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RHEOLOGICAL BEHAVIOR OF A CHITOSAN/XANTHAN GUM HYDROGEL

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Abstract. Chitosan, a cationic polymer from crustacean shells, has unique properties that have made it the subject of many studies when it comes to developing biopolymers based products. When in solution or in association (as a hydrogel) with polyanionic polymers, such as xanthan gum, chitosan becomes a versatile biopolymer having characteristics such as biodegradability, biocompatibility, bioadhesiveness, mechanical strength and gel capacity, which makes it attractive in a wide variety of applications. Hydrogel formed by polyelectrolyte complexation between chitosan and xanthan gum was studied. The ideal pH for the formation of this hydrogel was defined by the zeta potential data. The behavior of the solutions polysaccharides and the hydrogel were analyzed by oscillatory rheological measurements. Rheological data confirmed the viscoelastic characteristics of these hydrogels, with G' being always greater than G'' in the frequency range studied. Chitosan presented, at low frequencies, a liquid-like behavior while xanthan solution presented a gel-like behavior. In creep and recovery analysis, chitosan/xanthan hydrogel recovered in 49%.

Keywords: Rheology, Hydrogel, Polysaccharide, Polyelectrolyte, Xanthan, Chitosan

1. INTRODUCTION

Hydrogels are usually formed by the covalent cross-linking of linear hydrophilic polymers to form a network of material capable of absorbing water, yet still remaining insoluble (Alistair et.al., 2006). In recent years, much attention has been focused on biopolymer gels because of their biocompatibility and biological functions, and consequently their potential applications in the biomedical and pharmaceutical fields. When it comes to forming hydrogels with biopolymers for pharmaceutical and biomedical applications, chitosan has been highlighted.

Chitosan is a cationic biopolymer derived from crustacean's shell, with properties that make it object of several studies related to development of biopolymers products (Jennings and Bumgardner, 2016). When associated with anionic biopolymers, like xanthan gum, a hydrogel is formed, by polyelectrolyte complexation, with unique physical and chemical properties (Huang et.al., 2015). The process of polyelectrolyte complexation between chitosan and xanthan consists on the ionic interactions between the amino groups of chitosan and carboxyl groups of xanthan forming a hydrogel network.

Hydrogels are undoubtedly complex materials and their rheological characterization represents an important tool also to investigate their structural characteristics, even better if combined with other techniques, to individuate the conditions required for their existence and formation, to examine effects caused by changes in chemical and environmental parameters, as well as to evaluate their potential use and performance in various biomedical applications (Lapasin, 2016).

In this work, chitosan/xanthan hydrogel was formed in an ideal pH value established by the zeta potential measurement. Rheological behavior of chitosan solution, xanthan solution and chitosan/xanthan hydrogel were analyzed in a total solid content of 1.2% (m/v).

2. MATERIAL AND METHODS

2.1 Material

Chitosan, with medium molecular weight, and xanthan gum, produced from the bacteria *Xanthomonas Campestris*, were supplied by Sigma Aldrich (St Louis, EUA). Analytical-grade hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used for chitosan solubilisation and chitosan and xanthan neutralization.

2.2 Methods

2.2.1 Chitosan Deacetylation Degree

The deacetylation degree (DD) of chitosan was determined by UV spectrophotometry according to the method proposed by Liu et al. (2006).

2.2.2 Viscosimetry Molecular Weight

The molecular weight of chitosan and xanthan gum samples was determined using the Mark-Houwink-Sakurada (MHS) equation, as described in Eq. (1) (Masuelli, 2014).

$$[\eta] = k (\bar{M}_v)^\alpha \quad (1)$$

$[\eta]$ and \bar{M}_v are the intrinsic viscosity and viscosity molecular weight, respectively, while k and α are constants for given solute-solvent system and temperature. Six different concentrations solutions of chitosan (0.1-1 %, w/v) in 0.1 M acetic acid-0.2 M NaCl and six different concentrations solutions of xanthan gum (0,01 – 0,125%, w/v) in 0.1 M NaCl were prepared. The chitosan solution was filtered to remove insoluble materials. The Cannon-Fenske capillary viscometer was used to measure the flow time of the solutions through the capillary in a constant temperature bath at 25°C. Three measurements were made on each sample. The running times of the solution and solvent were recorded as seconds (s) and used to calculate intrinsic viscosity $[\eta]$. The literature values of k and α for chitosan are 2.10×10^{-5} and 1.32 (Kassai, 2007), respectively, and 2.78×10^{-5} and 1.28 for xanthan (Masuelli, 2014).

2.2.3 Zeta Potential (ζ)

The ζ -potential analyses was performed with a Zetasizer Nano (Nano-ZS, Malvern Instruments, UK). Samples (10 mL) were placed in a titler (MPT-2, Malvern Instruments, UK) containing 0.25 mol/L HCl, 0.25 mol/L NaOH, and 0.025 mol/L NaOH, and analyses were conducted as function of pH (from 2.0 to 7.5). Data of ζ -potential are average values for each pH measure, and the results are shown with the mean values and the standard deviation calculated ($p < 0.05$). The ideal pH for the hydrogel formation was established according to Weinbreck et al. (2004) methodology, as the product of the absolute value of ζ -potential of both macromolecules at each pH values.

2.2.4 Hydrogel Formation

Chitosan solutions were prepared by dissolving 3.0 g of chitosan in 150 mL of 0.1 M HCl. The solution was then neutralized with 0.1 M NaOH and distilled water was added for a total volume of 250 mL at pH 4.05, forming a solution with 1,2% (w/v). To obtain good solutions, the chitosan powder was dispersed by agitation with a magnetic stirrer until total solubilisation. Xanthan gum solution was prepared by adding 3 g of xanthan to 200 mL of distilled water and dispersion was done with a magnetic stirrer until total solubilisation. The solution was then neutralized with 0.1 M HCl and distilled water was added for a total volume of 250 mL at pH 4.05. Mixing 250 mL of the chitosan solution with 250 mL of the xanthan solution formed the chitosan-xanthan hydrogel (CX hydrogel). Agitation was conducted for 10 min at room temperature and the hydrogel formed was separated out by filtration following Ruvalcaba (2007) methodology.

2.2.5 Rheological Tests

The rheological tests were performed using a rotational rheometer (HAAKE-Mars III; Thermo Scientific, USA) to measure the steady, dynamic and transient properties of the solutions and the hydrogel. Flow measurements were carried out at a temperature of 25 °C. Parallel plate geometry was used: plate diameter = 35 mm, gap = 1 mm for the hydrogels and 0.5 mm for the solutions, and strain = 1 Pa. Strain sweep were carried out over strain between 0.01 and

100 Pa in three different frequencies (0.1, 1.0 and 3.0 Hz) in order to establish the linear viscoelastic region. Logarithmic frequency sweeps were carried out over frequencies between 0.01 and 100 s⁻¹ with a stress of 1 Pa. Temperature sweep was performed from 25°C to 85°C with the temperature rate increase of 5 °C/min. For creep and recovery analyses the stress was applied instantly and maintained for a period of 150 s, after removing stress, compliance was measured during 300 s. Data were visualized at HAAKE RheoWin[®] software (version 4.41.0000).

3. RESULTS AND DISCUSSION

3.1 Chitosan Deacetylation Degree

The chitosan deacetylation degree measured by UV spectrophotometry was found to be 82%. This value is in accordance with the information provided by Sigma Aldrich that states the DA is between 75-85%. A 82% of deacetylation means that 18% of the chitosan remain insoluble in acidic media, and this parameter determines most of the properties of chitosan, including solubility, extent of swelling in water, susceptibility to biodegradation, bioactivity, and biocompatibility, among others (Mendonza et. al., 2016).

3.2 Viscosimetry Molecular Weight

The flow times of the xanthan gum and chitosan solutions were measured and the relative and inherent viscosities calculated and used for the extrapolation of the $[\eta]$ values. The Table 1 summarizes the values of $[\eta]$ and molecular weight (M_v) of the samples.

Table 1: Experimental results for chitosan and xanthan intrinsic viscosity and molecular weight.

Polisaccharide	$[\eta]$ [cm ³ .g ⁻¹]	M_v [kDa]
Chitosan	965,5	652,5
Xanthan	1923,2	1403,7

Sangyong et. al (2014) classifies chitosan according to its molecular weight in three types: low molecular weight chitosan (less than 150 kDa), medium molecular weight chitosan (between 150 and 700 kDa) and high molecular weight chitosan (between 700 and 1000 kDa). For the chitosan used, the value is within the range of the average molecular weight, which confirms the information provided by the supplier (Sigma Aldrich).

The molecular weight of xanthan gum depends on the association between the chains, forming aggregates of several individual chains. The fermentation conditions are also a parameter that influences the molecular weight of the final product, which has been reported in the literature between 1000 and 20000 kDa (García-Ochoa et al, 2000).

3.3 Zeta Potential (ζ)

The ζ -potential variation with pH for the chitosan and xanthan gum dispersions are shown in Figure 1.

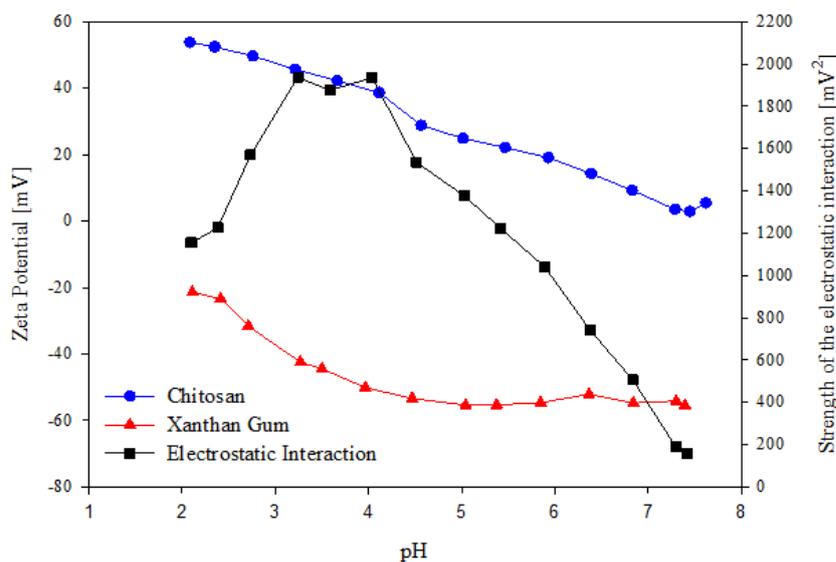


Figure 1: Zeta potential of chitosan (●) and xanthan (▲) dispersions and strength of the electrostatic interaction (■).

Chitosan dispersions displayed a weak charged polyelectrolyte behavior, exhibiting positive ζ -potential values at all pH values studied, with the positive charge decreasing considerably as pH reached a value of 7.5. This phenomenon is commonly attributed to the loss charge of the glucosamine segments (pKa 6.3–7.5) (Vaarum and Smidsrod, 2005). The zeta potential values of xanthan gum decreased from -21.0 mV to -55.0 mV by raising the pH from 2 to 7.5. The strength of the electrostatic interaction between oppositely charged polyelectrolytes was calculated as the product of the absolute value of ζ -potential of both macromolecules at each pH values. The highest point of the electrostatic interaction curve indicates the value 4.05 as being ideal for the formation of the xanthan gum /chitosan hydrogels. Therefore, solutions of chitosan and xanthan gum had their pHs adjusted to this value before being mixed for the formation of the hydrogels.

3.4 Rheological Tests

From the stress sweep analyses, 1 Pa was chosen as the stress, inside the linear viscoelastic region, for the subsequent analyses. The hydrogel behavior, as the chitosan and xanthan solutions behavior, during the frequency sweep are presented in Figure 2.

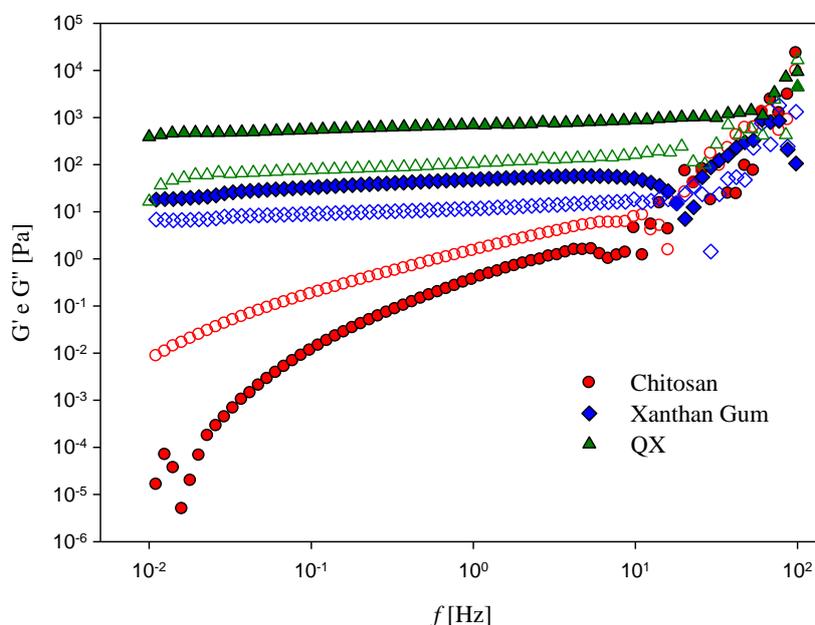


Figure 2: Behavior of chitosan, xanthan and CX hydrogel during frequency sweep. Closed symbols (\bullet , \blacklozenge , \blacktriangle) indicate G' and open symbols (\circ , \diamond , \triangle) indicate G'' .

Chitosan shows the behavior of a viscous liquid. Loss modulus (G'') is higher than storage modulus (G') and as f increases, G' increases more rapidly with respect to G'' , so a crossover of the G' and G'' curves can be detected. Both modulus are highly dependent of the frequency, typical behavior of a solution. Xanthan gum otherwise, presented a weak gel behavior. The storage modulus (G') is always greater than the loss modulus (G'') over almost the entire range of frequencies studied, meaning that the linear viscoelastic behavior of this xanthan gum solution is dominated by an elastic nature. In addition, both the storage and loss modulus are found to be only weak functions of frequency, indicating that a gel-like structure is present in these systems. From these results, it can be understood that concentrated xanthan gum solutions form a gel-like system, as indicated by a dominance of the elastic storage modulus over the viscous loss modulus (Picout and Ross-Murphy, 2003). Such a linear viscoelastic behavior may be attributed to the occurrence of attractive interactions between helices formed by rigid xanthan macromolecules, which can give rise to a weak three-dimensional network and a complex aggregation in solutions by means of physical entanglements and hydrogen bonding (Song et. al, 2006). The hydrogel also presented a gel-like behavior. G' is greater than G'' and both are weakly dependent of the frequency. Compared to the solutions, G' of CX hydrogel is more than ten times greater, showing that the elastic properties was improved by the electrostatic interaction between chitosan and xanthan gum that formed a highly interconnected gel-like network structure. Similar results were found for hydrogel formed by polyelectrolyte complexation between ovalbumin-chitosan (Xiong et. al., 2016), xyloglucan-chitosan (Simi and Abraham, 2010) and pectin-chitosan (Nordby et. al., 2003).

Figure 3 illustrates, in more details, the hydrogel behavior during the frequency sweep. G' is more than ten times greater than G'' for the most frequencies studied. The complex viscosity shows a slope close to -1, characteristic of a gel.

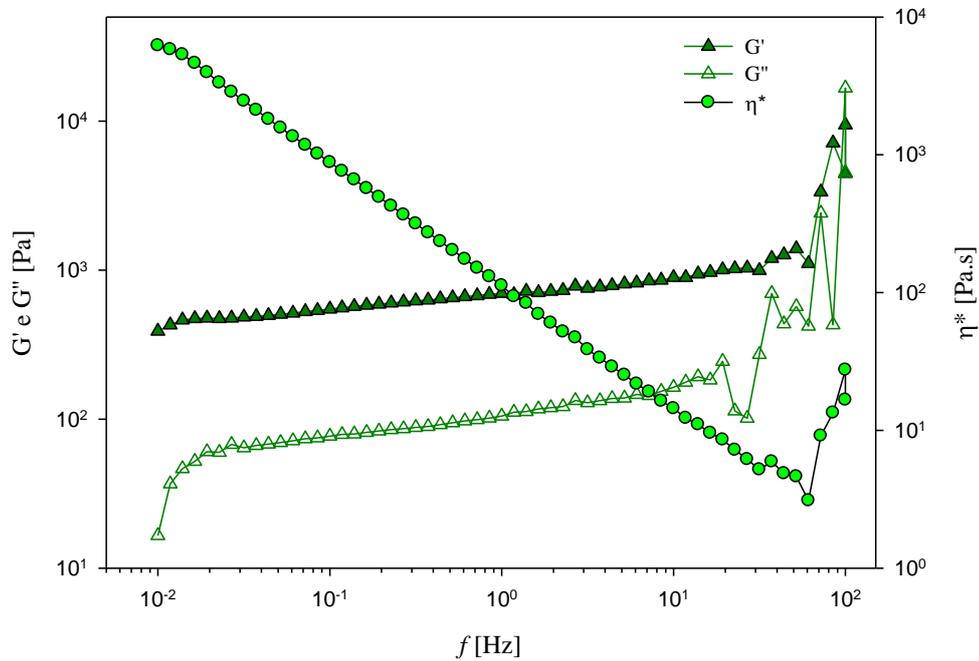


Figure 3: CX hydrogel behavior during frequency sweep.

Figure 4 illustrates the $\tan \delta$ values for both solutions and for the hydrogel. This parameter indicates the relation between G' and G'' . Values of $\tan \delta$ close to zero indicates that the elastic modulus is much higher than the loss modulus, values close to one indicates material in transition between solid and liquid behavior or vice-versa and, when $\tan \delta = \infty$ indicates a liquid-like behavior (Osswald and Rudolph, 2015). Chitosan solution behaved as a liquid-like solution, while xanthan and CX hydrogel presented $\tan \delta$ values smaller than 1, being $\tan \delta$ of the hydrogel almost twenty times minor than xanthan, indicating a more elastic behavior.

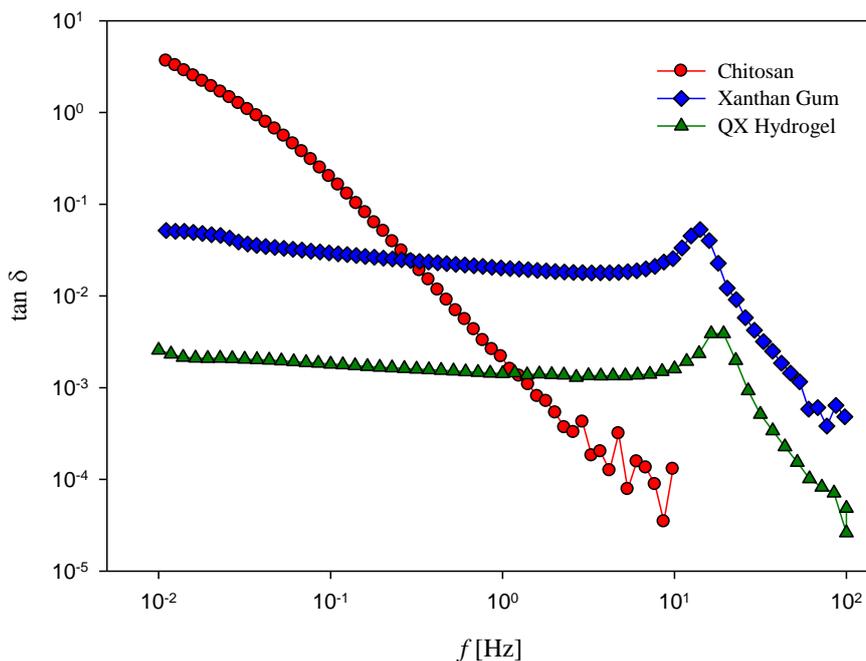


Figure 4: $\tan \delta$ of chitosan (●) and xanthan (◆) solutions and of CX hydrogel (▲).

The hydrogel was submitted to a temperature sweep and the result is illustrated in Figure 5. The hydrogel showed a stable behavior in a temperature sweep from 25°C to 60°C. The behavior of hydrogel was predominantly more elastic, with G' greater than G'' in the temperature interval studied. Above 60 °C, both moduli G' and G'' increased, this response to temperature might be attributed to the vibration and rotation energy of water increased obviously resulting in the escape of water molecules surrounding the polymers chain. Therefore, the polymer segments begin to associate with each other to form a more structured network hydrogel (Li et. al., 2010).

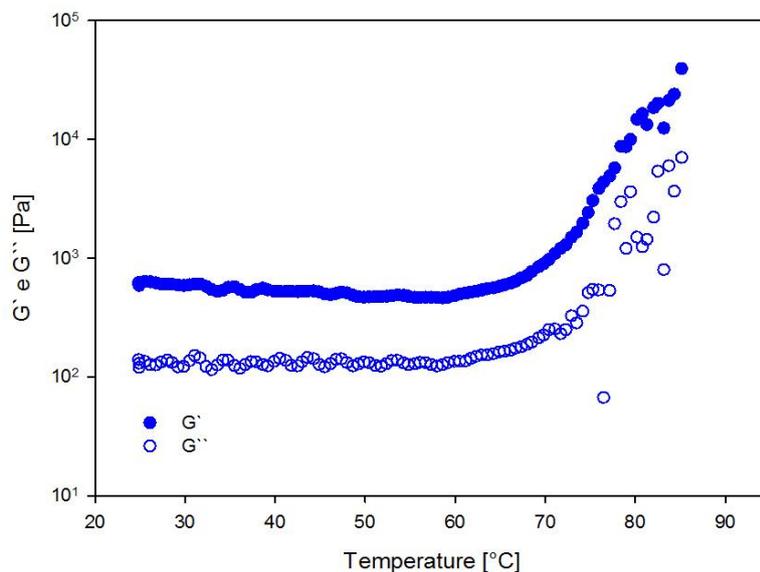


Figure 5: Temperature sweep of CX hydrogel. Open symbol indicate G' (●) and closed symbol indicate G'' (○).

In the creep and recovery curve, hydrogel presented a recovery of approximately 49% (Figure 6) when submitted to a stress of 1 Pa for 150 seconds. Compared to xanthan gum, the maximum compliance of the hydrogel ($0,0066710 \text{ Pa}^{-1}$) was much smaller than the xanthan compliance ($0,3307 \text{ Pa}^{-1}$). Smaller values of compliance indicate a more elastic behavior, confirming that the polyelectrolyte complexation improved the elastic properties of the biopolymers.

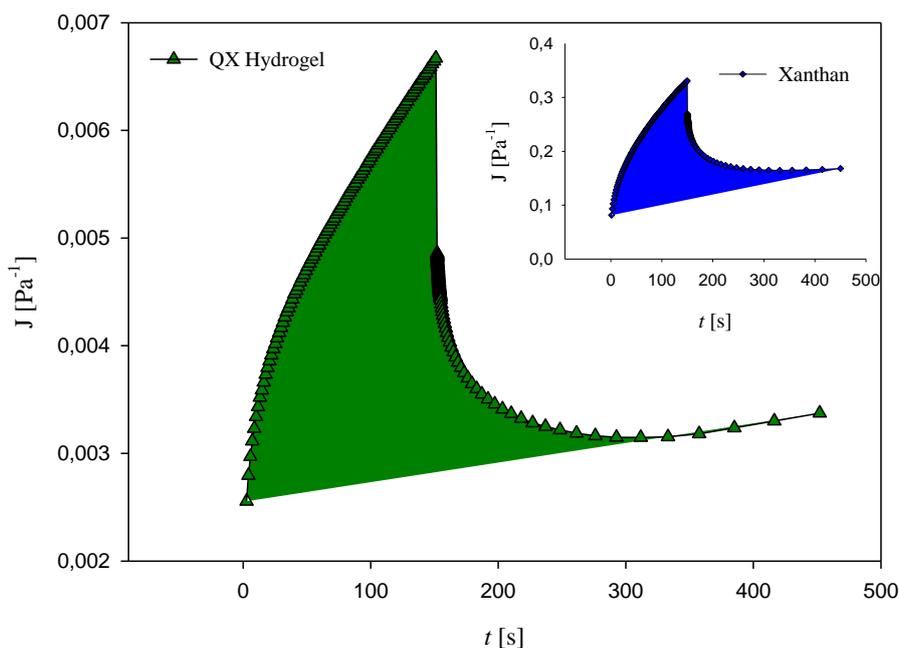


Figure 6: Creep and recovery curves of CX hydrogel (▲) and xanthan gum (◆).

4. CONCLUSIONS

Hydrogels formed by complexation of chitosan, a biopolymer cationic, and xanthan, a biopolymer anionic, have been rheologically analyzed. By the zeta potential measurement, the pH value, where could occur the highest electrostatic interaction, was around 4.05. In this pH, chitosan presented a viscous-like behavior, while xanthan and chitosan/xanthan hydrogel presented a gel-like behavior, with G' higher than G'' in the range of frequency studied. Temperature sweep showed a stable hydrogel in temperatures from 25 to 60°C. Data of creep and recovery test confirmed the viscoelastic behavior of the hydrogel observed in the oscillatory test. The process of polyelectrolyte complexation improved the elastic characteristics of the biopolymers solutions.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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