



Rheology and ^{11}B Nmr Analysis of a Xyloglucan-borate Complex

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Abstract: A xyloglucan-tetraborate complex was analyzed by rheology and ^{11}B NMR techniques. By rheology was demonstrated an increase in the viscoelasticity of the sample, but no gel formation. This behaviour is in agreement with ^{11}B NMR data that shows the presence of a BD type complex formed between borate ion and β -D-galactose units present in the lateral chain of the polysaccharide.

Keywords: xyloglucan, borate complex, rheology, ^{11}B NMR.

Introduction

It is known that borate ion, $\text{B}(\text{OH})_4^-$, reacts with suitable polyols in aqueous solution to form complexes of different kinds. Many compounds of biological importance contain hydroxyl groups in positions favorable for reaction with borate ion and among them are the polysaccharides. 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 [1]. These properties were observed for complexes with borate and different kinds of polysaccharides, but the most studied was guar, whose complexes with borate was utilized by the petroleum industry [2]. Complexation of xyloglucan (a natural polymer that have a (1 \rightarrow 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) from *Tamarindus indica* seeds and borate was observed [1,3]. Martin *et al.*(2003) [4] studied, by rheology, the complexes formed by borate ions and a xyloglucan obtained from seeds of a Brazilian tree *Hymenaea courbaril*, known as jatobá. In another work from the same group this complex was analysed also by thermogravimetry [5]. Now, we correlated the results obtained by rheology with the ^{11}B NMR data.

Materials And Methods

Rheological analysis

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

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. The samples, in the appropriate concentrations, were solubilised in water or sodium tetraborate solutions, by stirring for 16 h at 25°C.

^{11}B NMR analysis

The ^{11}B NMR spectrum was obtained on a BRUKER equipment, DRX 400 AVANCE (128,37 MHz), at 30°C. The samples (xyloglucan at 20 mg/ml, pH 9,8 and a standard of metil β -D-galactopyranoside at 100 mg/ml, pH 9,3) were solubilized in 0.02 mol/l boric acid prepared in D_2O solution, and 0.1 mol/l boric acid solution was utilized as an external standard. The pH of the samples, measured with a QUIMIS pHmeter, was adjusted with drops of 1 mol/l NaOH solution.

Results And Discussion

Polysaccharide source and chemical and physico-chemical characteristics

The source of xyloglucan (XG) was *Hymenaea courbaril* seeds, which biopolymer was previously characterized by Martin *et al.*(2003) [4] as shown in Table 1.

Rheological analysis

In the Fig. 1 one can observe the dynamic behaviour of XG (20 mg/ml) when solubilized in water or aqueous 3 mg/ml sodium tetraborate (XG-B) at the linear viscoelastic region. For the sample solubilized in sodium tetraborate the G' values were higher than those in water, the difference between two moduli becoming smaller with a crossover occurring at lower frequencies. This characterizes an increase in the solid behaviour, consistent with the increase of the sample viscosity observed at sight.

Table 1 – Chemical and physico-chemical characteristics of XG

Yield (from crude seeds)	40 %
Glu:Xyl:Gal relationship (mol %)	4.3:3:1
Total sugar (%)	80.4
Protein (%)	4.6
Moisture content (%)	15
Average molar mass [#] - M_w (g/mol)	461,500
<i>Intrinsic viscosity</i> [*] - $[\eta]$ (ml/g)	359.8 ml/g
<i>Huggins' constant</i> [*] - k'	0.52

[#] in 0.5 g/l aq. sodium nitrite containing 200 ppm of sodium azide

^{*} in KCl 0.1 mol/l

The behaviour of these samples can be discussed in terms of hindered reptation, in which each polymer chain is supposed to contain many association sites, introduced by hydrogen bonding, ion complexation or crystallisation. The chains are also entangled with each other and diffusion is only possible along the chain. This model considers dynamics in temporary networks which have binding points with relatively weak binding energies, so that some junctions break and form frequently in the time scale. The most salient feature of such reversible networks is their enhanced viscoelastic behaviour, compared with that of polymers which have only hydrogen bonding associating groups. This enhancement shows up a much longer relaxation time (slower diffusion) [6]. When this concept is applied to our system, borate ions give rise to this temporary networks increasing the viscoelasticity of the sample, as demonstrated in Fig. 1. The cross-over between G' and G'' occurs at lower frequencies (1 Hz) than for XG alone (10Hz). This can be due to times shorter than the lifetime of a cross-link and such networks behave as a viscoelastic solution. On longer time scales, the successive breaking of only a few cross-links allows the chain to diffuse along its confining tube [6].

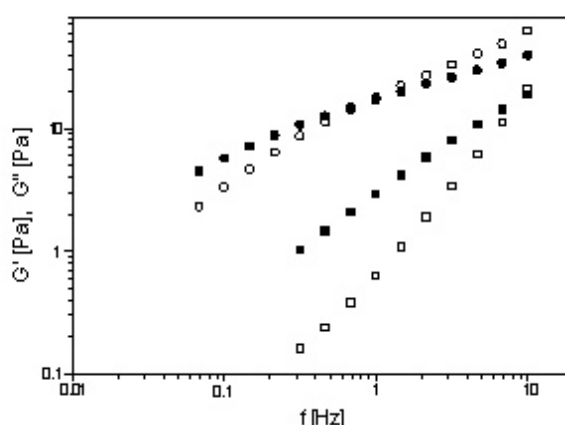


Figure 1 – Frequency sweeps, at 25°C, of XG 20 mg/ml solubilised in water (■) and in 3 mg/ml sodium tetraborate solution (●). G' open symbols, G'' full symbols.

¹¹B NMR analysis

The same complex XG-B studied by rheology was also studied by ¹¹B NMR and the signals were correlated with the methyl β -D-galactopyranoside used as a standard, once this technique provides a suitable analytical tool for structure elucidation of various diol borate esters in solution [7].

For the methyl β -D-galactopyranoside standard in pH 9.3 chemical shifts were obtained at δ -9.6, -13.5, -18.1, -21.7 and -22.9. In pH 11 similar chemical shifts were observed. In accord with data obtained by van Duin *et al.* [7], to several polyols and polyhydroxycarboxylates in aqueous alkaline solutions the shift at δ -9.6 can be attributed to a bidentate complex of BD₂ type, where a borate ion are complexed with four hydroxyl groups of two different diols. The shift at δ -13.5 can be attributed to a α,β -bidentate complex of BD type where a borate ion is complexed with two hydroxyl groups linked in contiguous carbon atoms in a diol molecule, and the shift at δ -18.1 to a α,γ -bidentate complex of BD type where a borate ion is complexed with two hydroxyl groups linked in alternate carbon atoms in a diol molecule. The shifts at δ -21.7 and -22.9 were not attributed yet, because these signals are not found in the literature.

For the XG-B complex was observed chemical shifts at δ -12.5, -13.5 and -21.5. Chemical shifts between δ -12.6 and -14.9 can be attributed to a α,β -bidentate BD complex. To attribute the signals at δ -12.5 and -21.5 are necessary complementary experiments.

The formation of a BD complex are in agreement with the data obtained by rheology where was observed an increase in the viscoelasticity of the sample, but not a gel behaviour. For the XG-B complex to behaviour itself like a gel it would be necessary the formation of a BD₂ complex between sugar units from different chains giving rise to a stable network which was not observed for our xyloglucan-borate complex.

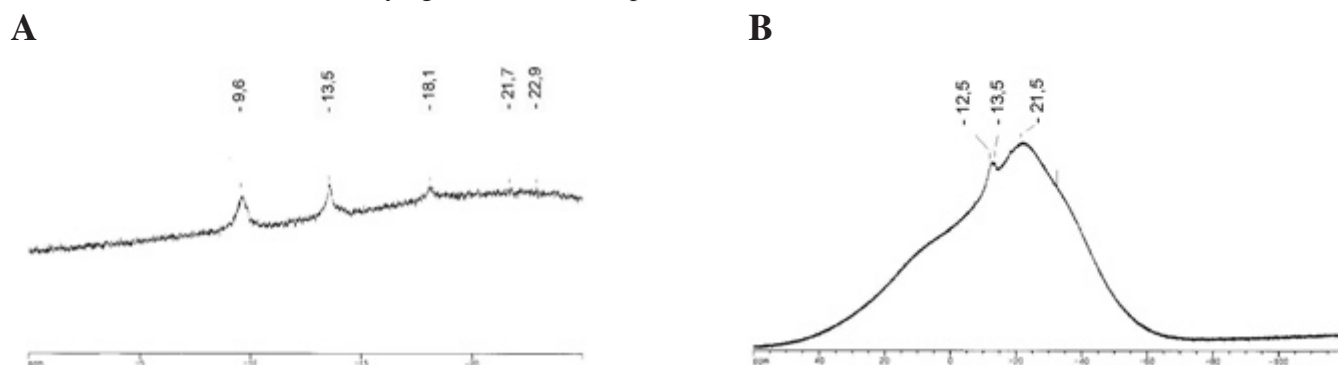


Figure 2 – ¹¹B NMR chemical shifts of methyl β-D-galactopyranoside, at pH 9.3 (A) and XG-B complex, at pH 9.8 (B), in D₂O at 30°C.

Conclusion

The XG-B complex obtained with the xyloglucan from the seeds of *H. courbaril* which has a Mw of 461,500 g/mol did not give rise to gel formation, but just promotes an increase in the viscoelastic behavior of the sample. This behaviour is in agreement with the ¹¹B NMR analysis where was observed only the formation of a complex type BD.

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