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Physico–chemical aspects of galactoxyloglucan from the seeds of *Hymenaea courbaril* and its tetraborate complex

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Abstract

A galactoxyloglucan hydrocolloid (HXGRP) (Glu:Xyl:Gal molar ratio, ~4:3:1) was obtained from cotyledons of seeds of *Hymenaea courbaril* by aqueous extraction and then purified (33% yield). Rheological measurements showed that HXGRP, in aqueous systems, interacts with tetraborate ions increasing both, viscosity and G′ values, also increasing the viscoelasticity of the system, but not forming a gel, as shown by Cox-Merz experiments. This interaction dependent of the amount of sodium tetraborate and the pH, and was analyzed in terms of hindered reptation. A temperature sweep of the HXGRP-tetraborate complex showed a characteristic cleavage of cross-links between polysaccharide and borate ions at high temperatures. An aqueous solution of HXGRP after heating at 85 °C for 2 h showed a decrease in its absolute viscosity.

Keywords: Galactoxyloglucan; *Hymenaea courbaril*; Sodium tetraborate; Rheology; Fluorescence analysis

1. Introduction

It is known that many water-soluble polymers form water-soluble gels when various cross-linking reagents are employed (Kesavan & Prud'homme, 1992).

Several studies involving borate and guar, hydroxypropylguar and other galactomannans have been carried out (Gey, Noble, Perez, & Taravel, 1988; Kesavan & Prud'homme, 1992; Noble & Taravel, 1990; Pezron, Ricard, Lafuma, & Audebert, 1988; Power, Rood, Peterson, & Boger, 1997; Rubinstein & Gliko-Kabir, 1995; Tayal, Pai, & Khan, 1999).

Since polysaccharides contain a multiplicity of hydroxyl groups, complex formation between borates or borate derivatives can occur with gel formation or an increase in the viscosity, with changes in other properties related to molecular size (Zittle, 1951). Gelation results from the cross-linking of different polymer chains, via the borate ion or sometimes parts of the same chain, in such a way that a three-dimensional network of connected chains is formed (Gey et al., 1988).

Cross-linked guar gels are used, for example, in the oil and gas industries to transport sand into artificially created fractures in oil-bearing rock in order to enhance petroleum productivity (Tayal et al., 1999).

Savur and Sreenivasan (1948) indicated that the xyloglucan from seeds of *Tamarindus indica* forms a highly viscous, gelatinous product when treated with small amounts of borax, thus resembling gum tragacanth. Zittle (1951), in a review, cited that the mucilage from tamarind seeds and borate ions forms a weak gel, with solutions becoming viscous and ropy. Ghose and Krishna (1946-cited by Rao & Srivastava, 1973) showed that a xyloglucan solution treated with small quantities of sodium tetraborate and then stirred, yields a semisolid gel, but no extensive studies involving rheology or other methods exist that characterize this interaction.

Xyloglucans were first found as amyloids in the cell walls of plant seeds (Hayashi, 1989; Kooiman, 1957). In cotyledons, they have a (1 → 4)-linked β-D-glucan backbone with α-D-xylosyl residues attached to O-6, and then terminal β-D-galactose attached to O-2 (Hayashi, 1989;

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Kooiman, 1961). In Brazil, xyloglucans have been studied since 1993, when Lima, Reicher, Corrêa, Ganter, and Sierakowski (1993) isolated one from crushed seeds of *Hymenaea courbaril*, known locally as 'jatobá'. Lima, Rechia, Ganter, Reicher, and Sierakowski (1995) isolated and studied some oligosaccharides obtained by enzymatic hydrolysis, which had a composition similar to that of *Tamarindus indica* seeds. Other studies have been carried out by our group (Freitas, 2000; Freitas, Gorin, Neves, & Sierakowski, 2003; Lima, Quoirin, Wollinger, Krüger, & Sierakowski, 2000; Martin, 1999; Martin, Obayashi, & Sierakowski, 2001; Martin, Souza-Lima, Gorin, Reicher, & Sierakowski, 2000; Souza-Lima, 1997; Vargas-Rechia et al., 1998) and Buckeridge and co-workers (Buckeridge et al., 1997; Franco, Rodrigues, Serra, Penegassi, & Buckeridge, 1996; Lima & Buckeridge, 2001; Tiné, Cortelazzo, & Buckeridge, 2000). We now characterize the interaction between the galactoxyloglucan from the seeds of *Hymenaea courbaril* and borate ions by rheological methods.

2. Materials and methods

2.1. General methods

Total sugar and protein contents of polymer were determined by the phenol-sulphuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and Hartree (1972) methods, respectively and that of moisture by that of Zobel and Stephen (1995). The monosaccharide composition was determined after total acid hydrolysis with 2 M TFA, for 8 h at 96 °C. Hydrolysates were reduced with sodium borohydride and acetylated with pyridine-acetic anhydride (1:1 v/v, 16 h at 25 °C). Gas chromatography was performed with a Hewlett Packard, model, 5890 A, series II chromatograph, using a DB-225 J and B Scientific column (0.25 mm i.d. × 30 m) with nitrogen as carrier gas (2 ml/min) and a flame ionization detector. Detector, injector and column temperatures were 300, 250 and 220 °C, respectively.

2.2. Polysaccharide source

Seeds of *H. courbaril* were collected in Ribeirão Preto, State of São Paulo, Brazil.

2.3. Galactoxyloglucan isolation and purification

Crushed seeds (65 g) were boiled in 300 ml of water for 15 min, peeled and then submitted to exhaustive aqueous treatment (1800 ml) at 25 °C. The combined extracts were precipitated with 2 vol. of ethanol. The crude precipitated polysaccharide was deproteinised by precipitation with Fehling solution (Jones & Stoodley, 1965). A solution of galactoxyloglucan (5 g/l) was centrifuged (7000 rpm, 45 min) and filtered successively through cellulose ester membranes with pore diameters of 3, 0.8 and 0.22 μm. The polymer solution was evaporated to a small volume (200 ml), treated with 2 vol. of ethanol and the precipitate (HXGRP, 33%) dried at room temperature.

2.4. Determination of average molar mass (M_w)

The d*n*/d*c* of HXGRP was determined using a Waters differential refractometer model 2410 at a wavelength of 546 nm, with at least 5 concentrations, between 1 and 0.1 mg/ml (filtered by Millipore filter 0.45 μm). The ratio of refractive index to concentration gave the constant d*n*/d*c* that is used to calculate the molar mass.

HXGRP (2 g/l) in 0.5 g/l aq. sodium nitrite containing 200 ppm of sodium azide was filtered through a 0.22 μm pore Millipore filter and injected into a GPC having 2000, 500, 250 and 120 ultragel columns. Detection was carried out using the Waters refractometer and a DAWN DSP-F Wyatt Technology model, controlled at a light scattering multiangle of 632.8 nm. The eluent at a flux of 0.6 ml/min was the same solution used to solubilize the HXGRP.

2.5. Rheological analyses

Static rheological measurements were performed in a BROOKFIELD LVDV-III rheometer, with a cone plate spindle (CP-40, CP-51 or CP-52) coupled to a BROOKFIELD TC-500 circulating bath that maintained the temperature at 25 °C.

Dynamic rheological analysis were performed in a HAAKE RS-75 rheometer with a cone plate sensor C 60/2° or DG 41, coupled to a circulating HAAKE DC-5 bath and HAAKE Peltier TC-80 heating system and in a TA Instruments AR 2000 rheometer with a Peltier system to control the temperature, utilizing two geometries (25 mm ETC parallel plate and 20 mm groove parallel plate). The heating-cooling rate of HXGRP aq. solutions was 1 °C/min and for HXGRP-tetraborate complex, it was 1 °C/min for the heating process, 0.5 °C/min for the cooling process from 85 to 50 °C and 1 °C/min from 50 to 5 °C, at a fixed frequency of 1 Hz.

For intrinsic viscosity determinations, the samples were solubilised in 0.1 mol/l aq. KCl or H_2O for approximately 2 h.

For other rheological measurements, the samples were solubilised in water or sodium tetraborate solution, by stirring, for 16 h at 25 °C. The HXGRP concentration was 20 mg/ml for the analyses with a variation of tetraborate concentration or pH, and 40 or 30 mg/ml for analyses of temperature and time variation. The concentrations were chosen because of the limitations of the equipment.

The tetraborate concentration was varied by using, 3, 5, 7 and 10 mg/ml of the sodium salt ($Na_2B_4O_7{\cdot}10\ H_2O$). In the study of the effect of pH, a fixed salt concentration of 3 mg/ml with 0.33 mg/ml of sodium borohydride was

utilized. The pH, measured with a QUIMIS pHmeter, before and after solubilization, was adjusted with 0.5, 1 and 3 mol/l NaOH or HCl.

When temperature was varied, the tetraborate concentration was 3 mg/ml.

2.6. Intrinsic viscosity determination

The absolute viscosity of the HXGRP solutions at different concentrations (0.4–2 mg/ml) was measured in a BROOKFIELD rheometer as described above and the value of reduced viscosity (η_{red}) was calculated. The plot of η_{red} versus concentration (C) was extrapolated to infinite dilution to determine the intrinsic viscosity [η]. The Huggin's constant (k') was obtained from the equation $\eta_{sp}/C = [\eta] + k'[\eta]^2 C$, where η_{sp} is the specific viscosity (Lapasin & Pricl, 1995).

2.7. Fluorescence assays

The pyrene fluorescence spectra of HXGRP solution (10 mg/ml), heated to 85 °C for 2 h and unheated were obtained using a fluorescence spectrophotometer HITACHI, model f-4500. The wavelength for pyrene excitation was 310 nm. The relative intensities of vibrational fluorescence bands were determined from an emission spectrum (360–460 nm, 5 nm slits).

3. Results and discussion

3.1. Isolation, purification, chemical and physico–chemical characterization of HXGRP

Exhaustive aqueous extraction from milled cotyledons of *H. courbaril* seeds yielded 40% of crude HXGRP. After deproteination by precipitation of HXGRP by Fehling solution and filtration using 3 and 0.22 μm membranes, the yield related to crude HXGRP was 75 and 33%, respectively.

The monosaccharide molar ratios, total sugar, protein and moisture content are shown in Table 1, where from the data obtained, it can be concluded that the purified component of the endosperm of *H. courbaril* seeds is a proteogalactoxyloglucan.

Table 1
Monosaccharide molar ratios, total sugar, protein and moisture content of HXGRP, following Millipore filtration

	Pore size	
	3 μm	0.22 μm
Glu:Xyl:Gal (mol%)	4.3:3:1	3.8:3:1
Total sugar (%)	80.4	81.6
Protein (%)	4.6	3.6
Moisture content (%)	15	12

The average molar mass (M_w) obtained by HPSEC-MALLS was 461,500 g/mol.

The intrinsic viscosity [η] was calculated using concentration values between 0.4 and 2 mg/ml from a plot of η_{red} versus C. Its values were 330.5 ml/g with k' (Huggin's constant) of 0.73 in an aqueous solvent and 359.8 ml/g with k' of 0.52 when the solvent was 0.1 mol/l aq. KCl solution. The Huggin's constant varied betwen 0.3 and 0.6 and depends on the molecular rigidity and thermodynamical system. It decreased in a good solvent and increased when intermolecular interactions increased (Milas, 1991). Thus, in HXGRP, 0.1 mol/l aq. KCl was a better solvent than water.

For polyelectrolyte polysaccharides, such as an oxidized scleroglucan, this different behaviour, on solubilisation in water or in salt, indicated a conformational change (Coviello, Dentini, Crescenzi, & Vincenti, 1995; Coviello et al., 1998), in the case of HXGRP, this needs to be further investigated.

3.2. Rheological analysis

In the flow curves for different concentrations of HXGRP, we can observe a similar behaviour to that of other polysaccharide systems. At low concentrations the behaviour is Newtonian, while at higher ones (10 mg/ml) it is slightly pseudoplastic, this characteristic being more pronounced with increasing concentration (data not shown).

Since one of the present objectives is verify if an interaction occurs between polysaccharide and tetraborate ions, the second step of the rheological investigation was to determine whether there was an increase in the viscosity of the system when HXGRP was solubilised in an aqueous sodium tetraborate. In the flow curves of Fig. 1, where HXGRP was solubilised in water and in aqueous 3 mg/ml

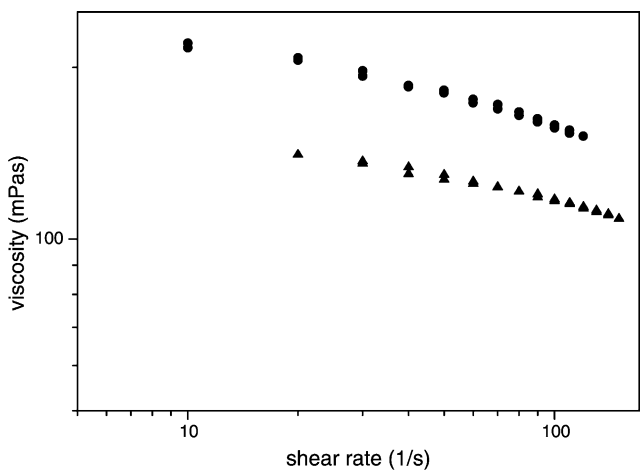

Fig. 1. Flow curves, at 25 °C, of HXGRP (15 mg/ml) in water (▲) and in aqueous sodium tetraborate 3 mg/ml (●).

sodium tetraborate, one can observe an increase in the viscosity of the resulting HXGRP-tetraborate complex. For better characterisation of this interaction, solutions of HXGRP or the HXGRP-tetraborate complex were submitted to dynamic rheological analysis. For this, strain sweep experiments were initially carried out with the objective of determine the linear viscoelastic region where G′ and G″ are independent of the strain. All subsequent experiments were carried out in this region with the deformation applied to the system varying between 3 and 5%.

In Fig. 2 one can observe the mechanical spectra of HXGRP solubilised in water at concentrations of 40 and 20 mg/ml. The behaviour at the two concentrations was that of a solution, since G″ (storage modulus) was higher than G′ (elastic modulus) at all frequency ranges. It can be observed that with the increasing frequency, the difference between the two moduli became smaller and finally crossed over. This behaviour is typical of concentrated polymer solutions (Lapasin & Pricl, 1995).

In Fig. 3 one can observe the dynamic behaviour of HXGRP (20 mg/ml) when solubilised in water or aqueous 3 mg/ml sodium tetraborate. For the sample solubilised in sodium tetraborate the G′ values were higher than those in water, the difference between the two moduli becoming smaller with a crossover occurring at lower frequencies. This characterises an increase in solid behaviour, consistent with the increase in sample viscosity.

This behaviour can be discussed in terms of the concept of reptation. Since it first emerged, the idea of reptation motion has rapidly gained great popularity in the polymer community (Fujita, 1990). This concept was introduced by de Gennes who discussed the Brownian motion of an unattached chain moving through a fixed network (Doi & Edwards, 1986). So, in concentrated polymeric systems, a polymer chain can be thought of as being confined within a tubelike region, which is constituted by neighbouring macromolecules, resulting in a limitation of its lateral

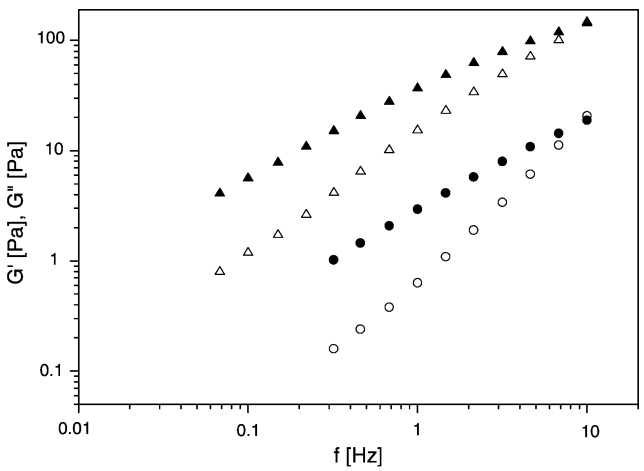

Fig. 2. Frequency sweeps, at 25 °C, of HXGRP 40 mg/ml (▲) and 20 mg/ml (●) in water. G′ open symbols, G″ full symbols.

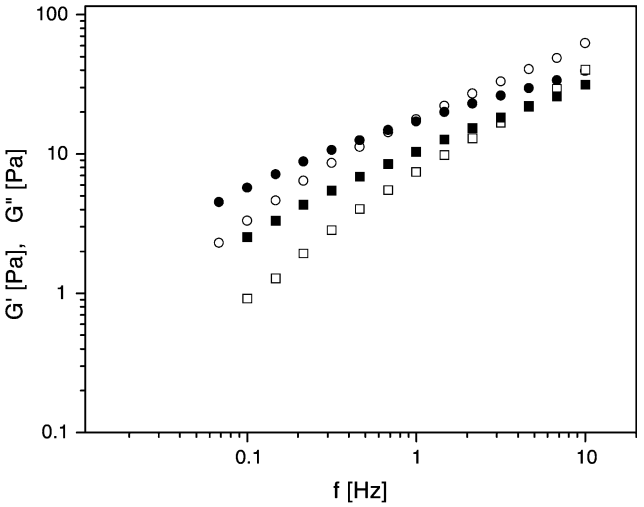

Fig. 3. Frequency sweeps, at 25 °C, of HXGRP 20 mg/ml in water (■) and in aqueous 3 mg/ml sodium tetraborate solution (●). G′ open symbols, G″ full symbols.

displacements so that it can only diffuse by a snakelike motion called reptation (de Gennes, 1971; Lapasin & Pricl, 1995).

In the model of hindered reptation introduced by Leibler, Rubinstein, and Colby (1991), each polymer chain is supposed to contain many association sites, induced by hydrogen bonding, ion complexation or crystallisation. The chains are also entangled with each other and diffusion is only possible along the chain (Leibler, Rubinstein, & Colby, 1991; Wientjes, Duits, Jongschaap, & Mellema, 2000).

This model considers dynamics in temporary networks which have binding points with relatively weak binding energies, so that junctions break and form frequently on the experimental time scale. The most salient feature of such reversible networks is their enhanced viscoelastic behaviour, compared with that of polymers which do not have associating groups. This enhancement shows up as a much longer relaxation time (slower diffusion) Leibler et al., 1991. When this concept is applied to our system, borate ions give rise to these temporary networks increasing the viscoelasticity of the sample, as demonstratred in Fig. 3. The cross-over between G′and G″ occurs at lower frequencies than for HXGRP alone. This can be due to times shorter than the lifetime of a cross-link and such networks behave as elastic rubber (gels). On longer time scales, the successive breaking of only a few cross-links allows the chain to diffuse along its confining tube (Leibler et al., 1991).

To check the stability of our samples, solubilised in water or in aqueous sodium tetraborate, and submitted to stress conditions, time sweeps were performed under a fixed frequency and deformation. Both values of G′, G″ and dynamic viscosity were constant when analysed over a period of six hours (data not shown). In the case of HXGRP solubilised in aqueous sodium tetraborate, this shows that the time of solubilisation was sufficient to establish all the cross-links of the system.

To show the influence of borate ion concentration on the rheological aspects of HXGRP-tetraborate complexes, the polysaccharide (20 mg/ml) was solubilised in water and 3, 5, 7 and 10 mg/ml sodium tetraborate solutions, and the dynamic rheological properties analysed.

In Fig. 4, one can observe the plot of G′ values versus sodium tetraborate concentration measured at three different frequencies. The G′ values increased up to a concentration of 7 mg/ml of sodium tetraborate, indicating that this is the concentration at which occurs maximum viscoelasticity. Also, that this behaviour is more pronounced at a frequency of 10 Hz and it is in agreement with the timelife of the cross-links (see above).

In studies on schizophyllan (Grisel & Muller, 1996), it was demonstrated that its rheological properties was largely affected by the addition of small quantities of borate ions and the higher the borax concentration, the stronger the gel became, although a plateau was not reached.

With the intention of increasing the viscoelasticity, the pH was adjusted to 9.83, 10.31 and 10.55. When the pH of a tetraborate solution increases, there is a higher availability of borate ions, increasing the number of cross-links (Power et al., 1997) and consequently the viscoelasticity of the mixture. The tan (δ) values that express the ratio G″/G′ (Naé, 1993) were utilised in an attempt to characterise the optimum behaviour. In Fig. 5, it can be observed that besides the pH elevation, the tan (δ) decrease was not so pronounced from pH 9.31 up. When the pHs where 9.83, 10.31 and 10.55, this difference did not practically occur, showing rheological stability over this range of pHs. Fig. 6 shows that the best pH for promoting the viscoelastic character of the mixture was 9.83. At this value and at higher ones the macroscopic aspect of the mixture was that of a gel that did not flow easily and could be manipulated. At pH 12.00, the mixture turns to a liquid with very low viscosity. In the complex hydroxypropylguar-tetraborate, G′ increased with increase of the pH up to pH 12.0 (Power et al., 1997). The different effect of pH between the two polymers can be due to ionisation of hydroxyl groups at high pH and/or a change in the molecular conformation (Noble, Turquois, & Taravel, 1990) of HXGRP.

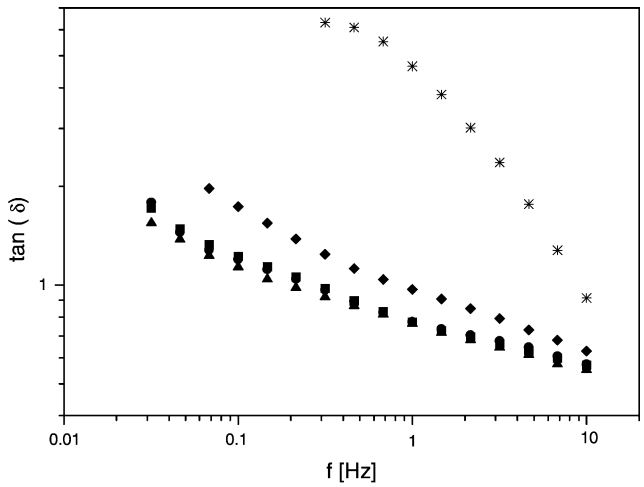

Fig. 5. Variation of tan (δ) values versus frequency with HXGRP 20 mg/ml in water, at pH 5.0 (*) and HXGRP (20 mg/ml)-tetraborate (3 + 0.33 mg/ml $NaBH_4$) complexes, at pHs 9.31 (◆), 9.83 (▲), 10.31 (■), 10.55 (●).

For hydroxypropylguar, Power et al. (1997), reported that at low pHs, the slope of the tan (δ) versus frequency curve is negative and that at high pHs it became positive, so that they could determine the gelling point of the system, namely the pH at which the tan (δ)-frequency gradient is zero. This was not possible for HXGRP, since the slope of tan (δ) versus frequency was negative even at pHs where the higher values for G′ were observed, since HXGRP-tetraborate did not form a gel and only a more viscoelastic solution.

To complement this observation, we examined an HXG obtained from seeds collected in another region of Brazil

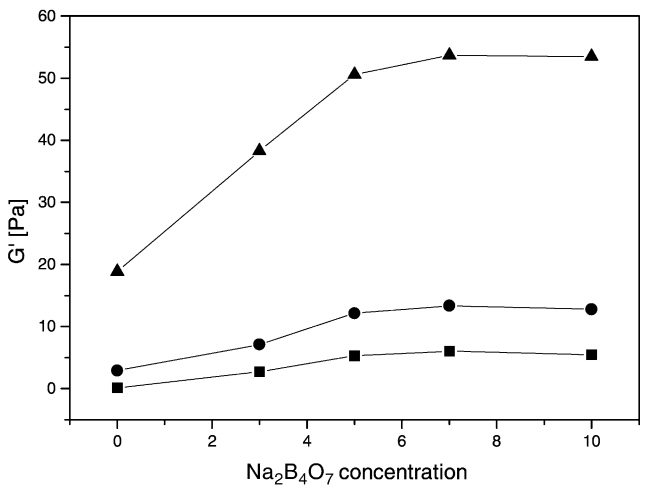

Fig. 4. Variation of G′ versus tetraborate concentration with HXGRP (20 mg/ml) at different frequencies. (▲) 10 Hz, (●) 1 Hz and (■) 0.316 Hz.

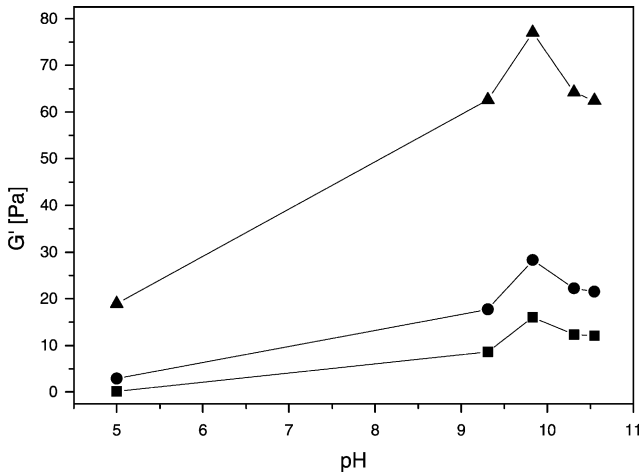

Fig. 6. Variation of G′ versus pH for HXGRP in water (pH 5.0) and HXGRP-tetraborate complexes in water at different frequencies. (▲) 10 Hz, (●) 1 Hz, (■) 0.316 Hz.

Table 2
Composition, M_w and R_g values of HXGFC

Glu:Xyl:Gal (mol%)	4:3:1
Total sugar (%)	80.7
Protein (%)	2
Moisture content (%)	14.8
M_w (g/mol)	1,528,000
R_g (nm)	83.7

(Foz do Chopin-PR) (Freitas et al., 2003). This sample denominated HXGFC had a greater molar mass than HXGRP (its monosaccharide molar ratios, total sugar, protein and moisture contents are summarised in Table 2).

The results obtained for HXGFC and HXGRP on solubilisation in water or in 7 mg/ml aqueous sodium tetraborate containing 0.33 mg/ml of sodium borohydride at pH 9.8 (conditions with the best viscoelastic behaviour), are shown in Fig. 7. It shows that the HXG with greater molar mass gives a more viscoelastic solution, demonstrating that viscoelasticity is correlated with the molar mass of the polysaccharide.

On performing Cox-Merz experiments with these two samples (Fig. 8), one can observe that the system does not really form a weak gel, having only the aspect of a viscoelastic solution, since the absolute viscosity is higher than the dynamic viscosity (Bot, Smorenburg, Vreeker, Pâques, & Clark, 2001; Han, Campanella, Guan, Keelong, & Hamaker, 2002; Roberts & Cameron, 2002).

To better characterise the HXGRP-tetraborate complex, we carried out a temperature-course of rheological changes during a process of heating and cooling. This is illustrated in Fig. 9, for 40 mg/ml HXGRP in water (A) and 30 mg/ml HXGRP in 3 mg/ml sodium tetraborate solution (B). In the analysis the concentration of the polysaccharide (without sodium tetraborate) was chosen because of equipment limitations.

Fig. 9(A), shows that both G′ and G″ values decreased during the heating process. On cooling, G″ increased with

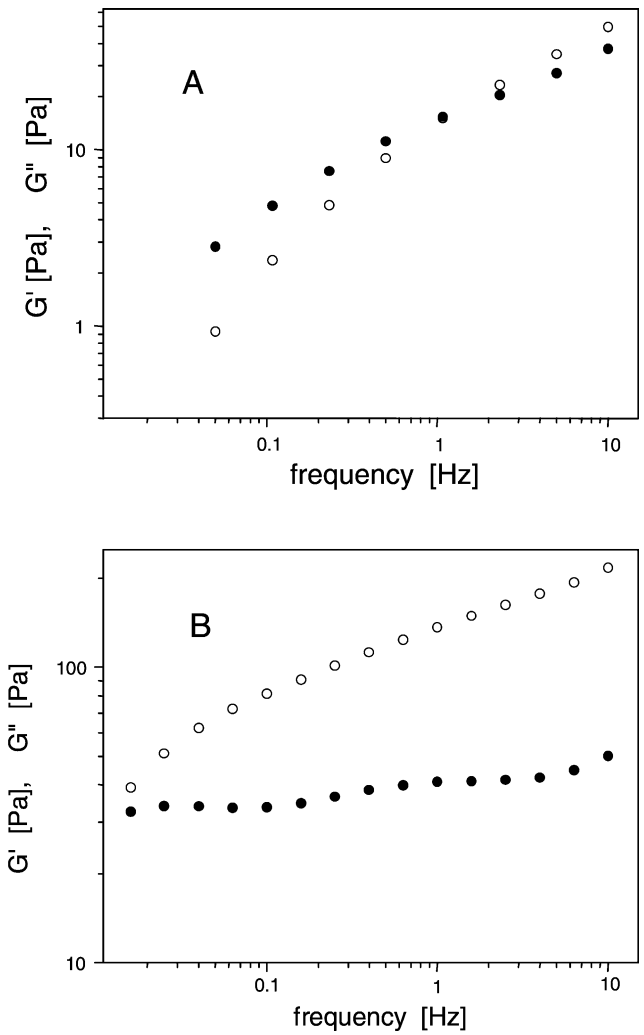

Fig. 7. Frequency sweeps, at 25 °C, of HXGRP (A) and HXGFC (B), both at 20 mg/ml, solubilized in sodium tetraborate 7 mg/ml, pH = 9.8. G′ open symbols, G″ full symbols.

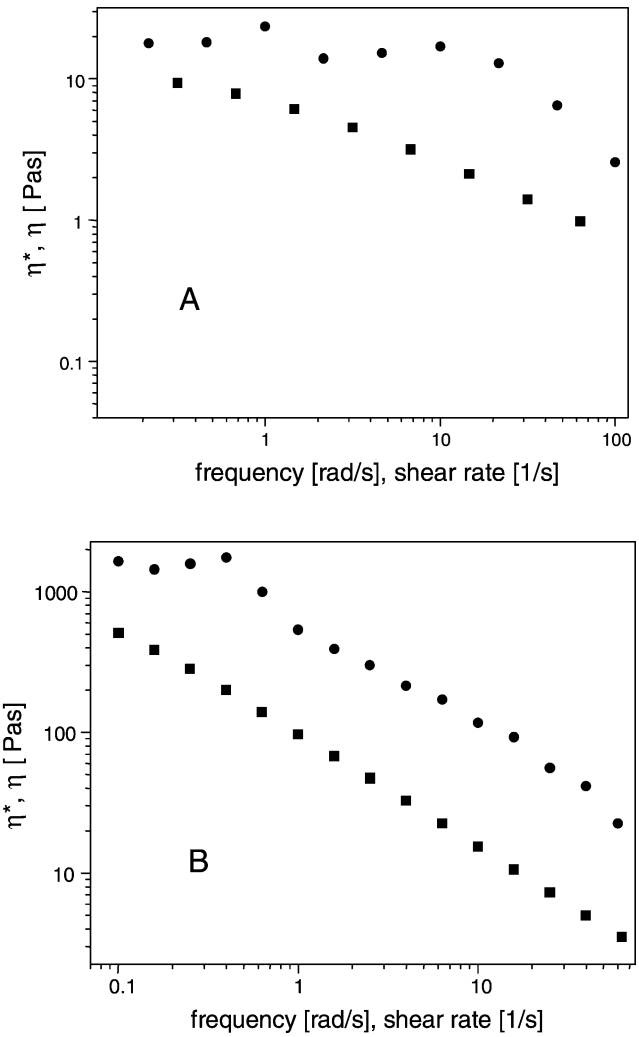

Fig. 8. Cox-Merz, plot of HXGRP (A) and HXGFC (B), both at 20 mg/ml, in aqueous sodium tetraborate 7 mg/ml, pH = 9.8. (●) absolute viscosity, (■) dynamic viscosity.

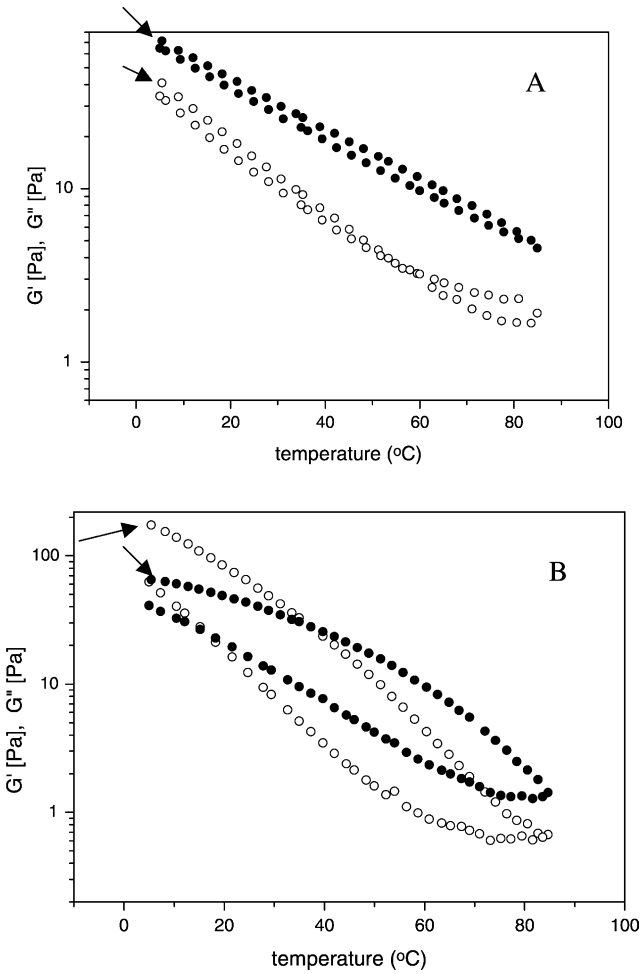

Fig. 9. Temperature sweep of HXGRP 40 mg/ml in water (A) at 1 Hz and 5% of deformation and aqueous HXGRP (30 mg/ml)-tetraborate (3 mg/ml) complex (B) at 1 Hz and 3% of deformation. G′ open symbols and G″ full symbols. Arrows indicate the beginning of the heating process.

values lower than found in the heating process, while G′ is greater, in the heating process up to a temperature of approximately 55 °C, when it became smaller as did G″. This increase in the storage modulus can indicate development of a higher rigidity of the molecule at this temperature.

In Fig. 9(B) one can see that the G′ values begin greater than those of G″ during the heating process up to ~35 °C, when its became lower. This can be explained since the equilibrium reactions for borate ions in water have positive activation energies, so that the number of cross-links decreases with temperature increase (Kesavan & Prud'homme, 1992). This can be attributed to the thermal breakage of the cross-links. It can also be observed, that on cooling the cross-linking increase, but G′ becomes greater than G″ at ~ 16 °C, but with a lower value than that observed in the initial process. When this sample was maintained at 5 °C for 2 h and then submitted to the same analysis, the G′ value does not return to the same value (data not shown).

The temperature curves of HXGRP with and without sodium tetraborate were obtained using different heating-cooling rates (5, 2.5, 1.0 and 0.5 °C/min) and the thermal hysteresis observed was greater the slower the proccess (data not shown).

The results presented above, are in accord with the mode of sample preparation. As it was difficult to solubilise HXGRP in tetraborate solution at room temperature, solubilization was attempted, by stirring at 60 °C, but the resulting complex had only a very low viscosity (data not shown). Another interesting observation, correlated with heating of the sample, is that when a sample of HXGRP was solubilised in water at 25 °C, heated to 85 °C for 2 h, and then cooled at 25 °C, its absolute viscosity decreased more than 20 fold when compared with that of an unheated sample.

Table 3
III, I and III/I intensity ratios of emission fluorescence of pyrene in water, HXGRP solution (10 mg/ml) heated to 85 °C, 2 h and cooled to room temperature and HXGRP not heated

	III	I	III/I
H_2O	19.95	35.22	0.57
HXGRP not heated	8.04	11.86	0.68
HXGRP heated	6.57	8.82	0.74

3.3. Pyrene probe experiments

The fluorescence emission spectrum of pyrene is sensitive to the polarity of the environment in which it is dispersed. In particular, the ratio of fluorescence intensities of two vibronic bands, termed III and I can serve as a sensor of hydrophobicity. Consequently, this property has been used by polymer chemists interested in the conformational behaviour of water-soluble polymers. In Table 3, one can observe the characteristic III/I ratio for water (Chee, Rimmer, Soutar, & Swanson, 2001), and for HXGRP heated to 85 °C for 2 h and then cooled to 25 °C and for unheated HXGRP. The III/I ratio values for the two samples are different indicating, that after heating, there is a more apolar environment than for in the unheated sample. This behaviour can be associated with the same modification, in the HXGRP solution that caused the decrease in its absolute viscosity, after heating, as described above.

To verify if there is an alteration in the conformation of the HXGRP molecule that can be correlated with this behaviour after heating it would be necessary to perform experiments, to evaluate T_m, using more sensitive techniques such as optical rotation, light scattering (LS), circular dicroism (CD) and differential scanning calorimetry (DSC).

4. Conclusions

Rheological measurements showed an increase in the viscosity of HXGRP solubilised in sodium tetraborate,

when compared with the same polymer concentration in water alone. Rheological dynamic measurements on HXGRP in water showed the solution behaviour and the interaction with sodium tetraborate, which makes the system more viscoelastic, indicating the existence of cross-links between tetraborate ions and HXGRP chains. This has been shown for other polysaccharides (Gey et al., 1988; Grisel & Muller, 1996; Kesavan & Prud'homme, 1992; Noble & Taravel, 1990), but without gel formation as shown by Cox–Merz experiments. The viscoelasticity of our system were influenced by the amount of sodium tetraborate and on increasing a salt concentration up to 7 mg/ml, a plateau was reached. pH also influenced the viscoelasticity and the optimum pH for the interaction between HXGRP and tetraborate ions at ~9.83, lower or higher pHs giving rise to decreases in its values. An alkaline pH increase the avaliability of borate ions and also promoted deprotonation of the hydroxyl group of the polysaccharide chain, necessary to form the complex whose formation and behaviour was analysed by hindered reptation. In temperature sweeps at fixed deformation and frequency, HXGRP, solubilised in sodium tetraborate, showed a characteristic breakage of cross-links between polysaccharides and tetraborate ions at high temperatures. An aqueous solution of HXGRP, after heating at 85 °C for 2 h, showed a decrease in its absolute viscosity. Fluorescence analysis utilizing a pirene probe, showed a change in the polarity of the system after submission to heating under the same conditions. However, further analysis are necessary to understand this behaviour.

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