of electrostatic interactions between these polymers and by the other intermolecular interactions between their chains, it is possible to suggest that the formation of the CHI-CMC complex occurred according to the schematic representation shown in Figure 3, showing the network formed by the two polysaccharides with the ionized groups along their chains.

We evaluated the variation of some parameters for preparing the samples obtained by the formation of the polyelectrolyte complex: CMC:CHI concentration of the polysaccharide solutions; volumetric ratio (CMC:CHI); order of adding the polymer solutions; and pH of the medium.

Based on the variation of the concentration of the polysaccharide solutions, it was possible to observe that the high viscosity of both the CMC and the CHI when using the concentration 3.0% m/v prevented PEC formation. Therefore, we reduced the concentration to 1.5% m/v, which consequently lowered the viscosity of the solutions and enabled obtaining the PEC. Therefore, there are no results for the samples with concentration of the solutions of 3.0% m/v. Table 1 shows the reaction conditions and results regarding formation of the PEC with solutions of both polysaccharides at concentration of 1.5% m/v.

At acid pH, chitosan presents protonated amine groups, while CMC has weakly charged carboxyl groups, i.e., there are few free charges to interact with the CHI. At basic pH,



Figure 3. Schematic representation of the formation of the PEC from the ionic polymers (a), and proposed mechanism for formation of the PEC based on CMC and CHI (b).

Table 1. Formation of the polyelectrolyte complex (PEC) according to the variation of the reaction conditions and integrity of each sample after the swellir	ıg
test (concentration of the solutions of CMC and CHI: 1.5% m/v)	

Valumatria ratia (CMC+CIII)	Order of adding the	e L La	Ecomotion of DEC	Integrity of PEC after	
volumetric ratio (CMC:CHI)	polysaccharide solutions	рн	Formation of PEC	swelling	
2:1			yes	sample disintegrated	
1:1	CHI added to CMC		yes	sample disintegrated	
1:2		4	yes	sample intact	
2:1		4	yes	sample disintegrated	
1:1	CMC added to CHI		yes	sample disintegrated	
1:2			yes	sample intact	
2:1			yes	sample disintegrated	
1:1	CHI added to CMC		yes	sample disintegrated	
1:2		5	yes	sample disintegrated	
2:1		5	yes	sample disintegrated	
1:1	CMC added to CHI		yes	sample disintegrated	
1:2			yes	sample disintegrated	
2:1			yes	sample disintegrated	
1:1	CHI added to CMC		yes	sample intact	
1:2		6	yes	sample intact	
2:1		6		sample intact	
1:1	CMC added to CHI		yes	sample intact	
1:2			yes	sample disintegrated	
2:1			yes	sample intact	
1:1	CHI added to CMC		yes	sample intact	
1:2		7	yes	sample intact	
2:1		7	yes	sample intact	
1:1	CMC added to CHI		yes	sample intact	
1:2			yes	sample intact	
2:1			yes	sample disintegrated	
1:1	CHI added to CMC		yes	sample intact	
1:2		0	yes	sample intact	
2:1		0	yes	sample disintegrated	
1:1	CMC added to CHI		yes	sample intact	
1:2			yes	sample intact	
2:1			no	_	
1:1	CHI added to CMC		no	_	
1:2		0	no	_	
2:1		7	no	-	
1:1	CMC added to CHI		no	-	
1:2			no	_	

<sup>a</sup>pH of the medium for preparing the PEC. CHI: chitosan; CMC: carboxymethylcellulose.

CHI is soluble only at pH up to 6.5-7.0; while CMC has ionized carboxyl groups.

As shown in Table 1, only when adjusting the pH of the system to 9 was it impossible to form PEC. When we mixed the polymers in the same beaker and homogenized the system, the polymer chains of both polysaccharides began to interact with each other, thus enabling PEC formation. At that moment, the pH values of the medium based on the mixture were 3.30 (CMC:CHI = 1:2), 3.86 (CMC:CHI = 2:1) and 3.55 (CMC:CHI = 1:1).

The adjustment of the pH results in the ionization of groups that, at the moment of mixing the polymer solutions, are not ionized. This fact influences the formation and stability of the PEC. The contrary can also happen, i.e., since the two polysaccharides have different molar mass values, parts of the polymer chain can exist that are not interacting with the other polymer, but that contain ionized groups, which can become neutral after correcting the pH.

Since the average viscometric molar mass of the CHI sample  $(1.1 \times 10^5)$  was greater than that of the CMC  $(4.8 \times 10^3)$ ,<sup>33</sup> at pH of 9, the carboxyl groups of the CMC had a high ionization degree. Because the sample had high substitution degree (SD = 2.23), stretching and repulsion occurred between the chains of the CMC due to the excess of negative charge. In turn, CHI, which initially managed to interact with the CMC, precipitated in the medium due to the strong basicity. Thus, it was not possible to achieve hydrogel formation at pH 9.

To confirm the presence of both polysaccharides in the samples, we performed FTIR analyses. The spectra had the main bands characteristic of CMC and CHI. Shifts of the band in the region of 3000 cm<sup>-1</sup> and of the band located at 1430 cm<sup>-1</sup> confirmed the occurrence of an interaction between the cationic groups of chitosan and the anionic groups of carboxymethylcellulose.<sup>47</sup> SI section (Figure S4 and Table S3) presents the spectra of the two PEC samples and a description of the absorption bands. It is not necessary to present the spectra of all samples since they had the same profile.

## Swelling kinetics of the polyelectrolyte complexes in water

Although we obtained PECs in a wide range of pH values, in many cases, the degree of interaction between the polymer chains was not sufficient to keep the sample intact (stable) when submitted to the swelling test (Table 1). This behavior can probably be attributed to the imbalance of the ionic groups in the two polysaccharides, thus preventing the formation of a stable material due to the absence of sufficiently strong ionic interactions. Here we defined a stable PEC as one that did not disintegrate after the swelling

test, due to the formation of sufficient ionic interactions to keep the sample stable.

The swelling process involves the absorption of water molecules on the surface of the lyophilized PEC, which penetrate inside the polymer network, initially hydrating the hydrophilic and polar groups, resulting in expansion of the material. Subsequently, the other molecules are absorbed by the PEC structure due to the osmotic force.<sup>48-50</sup>

Figure 4 shows two different samples, before and after the swelling test. In Figure 4a it is possible to observe that the sample with CMC:CHI = 1:1 at pH = 4 disintegrated after 1 h in the test, while Figure 4b shows that the sample with CMC:CHI = 1:1 at pH = 8 remained intact until the end of the swelling time (24 h).



**Figure 4.** Behavior of the PEC samples during the swelling test: (a) CMC:CHI = 1:1 at pH = 4; (b) CMC:CHI 1:1 at pH = 8.

The order of adding the polysaccharide solutions did not affect the integrity of the samples, because the swelling test results were similar in relation to this variable (Table 1). Therefore, the order of adding the solutions was standardized for the rest of the study as adding the CMC solution to the CHI solution (CMC:CHI).

Irrespective of the conditions utilized in preparing the polyelectrolyte complex, the samples that remained intact had SwD greater than 100%, and in the majority of these cases, the swelling was greater than 1000% (Table 2), so all of these hydrogel samples can be classified as superabsorbent materials.<sup>51</sup>

Table 2 and Figure S5 (SI section) show that the samples' SwD tended to decline with increasing pH. pH can influence the composition and stability of the PEC. In solutions with high acidity (polyacid) or basicity (polybase), the ionic state of the polyelectrolyte changes so that the net charge of a complex polyelectrolyte approaches zero. Under those conditions, the electrostatic forces responsible for the complexation cease to exist, so the complex can disintegrate into the individual polyelectrolytes.<sup>14</sup>

It is known that increasing the pH of the medium used to prepare a polyelectrolyte complex causes a reduction in the availability of protonated amine groups in chitosan, while the ionization of carboxyl groups of the CMC increases. Thus, when using the CMC:CHI volumetric ratio of 1:2, there is greater availability of  $NH_3^+$  ions, since there are more chitosan chains in the medium, so the formation of electrostatic interactions between the polymer chains also increases, resulting in a lower swelling degree of the PEC sample. The sample prepared at pH 8, using CMC:CHI 1:2, showed a maximum degree of swelling equal to  $441 \pm 184\%$  ( $8 \pm 2$  g), which was the lowest SwD value of all samples.

Table 2 shows the influence of varying the volumetric ratio of the polysaccharides on the SwD of the PEC (Table 2). It can be seen that irrespective of the pH of the medium, the highest swelling values were obtained for the samples prepared with CMC:CHI ratio = 1:1. Possible explanations for this result are a lower crosslinking degree between the polymer chains and/or the morphology of the samples, which might have regions with large voids in which water molecules are retained. Among these samples, the one with the highest SwD value (7628 ± 967% or 77 ± 10 g g<sup>-1</sup>) was the sample prepared at pH 6.

Both polysaccharides presented high substitution degrees, so a large number of ionizable groups were present, facilitating the occurrence of interactions between the respective polymer chains. Those interactions depend on the pH of the medium, since the pH value defines the quantity of ionized groups in each polymer, even though the CHI had higher molar mass than the CMC.<sup>17</sup>

With respect to the CMC:CHI volumetric ratio of 2:1, independent of the pH of the medium, the SwD values were lower (Table 2), indicating that at this volumetric ratio, the PEC might have been more strongly crosslinked. The highest swelling degree was obtained with the sample prepared in pH 6 (SwD =  $5085 \pm 454\%$  or  $52 \pm 5$  g).

When mixing the two polysaccharide solutions, irrespective of the volumetric ratio used, the pH of the medium was equal to 4. At this pH, the CMC was at its isoelectric point (Figure 2) and the CHI had ionized amino groups ( $NH_3^+$ ). Therefore, in the acid medium, the chitosan chains tended to spread apart due to the electrostatic repulsion caused by the protonated groups, in turn causing the chains to be more extended.<sup>52</sup>

The slow addition of the solution of NaOH 1 mol  $L^{-1}$ to adjust the pH resulted in the formation of carboxyl groups in the medium (COO<sup>-</sup>), originating from the CMC. In counterpart, there was a reduction of the free ionized amino groups. It has been reported in the literature<sup>32,33</sup> that the polymer chains of CHI (Mv =  $1.1 \times 10^5$  g mol<sup>-1</sup>) are longer than those of CMC (Mv =  $4.8 \times 10^3$  g mol<sup>-1</sup>), so the availability of amino groups tends to be higher than that of the carboxyl group. Therefore, it is possible to understand the influence of the larger quantity of CMC in the medium, favoring increased availability of carboxyl groups to interact electrostatically with the protonated groups (NH<sub>3</sub><sup>+</sup>) of CHI, resulting in greater crosslinking and thus lower swelling degree. Therefore, we believe that the gradual increase of the basicity of the medium, forming more carboxyl groups in the CMC's structure, led to a higher degree of crosslinking between the polymer chains, despite the reduction in the number of the protonated amino groups, i.e., the samples prepared with CMC:CHI volumetric ratio of 2:1 contained a smaller number of polymer chains of CMC than those prepared with ratio of 1:1 or 1:2 (CMC:CHI). Therefore, in the system with CMC:CHI volumetric ratio of 2:1, there was a greater quantity of -COO- groups, allowing the occurrence of an electrostatic interaction with the -NH<sub>3</sub><sup>+</sup> groups of chitosan, hence diminishing the swelling of the PEC.

Analysis of the samples with volumetric ratio of 1:1 in function of the difference between the size of the polymer chains, and thus the difference in the number of ionic groups in the two polysaccharides, indicated the probable existence

**Table 2.** Maximum compressive stress ( $\sigma_{max}$ ), swelling degree (SwD), morphological and thermal parameters of the PECs

	рН <sup>ь</sup>	$\sigma_{max}/MPa$	SwD / %	SwD <sup>c</sup> / (g g <sup>-1</sup> )	Morphological parameters Voids		- Thermal parameters <sup>d</sup>	
CMC:CHI <sup>a</sup>								
					Average size / mm	Quantity	T <sub>onset</sub> <sup>e</sup> / °C	T <sub>max</sub> <sup>f</sup> / °C
	6	$0.046 \pm 0.003$	$7628 \pm 967$	$77 \pm 10$	$2.09 \pm 0.8$	6000	258	290
1:1	7	$0.022 \pm 0.002$	$5061 \pm 50$	$52 \pm 1$	$2.06 \pm 0.3$	6200	270	295
	8	$0.060 \pm 0.010$	$1642 \pm 289$	$17 \pm 3$	$1.81\pm0.9$	14000	256	294
2:1	6	$0.008 \pm 0.001$	$5085 \pm 454$	$52 \pm 5$	$2.20 \pm 0.6$	10000	252	301
	7	$0.113 \pm 0.006$	$972 \pm 355$	$11 \pm 4$	$1.80 \pm 0.7$	7000	251	291
1:2	4	$0.073 \pm 0.010$	$3009 \pm 314$	31 ± 3	$2.40\pm0.4$	6000	256	288
	7	$0.055 \pm 0.006$	$2211 \pm 575$	$23 \pm 6$	$1.50\pm0.8$	34000	237	268
	8	$0.198 \pm 0.013$	$441 \pm 184$	$8 \pm 2$	$0.63 \pm 0.2$	3200	255	285

<sup>a</sup>Volumetric ratio of the polysaccharide solutions; <sup>b</sup>pH of medium for preparing the PEC; <sup>c</sup>SwD: swollen sample mass/dry sample mass (0.3 g); <sup>d</sup>equipment error =  $\pm 4$  <sup>o</sup>C; <sup>e</sup>temperature of start of degradation; <sup>f</sup>temperature of fastest degradation; CHI: chitosan; CMC: carboxymethylcellulose.

of a certain quantity of free amino groups in the CHI, i.e., without participation in the ionic crosslinking. This can cause an electrostatic repulsion between the groups, thus originating chitosan chain segments that are longer and more spread apart. In these chain segments, greater interaction can occur between the chitosan and the water molecules by means of hydrogen bonds, thus promoting a higher swelling degree.<sup>53</sup>

#### Compressive strength

The compressive strength test was performed only on the samples that remained intact after the swelling test. The values of maximum compressive stress ( $\sigma_{max}$ ) of the PECs are listed in Table 2.

According to the literature,<sup>34,53,54</sup> it is possible to obtain improved mechanical properties of polyelectrolyte complexes by increasing the availability of ionized polymer chains, which will form a network, resulting in higher compressive strength. Further evaluation of the data presented in the literature<sup>36,55</sup> indicated that satisfactory mechanical resistance of the material should be greater than 0.01 MPa. Thus, the sample prepared with CMC:CHI ratio of 2:1 and pH = 6 was not submitted to the next characterization step, since it had compressive strength of  $0.008 \pm 0.001$  MPa. This decision was based on the fact that because the polyelectrolyte complexes were superabsorbent, they had potential for application as soil conditioners (to increase water retention), thus helping to combat an important environmental problem caused by the use of large amounts of water to irrigate crops. The use of a superabsorbent PEC will make the soil near the roots moist for a longer period, enabling reducing the frequency of irrigation and the amount of water applied.

Figure 5 contains the stress-strain curves obtained in this study, indicating a tendency for concordance between the compressive strength and swelling degree results (Table 2), i.e., higher crosslinking between the CMC and CHI chains was associated with greater mechanical strength, and consequently with lower SwD. The sample prepared with CMC:CHI ratio of 1:2 and pH = 8 presented the highest compressive strength value  $(0.198 \pm 0.013 \text{ MPa})$ . Besides this, the deformation of the PEC was elastic, because the material returned to its initial conformation when the force was removed. This occurred because when a force is applied to compress a PEC, the polymer chains are rearranged to accommodate the deformation.<sup>56</sup> That characteristic is important due to the intended use of the sample prepared in this study (soil conditioner). After application, the soil will exert stress on the PEC, and it is desirable for the material to remain intact to assure greater durability.



Figure 5. Curves of maximum compression ( $\sigma_{max}$ ) versus deformation ( $\epsilon$ ) of the PECs.

#### Characterization of morphology

The morphology of the PEC was determined by SEM. Figure 6 presents the micrographs of the samples with magnification of 100×, along with the graphs referring to the static treatment, obtained by the ImageJ software,<sup>38</sup> presenting the relation between the size of the voids *versus* their distribution. We decided to use low magnification because we wanted to analyze a larger surface area of each sample. Table 2 reports the size and distribution of the voids in each PEC sample. Therefore, the analysis of the morphology was based on statistical analysis along with visual observation.

It is known that an acid pH value favors the protonation of the amino groups (NH<sub>3</sub><sup>+</sup>) of CHI, while the carboxyl groups of CMC become less anionic. Based on this, the pH range where the best crosslinking between the polysaccharides should theoretically occur is between the  $pK_a$  values of the polymers, i.e., between 5 and 7. Thus, we expected an increase of pH to be associated with a reduction of the voids in the samples, due to the greater electrostatic interaction between the polymer chains. That assumption was in line with the results of the swelling degree and compressive strength tests, where higher pH tended to diminish the SwD value, and hence increased the mechanical resistance. However, other parameters cannot be disregarded, such as the substitution degree of CMC (2.23, considered to be a high value) and the deacetylation degree of CHI (92%), since these can also significantly influence the morphology of the PEC. Therefore, we analyzed parameters such as a structure with relatively uniform distribution of voids and a greater surface contact area, to enable better interaction with the water molecules during swelling.

At the moment of mixing the polymer solutions, the pH was near 4, i.e., the CHI chains were protonated  $(NH_{3}^{+})$ 



**Figure 6.** Influence of pH of the medium used to prepare the PEC on the morphology of the samples: (a) CMC:CHI = 2:1 and pH = 6; (b) CMC:CHI = 2:1 and pH = 7; (c) CMC:CHI = 1:1 and pH = 6; (d) CMC:CHI = 1:1 and pH = 7; (e) CMC:CHI = 1:1 and pH = 8; (f) CMC:CHI = 1:2 and pH = 4; (g) CMC:CHI = 1:2 and pH = 7; (h) CMC:CHI = 1:2 and pH = 8.

and the CMC chains were neutral (–COOH). As NaOH was added to raise the pH to 8, the CMC chains were ionized by means of the COO<sup>-</sup> groups, thus allowing ionic crosslinking with the  $NH_3^+$  group.

In turn, the CHI chains are longer than those of the CMC, so some segments of the CHI chains had no counterpart CMC chains with which to interact. As the pH became basic, the quantity of protonated groups of CHI decreased, becoming neutral, permitting the occurrence of associations of the chains, reducing the number of voids caused by the electrostatic repulsion when they were protonated. Those associations occurred due to the presence of new intermolecular interactions by means of hydrogen bonds, reducing the SwD and increasing the mechanical resistance of the material.

Based on these observations, the sample prepared with

CMC:CHI volumetric ratio = 1:2 at pH 8 continued to be the best (Figure 6h and Table 2).

#### Thermal stability

Table 2 reports the values of  $T_{onset}$  and  $T_{max}$  for the PEC that did not disintegrate after the swelling test. There was a reduction of the  $T_{onset}$  values of the materials in relation to the pure polysaccharides (CMC = 282 °C and CHI = 288 °C). That fact can be explained by the formation of new interactions between the PEC structures, whether hydrogens bonds and/or electrostatic interactions between the COO<sup>-</sup> and NH<sub>3</sub><sup>+</sup> groups present in the structures of CMC and CHI, respectively.<sup>13,57-59</sup> These new attraction forces need greater energy to overcome in comparison with the covalent bonds existing in the polymers' structures. This

can explain the reduction of the  $T_{onset}$  values of the PEC samples prepared in this study.

Figure 7 presents the TG and DTG curves of two hydrogel samples. The choice of these two samples was based on the intention to apply the PEC as a soil conditioner, and also by the previous experiments. According to the swelling test, the sample with parameters CMC:CHI = 1:1/pH = 6 presented the highest SwD (7628 ± 967%), and according to the compressive strength test, sample CMC:CHI = 1:2 and pH = 8 had the highest  $\sigma_{max}$  value (0.198 ± 0.013 MPa). The profile of the curves shows that sample CMC:CHI= 1:2 and pH = 8 had the greatest thermal stability.

The analysis of the results obtained indicated that the best sample was that prepared with CMC:CHI ratio of 1:2, pH 8, and order of adding solutions of CMC (1.5% m/v) before CHI (1.5% m/v). Based on this conclusion and the fact that the CHI presented high deacetylation degree (92%) and greater average viscometric molar mass (Mv =  $1.1 \times 10^5$ ) than



Figure 7. TG and DTG curves of the PEC prepared with the conditions CMC:CHI = 1:1 and pH = 6, and CMC:CHI = 1:2 and pH = 8.

the CMC (Mv =  $4.8 \times 10^3$ ), which presented high substitution degree (2.23%), we propose a representation of the interactions that formed the PEC in this sample (Figure 8).



Figure 8. Proposed mechanism of interaction between the polysaccharides in the formation of the PEC at pH = 8 and CMC:CHI ratio = 1:2. CMC solution (1.5% m/v); CHI solution (1.5% m/v); Mv<sub>CHI</sub> =  $1.1 \times 10^5$ ; Mv<sub>CMC</sub> =  $4.8 \times 10^3$ ).

# Conclusions

It was possible to obtain superabsorbent PECs with good compressive strength through the formation of a polyelectrolyte complex between carboxymethylcellulose with high substitution degree (SD = 2.23) and chitosan with high deacetylation degree (DD = 92%), at basic pH, without the addition of a crosslinking agent, having potential application as a soil conditioner. We verified a significant variation in the morphology of the samples with respect to the size and number of voids when varying the reaction conditions. The compressive strength test indicated that the polyelectrolyte complex sample with the highest quantity of chitosan and prepared at pH 8 had the best crosslinking and thus the greatest mechanical resistance. The thermal analysis indicated a reduction of the value of T<sub>onset</sub> of the PECs in relation to the pure polysaccharides, due to the formation of new interactions in the PEC, which required less energy to overcome in comparison with the covalent bonds in the structures of the polysaccharides. The best experimental conditions were CMC:CHI ratio = 1:2 and pH 8 because that sample presented the highest compressive strength among the PECs produced ( $0.198 \pm 0.013$  MPa), as well as the greatest thermal stability, and swelling degree of  $441 \pm 184\%$ .

# **Supplementary Information**

Supplementary information (FTIR spectra of polysaccharides and PECs, <sup>1</sup>H NMR spectrum of CMC, thermograms of pure polysaccharides) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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## References

- Anitha, A.; Sowmya S.; Kumar, P. T. S.; Deepthi, S.; Chennazhi, K. P.; Ehrlich, H.; Tsurkan, M.; Jayakumar, R.; *Prog. Polym. Sci.* 2014, *39*, 1644. [Crossref]
- Prashanth, K. V. H.; Tharanathan, R. N.; *Trends Food Sci. Technol.* 2007, 18, 117. [Crossref]
- Gull, N.; Khan, S. M.; Khalid, S.; Zia, S.; Islam, A.; Sabir, A.; Sultan, M.; Hussain, F.; Khan, R. U.; Butt, M. T. Z.; *Int. J. Biol. Macromol.* 2020, *164*, 4370. [Crossref]

- Gull, N.; Khan, S. M.; Butt, O. M.; Islam, A.; Shah, A.; Jabeen, S.; Khan, S. U.; Khan, A.; Khan, R. U.; Butt, M. T. Z.; *Int. J. Biol. Macromol.* 2020, *162*, 175. [Crossref]
- Borges, R.; Wypych, F.; J. Braz. Chem. Soc. 2019, 30, 326. [Crossref]
- Frachinia, E. C. G.; Petri, D. F. S.; J. Braz. Chem. Soc. 2019, 30, 2010. [Crossref]
- Gonzaga, M. L. C.; Campelo, M. S.; Saraiva, K. B.; Santos, A. Q.
   S.; Leal, L. K. A. M.; Ricardo, N. M. P. S.; Soares, S. A.; Ribeiro,
   M. E. N. P.; *J. Braz. Chem. Soc.* **2020**, *31*, 990. [Crossref]
- Vásconez, M. B.; Flores, S. K.; Campos, C. A.; Alvarado, J.; Gerschenson, L. N.; *Food Res. Int.* 2009, 42, 762. [Crossref]
- Coimbra, P.; Ferreira, P.; de Sousa, H. C.; Batista, P.; Rodrigues, M. A.; Correia, I. J.; Gil, M. H.; *Int. J. Biol. Macromol.* 2011, 48, 112. [Crossref]
- Yoshida, C. M. P.; Bastos, C. E. N.; Franco, T. T.; *Food Sci. Technol.* 2010, 43, 584. [Crossref]
- Sainitya, R.; Sriram, M.; Kalyanaraman, V.; Dhivya, S.; Saravanan, S.; Vairamani, M.; Sastry, T. P.; Selvamurugan, N.; *Int. J. Macromol.* 2015, *80*, 481. [Crossref]
- Sannino, A.; Demetri, C.; Madaghiele, M.; *Materials* 2009, 2, 353. [Crossref]
- Costa, M. P. M.; Prates, L. M.; Baptista, L.; Cruz, M. T. M.; Ferreira, I. L. M.; *Carbohydr. Polym.* **2018**, *198*, 51. [Crossref]
- Das, B.; Tsianou, M.; Adv. Colloid Interface Sci. 2017, 244, 71. [Crossref]
- Folchman-Wagner, Z.; Zaro, J.; Shen, W.-C.; *Molecules* 2017, 22, 1089. [Crossref]
- Liu, X.; Tang, C.; Han, W.; Xuan, H.; Ren, J.; Zhang, J.; Ge, L.; *Colloids Surf.*, A 2017, 529, 1016. [Crossref]
- Insua, I.; Wilkinson, A.; Fernandez-Trillo, F.; *Eur. Polym. J.* 2016, *81*, 198. [Crossref]
- Rosca, C.; Popa, M. I.; Lisa, G.; Chitanu, G. C.; *Carbohydr. Polym.* 2005, 62, 35. [Crossref]
- Altam, A. A.; Zhu, L.; Huang, W.; Huang, H.; Yang, S.; Carbohydr. Polym. Technol. Appl. 2021, 2, 100100. [Crossref]
- Zhao, J.; Xing, T.; Li, Q.; Chen, Y.; Yao, W.; Jin, S.; Chen, S.; J. Appl. Polym. Sci. 2020, 137, e48980. [Crossref]
- Shang, J.; Shao, Z.; Chen, X.; *Biomacromolecules* 2008, 9, 1208. [Crossref]
- Chen, P.; Xie, F.; Tang, F.; Mcnally, T.; *Compos. Sci. Technol.* 2020, 189, 108031. [Crossref]
- Ferreira, D. C. M.; Ferreira, S. O.; Alvarenga, E. S.; Soares, N. F. F.; Coimbra, J. S. R.; Oliveira, E. B.; *Carbohydr. Polym. Technol. Appl.* 2022, *3*, 100197. [Crossref]
- Sabadini, R. C.; Fernandes, M.; de Zea Bermudez, V.; Pawlicka, A.; Silva, M. M.; *J. Appl. Polym. Sci.* 2022, *139*, e52998. [Crossref]
- Martino, A. D.; Khan, Y. A.; Durpekova, S.; Sedlarik, V.; Elich,
   O.; Cechmankova, J.; *J. Cleaner Prod.* 2021, 285, 124848.
   [Crossref]

- Iftime, M. M.; Ailiesei, G. L.; Ungureanu, E.; Marin, L.; *Carbohydr. Polym.* 2019, 223, 115040. [Crossref]
- Ghobashy, M. M.; El-Damhougy, B. K.; El-Wahab, H. A.; Madani, M.; Amin, M. A.; Naser, A. E. M.; Abdelhai, F.; Nady, N.; Meganid, A. S.; Alkhursani, S. A.; Alshangiti, D. M.; *Polym. Adv. Technol.* 2021, *32*, 514. [Crossref]
- Bauli, C. R.; Lima, G. F.; de Souza, A. G.; Ferreira, R. R.; Rosa, D. S.; *Colloids Surf.*, A 2021, 623, 126771. [Crossref]
- Campana-Filho, S. P.; de Britto, D.; *Quim. Nova* 2009, *32*, 1461. [Crossref]
- Kono, H.; Oshima, K.; Hashimoto, H.; Shimizu, Y.; Tajima, K.; Carbohydr. Polym. 2016, 150, 241. [Crossref]
- Tian, F.; Liu, Y.; Hu, K.; Zhao, B.; *Carbohydr. Polym.* 2004, 57, 31. [Crossref]
- da Costa, M. P. M.; Delpech, M. C.; Ferreira, I. L. M.; Cruz, M. T. M.; Castanharo, J. A.; Cruz, M. D.; *Polym. Test.* 2017, 63, 427. [Crossref]
- da Silva, G. M.; da Rocha, R. F. P.; da Costa, M. P. M.; Ferreira, I. L. M.; Delpech, M. C.; *J. Mol. Liq.* 2018, 268, 201. [Crossref]
- Maciel, D. J.; Ferreira, I. L. M.; *Mater. Lett.* 2018, 229, 142. [Crossref]
- da Costa, M. P. M.; Ferreira, I. L. M.; Cruz, M. T. M.; *Carbohydr. Polym.* 2016, *146*, 123. [Crossref]
- da Costa, M. P. M.; Rebelo, K.; Ferreira, I. L. M.; Cruz, M. T. M.; *J. Appl. Polym. Sci.* 2022, *139*, 51776. [Crossref]
- 37. ASTM D 1621-00: Standard Test Method for Compressive Properties of Rigid Cellular Plastics, Philadelphia, 2017.
- Rasband, W. S.; *ImageJ*, version; U. S. National Institutes of Health, Bethesda, Maryland, USA, 2015.
- Silverstein, R. M.; Webster, F. X.; Kiemle, D.; Spectrometric Identification of Organic Compounds, 7<sup>th</sup> ed.; John Wiley & Sons: New York, USA, 2005.
- Ho, F. F. L.; Kloslewlcz, D. W.; Anal. Chem. 1980, 52, 913. [Crossref]
- Caraschi, J. C.; Campana-Filho, S. P.; *Polímeros* 1999, 9, 70. [Crossref]
- Singh, P.; Medronho, B.; Alves, L.; da Silva, G. J.; Miguel, M. G.; Lindman, B.; *Carbohydr. Polym.* 2017, *175*, 87. [Crossref]
- Elgadir, M. A.; Uddin, M. S.; Ferdosh, S.; Adam, A.; Chowdhury, A. J. K.; Sarker, M. Z. I.; *J. Food Drug Anal.* 2015, 23, 619. [Crossref]

- BeMiller, J. N.; *Carbohydrate Chemistry for Food Scientists*, 3<sup>rd</sup> ed.; Elsevier: London, UK, 2018.
- Garcia, J. A. F.; de Moura, M. R.; Aouada, F. A.; *Quim. Nova* 2019, 42, 831. [Crossref]
- Sorlier, P.; Denuzière, A.; Viton, C.; Domard, A.; Biomacromolecules 2001, 2, 765. [Crossref]
- Ostrowska-Czubenko, J.; Gierszewska-Druzynska, M.; Carbohydr. Polym. 2009, 77, 590. [Crossref]
- Ullah, F.; Othman, M. B. H.; Javed, F.; Ahmad, Z.; Akil, H. M.; Mater. Sci. Eng. C 2015, 57, 414. [Crossref]
- Hoffman, A. S.; Adv. Drug Delivery Rev. 2002, 64, 18. [Crossref]
- Froix, M. F.; Nelson, R.; *Macromolecules* 1975, 8, 726. [Crossref]
- Guilherme, M. R.; Aouada, F. A.; Fajardo, A. R.; Martins, A. F.; Paulino, A. T.; Davi, M. F.; Rubira, T. A. F.; Muniz, E. C.; *Eur. Polym. J.* 2015, 72, 365. [Crossref]
- Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Gurny, R.; *Eur. J. Pharm. Biopharm.* 2004, *57*, 35. [Crossref]
- Davydova, V. N.; Yermak, I. M.; *Biophysics* 2018, 63, 501. [Crossref]
- 54. Ahmed, E. M.; J. Adv. Res. 2015, 6, 105. [Crossref]
- Campese, G. M.; Tambourgi, E. B.; Guilherme, M. R.; de Moura, M. R.; Muniz, E. C.; Youssef, E.; *Quim. Nova* 2007, *30*, 1649. [Crossref]
- Callister, W. D.; *Materials Science and Engineering: An Introduction*, 5<sup>th</sup> ed.; John Wiley & Sons: New York, USA, 1999.
- Fajardo, A. R.; Lopes, L. C.; Pereira, A. G. B.; Rubira, A. F.; Muniz, E. C.; *Carbohydr. Polym.* **2012**, *87*, 1950. [Crossref]
- Hartig, S. M.; Greene, R. R.; Dasgupta, J.; Carlesso, G.; Dikov, M. M.; Prokop, A.; Davidson, J. M.; *Pharm. Res.* 2007, 25, 2353. [Crossref]
- Kulkarni, A. D.; Vanjari, Y. H.; Sancheti, K. H.; Patel, H. M.; Belgamwar, V. S.; Surana, S. J.; Pardeshi, C. V.; *Artif. Cells, Nanomed., Biotechnol.* 2016, 44, 1615. [Crossref]

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