

# Transient and quasi-permanent networks in xyloglucan solutions



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## ARTICLE INFO

### Article history:

Received 6 February 2015

Received in revised form 4 April 2015

Accepted 27 April 2015

Available online 8 May 2015

### Keywords:

Xyloglucan

Gel

Viscosity

Transient network

## ABSTRACT

Viscoelastic properties of aqueous solutions of xyloglucan extracted from *Hymenaea courbaril* seeds (Jatobá gum) were investigated by rheology over a wide range of concentrations and temperatures. The polymer was characterized in dilute solutions by light scattering measurements and size exclusion chromatography. Xyloglucan formed, in semi-dilute solutions ( $C$  0.3 wt%), a transient network with cross-links characterized by a broad distribution of lifetimes, independent of the temperature and concentration. Progressively, at higher temperatures ( $>60^\circ\text{C}$ ), a second much weaker quasi-permanent network was formed and attributed to the exchange of intra- to inter-chain bonds. The stiffness of the second network increased with decreasing temperature, but it could be easily broken by applying a relatively weak shear stress and is readily reversible on re-heating, and partially reversible on resting at  $20^\circ\text{C}$ .

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## 1. Introduction

Xyloglucan is a polysaccharide that has a structural function in the cell wall of plants and works as a reserve of carbohydrate in seeds of some species of dicotyledoneae. The xyloglucan found in seeds has principally the monosaccharides D-glucose, D-xylose and D-galactose, usually at molar proportions of 4:3:1, respectively. The backbone of xyloglucan is composed by units of D-glucopyranose (G), linked  $\beta$ -(1  $\rightarrow$  4), with substitutions at glucose O-6 by  $\alpha$ -D-xylopyranose (X), and some xyloses can be also substituted at O-2 by  $\beta$ -D-galactopyranose (L) (Hayashi, 1989; Reid, 1985).

Tamarind xyloglucan (also denominated Tamarind gum) isolated from *Tamarindus indica* L. seeds is used as a thickening agent in food and pharmaceutical industries. Xyloglucan can also be isolated from seeds of *Hymenaea courbaril* as an alternative source and is called Jatobá gum. The structure of xyloglucan chains from these two different sources is close, except that Tamarind gum only contains sequences with two or three consecutive X units, whereas Jatobá gum also contains sequences with four consecutive X units (Fig. 1) (Buckeridge et al., 1997; Freitas et al., 2005).

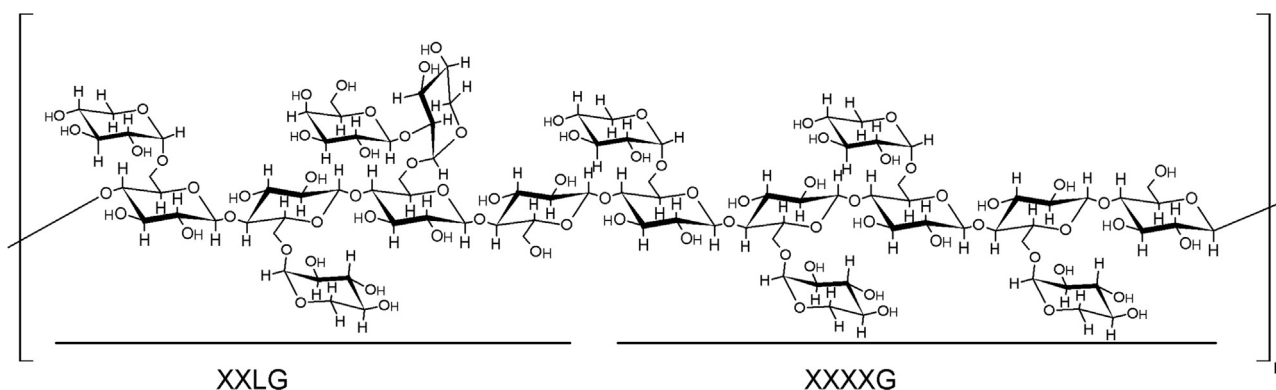
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Because of the use of xyloglucan as a thickening agent several authors have studied the viscoelastic properties of xyloglucan aqueous solutions as a function of the concentration (Khounvilay & Sittikijyothin, 2012; Martin, Freitas, Obayashi, & Sierakowski, 2003; Wang, Ellis, Ross-Murphy, & Burchard, 1997). The viscosity of semi-dilute solutions, i.e. above the overlap concentration of xyloglucan, was found to increase strongly with increasing concentration and shear thinning was observed for the more viscous solutions. The frequency dependence of the shear moduli was typical for viscoelastic polymer solutions, with a solid-like behavior at high oscillation frequencies and liquid-like behavior at low frequencies. Wang et al. (1997) suggested that the rheology of semi-dilute xyloglucan solutions was caused by entanglement of the polymeric chains.

Sims et al. (1998) compared the viscosity of xyloglucan originating from different species of plants, including Nicotiana, Apple pomace and Tamarind. They reported that the viscosity at a given polymer concentration strongly depended on the origin of the xyloglucan, due to differences in the molar mass. They noted that the viscosity decreased with increasing temperature, but did not depend significantly on the pH.

Though the viscosity of aqueous xyloglucan solutions has been investigated in some detail, the concentration and temperature dependence of the frequency dependent shear moduli has not yet been studied systematically. Therefore it has not been possible to fully understand the dynamic mechanical properties of xyloglucan solutions.



**Fig. 1.** Schematic representation of two oligosaccharide segments in the xyloglucan chain, the backbone is a  $\beta$ -(1  $\rightarrow$  4) D-glucopyranose (G), with substitutions at O-6 by  $\alpha$ -D-xylopyranose (X), and at O-2 by  $\beta$ -D-galactopyranose (L). For the oligosaccharide nomenclature used see Fry (1989).

The aim of the investigation presented here was to characterize and elucidate the rheology of xyloglucan in aqueous solution. We studied solutions of xyloglucan from *H. courbaril* seeds, over a wide range of temperatures (10–90 °C) and concentrations (0.1–5 wt%) using continuous and oscillatory shear rheology. The structure of the polymers in dilute solution was characterized by light-scattering and size exclusion chromatography. The viscoelastic behavior of the xyloglucan solutions will be discussed in comparison with similar behavior reported for guar gum (Wientjes, Duits, Jongschaap, & Mellema, 2000) and hydroxylpropyl methyl cellulose (HPMC) (Shahin, Nicolai, Benyahia, Tassin, & Chassenieux, 2013).

## 2. Materials and methods

### 2.1. Material

Seeds of *H. courbaril* were obtained from Project “matas nativas”, Itatinga, São Paulo, Brazil. The xyloglucan was obtained by aqueous extraction at 25 °C of pooled and milled seeds. Each viscous extract was centrifuged at 10,000  $\times$  g for 20 min at 20 °C, and the supernatant was collected. The xyloglucan gum was obtained after its precipitation with two volumes of 96% ethanol, washed with 96% ethanol and with one volume of acetone (Freitas et al., 2005).

The carbohydrate content was determined according Freitas et al. (2005), as 90% of total carbohydrates, 2% of proteins and 8% moisture.

### 2.2. Characterization

After extraction and chemical characterization, the xyloglucan oligosaccharide composition was determined using the enzyme cellulase (*endo*-1,4- $\beta$ -D-glucanase/EGII), from *Trichoderma longibrachiatum*, that was purchased from Megazyme (Bray Co., Wicklow, Ireland) and was used without further purification.

Quantification of oligosaccharides obtained after enzymatic digestion was done by high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC) analysis using a Thermo Scientific ICS-5000 system with a Carbo-pack PA-100 column (Thermo Scientific Dionex, Sunnyvale, CA, USA), ED gold electrode and an amperometric pulse detector (PAD). The eluent used was NaOH 88 mmol L<sup>-1</sup> with a gradient of NaOAc 1 mol L<sup>-1</sup> from 7 to 15% (v/v), flow rate of 0.9 mL min<sup>-1</sup> at 30 °C. The data were treated with the Chromeleon 7 program (Thermo Scientific Dionex, Sunnyvale, CA, USA), used to identify and quantify the oligosaccharides composition.

The macromolecular characterization of xyloglucan was also performed. Weight average molar mass ( $M_w$ ) and dispersity

( $\bar{D} = M_w/M_n$ ) were determined by size exclusion chromatography (SEC) at room temperature with a Tosho G6000PW column. The light scattering detector was a Dawn EOT<sup>TM</sup> (Wyatt technology, Santa Barbara, CA, USA) and the refractive index was measured using a Shodex RI 71 (Showa Denko K.K., Tokyo, Japan). A volume of 100  $\mu$ L of the 0.05 wt% xyloglucan solutions was injected using an automatic injection system (Autoinjector 234, Gilson, Middleton, WI, USA). The system was eluted with 0.1 mol L<sup>-1</sup> NaNO<sub>3</sub> at pH 7, with a flow rate of 1 mL min<sup>-1</sup>. The data were analyzed using the Software ASTRA 6.1.1.

Light scattering (LS) measurements were done at 20 °C over a range of scattering wave vectors ( $6.39 \times 10^6 - 2.55 \times 10^7$  m<sup>-1</sup>) using an ALV-CGS3 equipment (ALV-GmbdH Langen, Germany). From these experiments  $M_w$ , the z-average radius of gyration ( $R_g$ ) and the second virial coefficient ( $A_2$ ) were determined following standard methods. Briefly, samples were prepared at different concentrations (0.01, 0.0275, 0.05, 0.10 and 1.00 wt%) and filtered through 0.2  $\mu$ m pore-size Anotop® filters. From the excess scattering intensity normalized by that of a toluene standard ( $I_r$ ),  $M_w$ ,  $R_g$  and  $A_2$  were determined using the following expression (Brown, 1993; Nicolai, 2007):

$$\frac{KC}{I_r} = \frac{1}{M_w} + \frac{(R_g)^2}{3M_w} q^2 + 2A_2C$$

where  $K$  is an optical constant appropriate for vertically polarized incident light:

$$K = \frac{4\pi^2 n^2}{\lambda^4 N_a} \left( \frac{\partial n}{\partial C} \right)^2 \left( \frac{n_s}{n} \right)^2 \frac{1}{R_s}$$

with  $n$  is the refractive index of the solvent and  $n_s$  and  $R_s$  the refractive index and Rayleigh scattering of the toluene, respectively.  $N_a$  is Avogadro's number, and  $\lambda$  is the wavelength of the laser (632.8 nm). The refractive index increment of xyloglucan is  $\partial n/\partial C = 0.113$  mL g<sup>-1</sup> (Freitas, Martin, Paula, Feitosa, & Sierakowski, 2004).

## 3. Rheology

The xyloglucan powder was dissolved at 20 °C in ultrapure water (Millipore system, Millipore) to which 200 ppm of NaN<sub>3</sub> was added as a bacteriostatic agent. The xyloglucan solutions from 0.1 wt% to 5 wt% were kept at 20 °C for at least 48 h, in the solvent prior to analysis, and the pH of solutions was 6.8 in all experiments. All the concentrations are expressed in weight %.

Oscillatory and continuous shear experiments were done using a rheometer AR 2000 Advanced Rheometer, TA instruments (New Castle, DE, USA). In both cases a cone and plate geometry was used

(40 mm diameter, 2° cone). For oscillatory shear measurements, the imposed stress was chosen to be within the linear response regime unless otherwise specified. A plate-plate geometry was also used with various gaps in order to test if there was an effect of the geometry on the measurements, but no significant influence was observed.

The experiments as a function of the temperature were done by heating the samples from 10 °C to 90 °C in steps of 10 °C, and subsequently cooling to 20 °C. The measurements were done after 1 h at each temperature. To avoid samples evaporation, they were covered with a layer of mineral oil.

In order to obtain the steady state shear viscosity at low shear rates the strain was measured as function of time after imposing different values of the shear stresses between 0.1 and 50 Pa until steady shear flow was observed.

## 4. Results

The oligosaccharide composition of the xyloglucan gum used for this study was characterized as described in the materials and methods section, see Table 1. It contained a new sequence with four consecutive X units that was not present in xyloglucan from other plants like Tamarind. The composition was similar, but not identical, to results previously reported for xyloglucan isolated from *H. courbaril* seeds. Some variation in the oligosaccharide composition has been observed, depending of the collecting place of the *H. courbaril* seeds (Freitas et al., 2005).

Size exclusion chromatography and light scattering measurements were done to characterize the xyloglucan in aqueous solution. A chromatogram is shown in Fig. S1 of the supplementary information. The  $q$ -dependence of  $KC/I_r$  at different concentrations and the concentration dependence of  $KC/I_r$  at  $q \rightarrow 0$  are shown in Fig. S2. The values of  $M_w$ ,  $R_g$  and  $A_2$  derived from these measurements are given in Table 1. A positive value of  $A_2$  was found indicating that water is a good solvent for xyloglucan. We may estimate the overlap concentration ( $C^*$ ) of the xyloglucan chains as:  $C^* = 3M_w/(N_a 4\pi R_g^3)$ , with  $N_a$  Avogadro's number. Using the values of  $M_w$  and  $R_g$  from light scattering we find  $C^* = 0.3$  wt%.

**Table 1**  
Oligosaccharide composition and macromolecular characteristics of the xyloglucan used for this study.

	SEC <sup>a</sup>	LS <sup>b</sup>
$M_w/10^6$ (g mol <sup>-1</sup> )	3.0 ± 0.4	2.9 ± 0.04
$\bar{D}^c$	1.3	–
$R_g$ (nm) <sup>d</sup>	–	75.5
$C^*$ (wt%) <sup>e</sup>	–	0.3
$A_2$ (cm <sup>3</sup> mol/g <sup>2</sup> ) <sup>f</sup>	–	$2.0 \times 10^{-4}$
Oligosaccharides <sup>g</sup>	Mean ± SD (%) <sup>h</sup>	
XXG	1.5 ± 0.1	
XXXG	2.9 ± 0.2	
XLXG	–	
XXLG	36.3 ± 3.3	
XLXLG	17.4 ± 1.0	
XXXLG	18.0 ± 1.0	
XLXXG	16.9 ± 0.9	
XXXLG	10.4 ± 0.5	

<sup>a</sup> SEC, size exclusion chromatography at concentration of 0.05 wt%.

<sup>b</sup> LS, light scattering; Using the Zimm formalism and concentrations from 0.01 to 0.11 wt%.

<sup>c</sup>  $\bar{D}$ , is the dispersity ( $M_w/M_n$ ).

<sup>d</sup>  $R_g$ , is the root-mean square radius of gyration.

<sup>e</sup>  $C^*$  is the overlap concentration obtained from  $C^* = 3M_w/(N_a 4\pi R_g^3)$ .

<sup>f</sup>  $A_2$ , second virial coefficient.

<sup>g</sup> The standard oligosaccharide nomenclature (Fry, 1989)

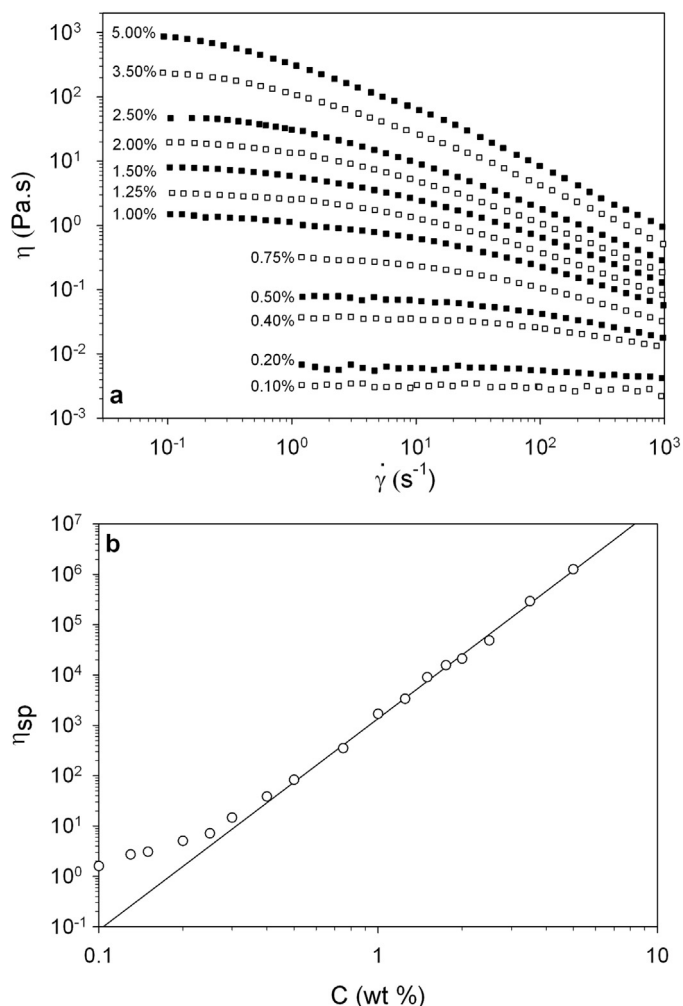
<sup>h</sup> The mean and standard deviation of three independent analyses.

## 5. Strong transient network

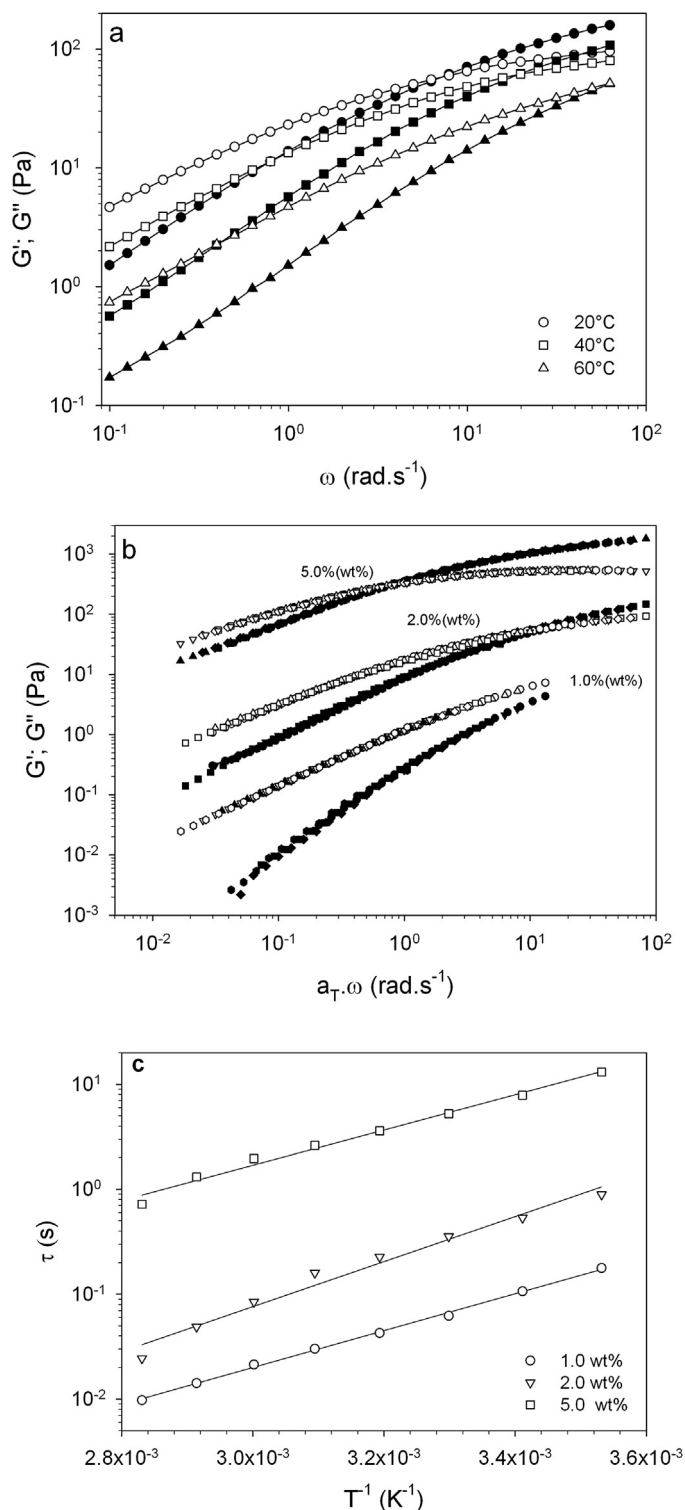
The viscosity as a function of the shear rate ( $\dot{\gamma}$ ) was determined for xyloglucan solutions at 20 °C, for concentrations ranging from 0.1 wt% to 5 wt%, see Fig. 2a. At 0.1 and 0.2 wt% the flow was Newtonian up to  $\dot{\gamma} = 10^3$  s<sup>-1</sup>, but at higher concentrations, a shear thinning behavior was measured. Shear thinning started at lower shear rate with increasing polymer concentration.

The specific viscosity ( $\eta_{sp} = \eta_0/\eta_s$ ) was calculated using the limiting value at low shear rates ( $\eta_0$ ) and the solvent viscosity ( $\eta_s$ ) and is plotted as a function of the concentration in Fig. 2b. The specific viscosity increased steeply with increasing concentration for  $C > C^*$  (0.3 wt%) where it can be well described by a power law:  $\eta_{sp} \propto C^{4.2 \pm 0.1}$ .

The frequency dependence of the storage ( $G'$ ) and loss ( $G''$ ) shear moduli at different temperatures up to 60 °C are shown in Fig. 3a for a xyloglucan solution at  $C = 2$  wt%. Similar results were obtained at other concentrations between 1 wt% and 5 wt%. Master curves could be obtained at all concentrations by frequency-temperature superposition using horizontal shift factors only, see Fig. 3b. Good superposition was found at high frequencies and temperatures up to 60 °C. When xyloglucan solutions were heated above 60 °C the shear moduli decreased less strongly at low frequencies. The origin of this phenomenon will be discussed hereafter.



**Fig. 2.** (a) Flow curves of xyloglucan solutions at 20 °C and at different concentrations indicated in the figure. (b) Specific viscosity ( $\eta_{sp}$ ) as a function of concentration for xyloglucan solutions, at 20 °C. The solid line represents a linear least squares fit to the results for  $C > 0.3$  wt%.

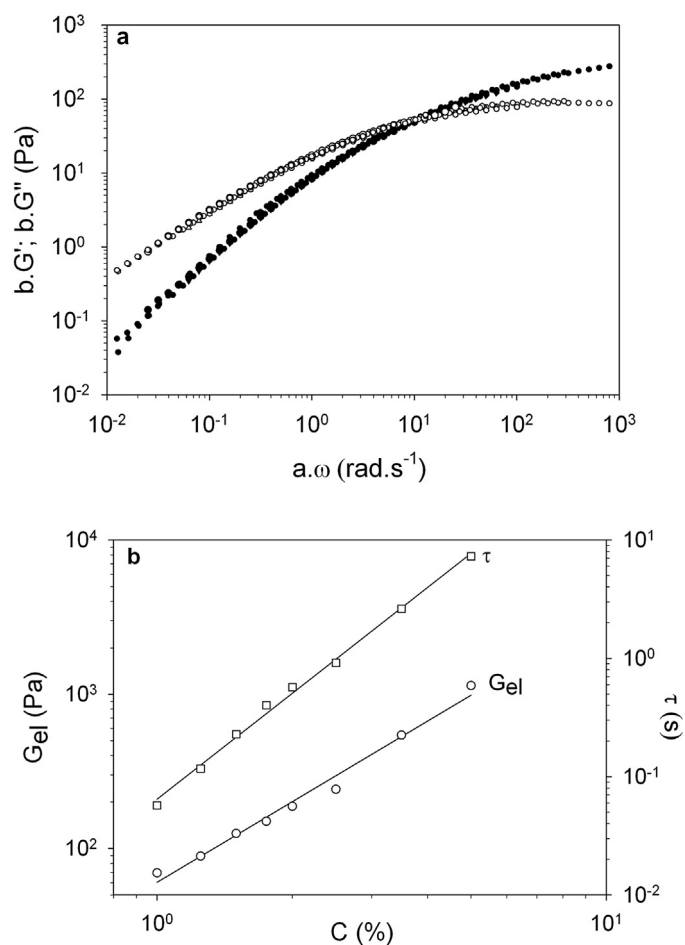


**Fig. 3.** (a) Frequency dependence of the storage (filled symbols) and loss (open symbols) moduli of a xyloglucan solution at  $C=2$  wt% at 20, 40 and 60 °C. (b) Master curves obtained by frequency-temperature superposition (10–60 °C) of xyloglucan solutions at different concentrations. Storage (filled symbols) and loss (open symbols) moduli. The reference temperature is 20 °C. (c) Arrhenius representation of the temperature dependence of the relaxation time of xyloglucan solutions at different concentrations. The solid lines represent linear least squares fits to the data.

The frequency dependence of  $G'$  and  $G''$  is typical for viscoelastic polymer solutions with a predominantly elastic behavior at high frequencies and viscous behavior at low frequencies. We define a characteristic relaxation time ( $\tau$ ), as the inverse of the frequency at which  $G'$  and  $G''$  cross ( $\tau = \omega_c^{-1}$ ). An Arrhenius representation of the temperature dependence of  $\tau$ , see Fig. 3c, shows that the relaxation is an activated process ( $\tau \propto \exp(E_a/RT)$ ) with an apparent activation energy  $E_a = 37 \pm 4 \text{ kJ mol}^{-1}$  that does not depend on the concentration.

For entangled polymer solutions the temperature dependence of the relaxation time is proportional to the solvent viscosity. Here we find that the temperature dependence of the relaxation time is much stronger. The stronger temperature dependence can be explained by the formation of cross-links with a finite lifetime that slow down Brownian diffusion of the chains. We therefore suggest that xyloglucan forms a transient network, i.e. a network with transient cross-links (Tanaka & Edwards, 1992). The cross-links were formed by specific interactions between the chains. The observation that no vertical shifts were needed to obtain the master curves implies that the cross-link density did not depend significantly on temperature.

Master curves obtained at different concentrations could be superimposed using both horizontal and vertical shift factors, see Fig. 4a. Fig. 4b shows that both, the relaxation time ( $\tau$ ) and the high



**Fig. 4.** (a) Master curves of the frequency dependence of storage (filled symbols) and loss (open symbols) moduli for xyloglucan solutions obtained by frequency-concentration superposition of individual master curves at eight different concentrations ranging between 1 wt% and 5 wt%. The reference concentration is 2 wt%. (b) Concentration dependence of  $G_{el}$  (Pa) and  $\tau$  (s) for xyloglucan solutions at 20 °C.



frequency elastic modulus ( $G_{el}$ ) increased with increasing concentration.

The values of  $\tau$  and  $G_{el}$  at lower concentrations, for which the crossover of  $G'$  and  $G''$  was outside the frequency experimental window were deduced from the shift factors. Here we arbitrarily define  $G_{el}$  as  $G'$  at  $100\omega_c$ . The concentration dependences of  $\tau$  and  $G_{el}$  between 1 wt% and 5 wt% can be described by power laws:  $\tau \propto C^{2.8 \pm 0.1}$ ,  $G_{el} \propto C^{1.7 \pm 0.1}$ , see Fig. 4b. The exponents are incompatible with that of semi-dilute entangled polymer solutions for which  $\tau \propto C^{1.6}$ ,  $G_{el} \propto C^{2.2}$  (Rubinstein & Colby, 2003) and corroborate the idea that a transient network is formed by specific interactions between the chains. Since  $\eta \propto G_{el}\tau$ , it follows that the power law exponent describing the concentration dependence of the viscosity (4.2) should be equal to the sum of the exponents for the concentration dependencies of  $\tau$  and  $G_{el}$  (4.5), which is the case here within the experimental uncertainty.

If we consider that the high frequency elastic modulus is due to rubber elasticity of the transient network then  $G_{el} \approx \nu RT$  (Rubinstein & Colby, 2003) with  $R$  the gas constant,  $T$  the absolute temperature and  $\nu$  the molar concentration of elastically active chains. We find that the average molar mass between cross-links ( $M_e = CRT/G_e$ ) decreased from  $3.5 \times 10^5$  g/mol at 1 wt% to  $1.0 \times 10^5$  g/mol at 5 wt%. Comparison with the weight average molar mass of the xyloglucan chains shows that the average number of transient cross-links per chain increased from 6 to 21 between  $C = 1$  wt% and  $C = 5$  wt%.

We have compared the dynamic complex viscosity ( $\eta^*$ ) deduced from oscillatory shear as a function of the frequency with the viscosity measured as a function of the shear rate, see Fig. S3 of the supplementary information. The results superimposed for  $C < 1$  wt%, but at higher concentrations  $\eta$  decreased more strongly with increasing shear rate than  $\eta^*$  with increasing frequency, as was already shown by Khounvilay and Sittikijyothin (2012). The implication is that the structure, and therefore the relaxation process of the xyloglucan solution, was modified above a critical flow rate that decreased with increasing concentration from  $20 \text{ s}^{-1}$  at  $C \leq 2$  wt% to  $0.3 \text{ s}^{-1}$  at  $C \geq 2.5$  wt%.

## 6. Weak quasi-permanent network

We mentioned before that when xyloglucan solutions were heated above  $60^\circ\text{C}$  the shear moduli decreased less strongly with decreasing frequency than for unheated solutions. This effect was much more pronounced when the heated solutions were cooled back to  $20^\circ\text{C}$ . The frequency dependence of  $G'$  and  $G''$  at different temperatures, measured after cooling to  $20^\circ\text{C}$ , is shown in Fig. 5 for a xyloglucan solution at  $C = 2$  wt%. Similar results were obtained at other concentrations between 1 wt% and 5 wt% (results not shown). When xyloglucan solutions were heated up to  $50^\circ\text{C}$ , and cooled to  $20^\circ\text{C}$  before measuring, no significant difference was observed compared to unheated sample. However, for solutions that were heated at  $90^\circ\text{C}$  the values of  $G'$  and  $G''$  at  $20^\circ\text{C}$  decreased in a less extend with decreasing frequency at low frequencies (see Fig. 5).

We suggest that this behavior was caused by the formation of a weak quasi-permanent network during heating. The expression “quasi-permanent” is used here to indicate that the cross-links either have a very long life time or are permanent. The stiffness of this network increased with decreasing temperature, which explains why  $G'$  at low frequencies was larger at lower temperatures. The stiffness of the weak network also increased with increasing polymer concentration, but it was in all cases more than two orders of magnitude lower than that of the transient network. This implication is that only a small fraction of the xyloglucan chains was involved in the formation of the weak quasi-permanent

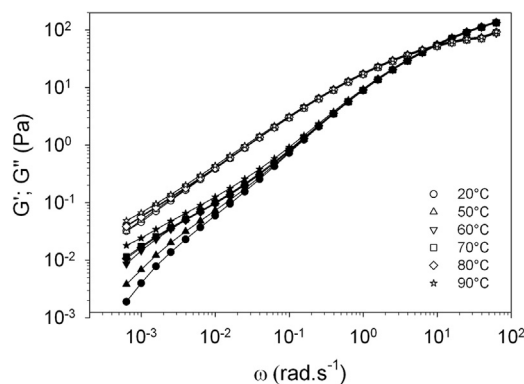


Fig. 5. Frequency dependence of storage (filled symbols) and loss (open symbols) moduli of xyloglucan solutions at 2 wt%, measured at  $20^\circ\text{C}$  after being heated at the different temperatures indicated in the legend.

network. The frequency dependence of the moduli before and after heating were the same at high frequencies where we measure the response of the strong transient network. This means that the formation of the weak quasi-permanent network did not have much impact on the strength or the relaxation of the strong transient network.

Repetitions with different heating times showed that the weak network was formed within a few minutes and that the moduli remained constant at longer heating times. However, the stiffness of the weak quasi-permanent network decreased with decreasing heating temperature. It was barely perceptible at  $60^\circ\text{C}$  and could not be detected at lower temperatures.

The dynamic viscosity at  $20^\circ\text{C}$  was determined as a function of increasing shear rate for a solution of xyloglucan at 2 wt% before and after heating to  $90^\circ\text{C}$ , see Fig. 6. For the unheated solution the viscosity was constant at low frequencies. However, for the heated solution the dynamic viscosity was much higher at low shear rates, which corroborates the observation that the shear moduli at low frequencies were higher for the heated solutions. The cross-links that were formed by heating were broken by the flow. The fraction of surviving cross-links decreased with increasing flow rate leading to a decrease of the dynamic viscosity until for  $\dot{\gamma} > 1$  it was the same as for the unheated solution.

In an attempt to establish whether the weak network was a solid with a yield stress or a viscous liquid with a finite zero-shear viscosity, the deformation under shear was measured as a function

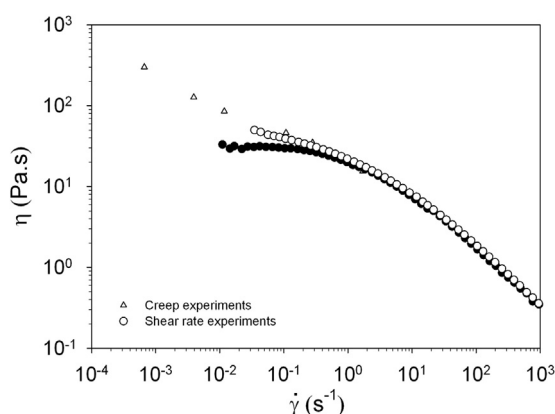
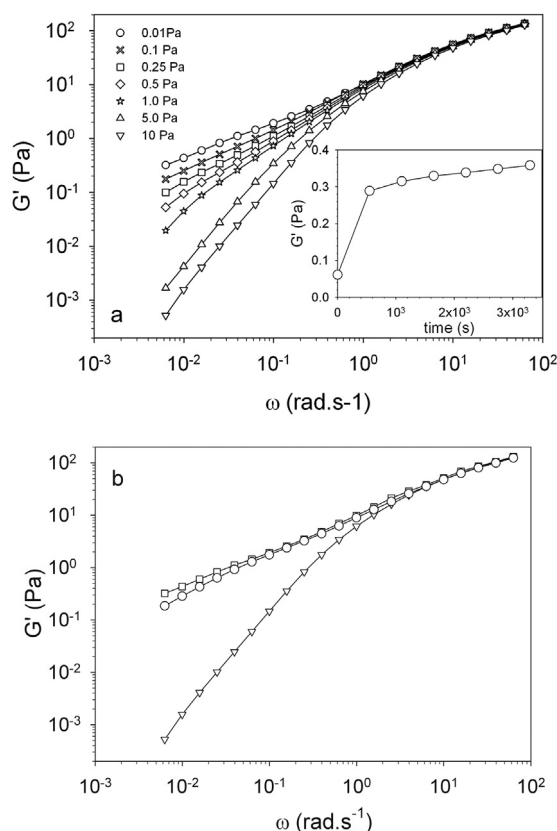


Fig. 6. Viscosity as a function of shear rate at  $20^\circ\text{C}$  for a xyloglucan solution at 2 wt% before (closed symbols) and after heating (open symbols) at  $90^\circ\text{C}$ . The values at low shear rates for the heated solution were obtained from creep measurements (open triangles).

of time at different shear stresses ( $\sigma$ ), see Fig. S4 of the supplementary information. For  $\sigma > 1$  Pa, regular shear flow was observed rapidly but at lower shear rates initially creep was observed and regular shear flow was recovered only after a time that increased with decreasing shear stress. For  $\sigma < 0.2$  Pa regular shear flow could no longer be detected within 15 h after the start of the experiment. Therefore we cannot tell whether the weak network was a permanent network with very low yield stress or a transient network with a very long terminal relaxation time. For this reason we used the expression quasi-permanent for denominating this network.

The effect of increasing the applied shear stress on the frequency dependent shear modulus is shown in Fig. 7a for a xyloglucan solution at  $C = 2$  wt% that was heated to  $90^\circ\text{C}$  and subsequently cooled to  $20^\circ\text{C}$ . The effect of the quasi-permanent network on the modulus at low frequencies disappeared when high stress was applied showing that the crosslinks of the quasi-permanent network had been broken.

The quasi-permanent network recovered only partially after breakage at  $20^\circ\text{C}$ , which is illustrated in the insert of the Fig. 7a, where the evolution with time of  $G'$  at  $\omega = 0.0628 \text{ rad s}^{-1}$  and at  $\sigma = 0.01$  Pa is shown after the solution had been sheared at  $\sigma = 10$  Pa. At  $20^\circ\text{C}$  recovery was still far from complete after 2 h. However, the original value of  $G'$  could be completely recovered quickly by re-heating the sample to  $90^\circ\text{C}$ , see Fig. 7b. These experiments show that crosslinks of the quasi-permanent network can be easily broken by shear stress. They reform only slowly and partially at  $20^\circ\text{C}$ , but rapidly at  $90^\circ\text{C}$ .



**Fig. 7.** (a) Frequency dependence of  $G'$  at  $20^\circ\text{C}$  obtained at different shear stresses for a xyloglucan solution at  $C = 2.0$  wt% after heating to  $90^\circ\text{C}$ . The insert shows the evolution with time of  $G'$  at  $\omega = 0.0628 \text{ rad s}^{-1}$ ,  $\sigma = 0.01$  Pa after shearing the solution at  $\sigma = 10$  Pa. (b) Frequency dependence of  $G'$  at  $20^\circ\text{C}$  for a heated xyloglucan solution at  $C = 2.0$  wt% at  $\sigma = 0.01$  Pa (squares) and at  $\sigma = 10$  Pa (triangles). These results are compared with those obtained after the solution that was sheared at  $\sigma = 10$  Pa was reheated to  $90^\circ\text{C}$  and then cooled to  $20^\circ\text{C}$  (circles).

The effect of heating on the structure of the polymers was evaluated by light scattering after cooling the solutions to  $20^\circ\text{C}$  and dilution. Heating up to  $70^\circ\text{C}$  did not have a significant effect on the scattering intensity. However, when solutions at  $C = 2$  wt% were heated at  $90^\circ\text{C}$ , we observed a strong increase of the scattering intensity measured at  $20^\circ\text{C}$  indicating that large aggregates had been formed, see Fig. S2c of the supplementary information. The aggregates could be removed or broken-up by filtration through  $0.22 \mu\text{m}$  pore size filter. The scattering intensity of the filtered solutions was the same as for the unheated solution, which means the weight fraction of the aggregated xyloglucan that was retained by the filtration was negligible.

The presence of aggregates in dilute xyloglucan solutions has already been reported in the literature (Freitas et al., 2005; Picout, Ross-Murphy, Errington, & Harding, 2003). The latter also reported increased scattering from large aggregates after heating and showed that they could be removed by filtration. Based on the dynamic mechanical properties discussed above, we speculate that these large aggregates were formed by the same bonds that led to the quasi-permanent weak network.

## 7. Discussion

Wang et al. (1997) suggested that relaxation of the imposed stress in xyloglucan solutions is due to reptation of non-interacting entangled polymer chains. For such a process the relaxation time is expected to be simply proportional to the solvent viscosity. However, the present investigation showed that the relaxation time was much more strongly dependent of the temperature, which indicates that there are specific attractive interactions, perhaps hydrogen bonds, between the sections of different xyloglucan chains in aqueous solution. The dynamic mechanical measurements of the unheated solutions can be well understood if it is assumed that a network is formed by the xyloglucan chains with cross-links that have a finite lifetime. According to Hayashi (1989), association between xyloglucan chains is not possible due to the large number of side chains. However, the xyloglucan also presents bare galactose moieties that can interact. Specific interactions were clearly observed after enzymatic modification of xyloglucan with  $\beta$ -galactosidase leading to an increased amount of free galactose moieties (Brun-Graeppi et al., 2010; Busato, Reicher, Domingues, & Silveira, 2009; Nisbet et al., 2006; Shirakawa, Yamatoya, & Nishinari, 1998). In these cases the interaction leads to formation of strong permanent gels after heating.

Observations similar to those reported here for xyloglucan were reported in the literature for aqueous solutions of guar gum (Wientjes et al., 2000) and HPMC (Shahin et al., 2013). For both systems the polysaccharide chains formed a relatively weak quasi-permanent network within a strong transient network. The frequency dependent shear moduli at higher frequencies corresponded to the response of the transient network, while the weak network manifested itself by a weak frequency dependence of  $G'$  at low frequencies. Also for these polysaccharides, the shear moduli obtained at different temperatures and concentrations could be superimposed at higher frequencies and the master curves were characteristic for a viscoelastic liquids with a broad distribution of relaxation times.

The properties of the strong transient network are remarkably similar for these three different polysaccharides. For all of them, the temperature dependence of the relaxation time was stronger than that of the solvent viscosity. The power law concentration dependence of  $\tau$  was stronger than for non-interacting entangled polymers and characterized by power law exponents that were close: 2.5, 2.8 and 2.8 for HPMC, guar and xyloglucan, respectively. The power law concentration dependence of  $G_{el}$  was

for all systems weaker than for non-interacting entangled polymers and characterized by the exponents 1, 1.8 and 1.7 for HPMC, guar and xyloglucan, respectively. The close correspondence of the concentration dependence of guar and xyloglucan is particularly striking.

Shahin et al. (2013) suggested that the relaxation of the HPMC transient network could be interpreted in terms of relaxation by reptation of entangled chains that form transient binary cross-links with a broad distribution of relaxation times (Rubinstein & Colby, 2003). However, Wientjes et al. (2000) concluded that their results for guar were not quantitatively compatible with this model.

The origin of the weak quasi-permanent network was interpreted differently for HPMC and guar. Shahin et al. (2013) suggested that it was caused by the formation of long rigid fibrils by a fraction of the HPMC chains that cross-linked to form the network. The fraction of chains that formed the fibrils was very small at room temperature, but increased with increasing temperature causing an increase of the elastic modulus of the quasi-permanent network. We will not consider here the behavior of HPMC above the critical temperature where it micro-phase separates.

Wientjes et al. (2000) proposed that two types of bonds were formed between the guar chains. The first type was relatively short lived and responsible for the formation of the transient network. The second type of cross-link was long lived and formed the weak quasi-permanent network. The observed increase of the elastic modulus of this network with increasing temperature suggests that the density of cross-links increased. However, if one considers the very small absolute values of the moduli, it follows that only a small fraction of the chains was involved in the network as was also the case for xyloglucan.

From this brief discussion of the results on HPMC and guar it is clear that the behavior of these polysaccharides is very similar to those obtained for xyloglucan reported here. The main differences are that xyloglucan formed the weak network only after heating and that its elastic modulus decreased with increasing temperature. We can only speculate about the physical origin of the two types of bonds leading to the two types of networks. Based on the temperature dependence Wientjes et al. (2000) suggested that the permanent bonds were caused by hydrophobic interactions. The opposite temperature dependence found for xyloglucan suggests that hydrogen bonds are involved. Interestingly, strong permanent hydrogels are formed both by guar (Wientjes et al., 2000) and xyloglucan (Brun-Graeppi et al., 2010; Busato et al., 2009; Nisbet et al., 2006; Shirakawa et al., 1998) when a significant fraction of galactose side chains has been removed enzymatically, which allows for stronger interaction between the backbones. In the case of modified guar, the gels formed when the systems were cooled, while for modified xyloglucan they formed upon heating. The opposite temperature dependence reflects the dependence of the stiffness of the weak network for unmodified guar and xyloglucan. This observation hints at the possibility that the behavior of unmodified guar and xyloglucan is caused by interaction between rare parts of the backbone that are devoid of galactose side chains. We speculate that unmodified xyloglucan formed the weak network only after heating, because weakening of the bonds by heating allowed exchange of the bonds between overlapping chains leading to the formation of a sparsely cross-linked percolating network.

It appears that the formation of two types of bonds in semi-dilute polysaccharide solutions leading to the formation of a transient and a quasi-permanent network, is a feature shared by different polysaccharides. However, the detailed characteristics are different, indicating that the mechanism of the transient and quasi-permanent network formation is specific for each type of polysaccharide.

## 8. Conclusion

In aqueous solution bonds with finite lifetime are formed between xyloglucan chains, which leads to formation of a transient network in semi-dilute solutions. The high frequency elastic modulus of this transient network is independent of the temperature, but the terminal relaxation time is characterized by an apparent activation energy of  $37 \text{ kJ mol}^{-1}$ . Both the elastic modulus and the relaxation time increase with increasing concentration following power laws. The viscoelastic relaxation is characterized by a broad distribution of relaxation times independent of the temperature and the concentration, that is probably caused by a distribution of bond strengths.

At temperatures above  $60^\circ\text{C}$ , a weak quasi-permanent network is formed by a small fraction of xyloglucan chains. The elastic modulus that is formed during heating increases with decreasing temperature during cooling after heat treatment. The weak bonds are easily broken by shear flow and recover only partially at room temperature. The visco-elastic behavior of semi-dilute xyloglucan solutions reflecting the presence of a transient and a quasi-permanent network is similar to that of other polysaccharides, in particular guar.

## Acknowledgements

We acknowledge the Brazilian funding agencies CNPq (Conselho Nacional de Pesquisa, process nº 477275/2012-5), Rede Nanobiotec/Capes-Brazil, project 34 and Nanoglicobiotec-Ministry of Science and Technology/CNPq nº 564741/2010-8. Rilton Alves de Freitas has a post-doctoral scholarship from CNPq (246301/2013-9).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carbpol.2015.04.066>

## References

- Brown, W. (1993). *Light scattering: Principles and development*. Oxford: Clarendon Press.
- Brun-Graeppi, A. K. A. S., Richard, C., Bessodes, M., Scherman, D., Narita, T., Ducouret, G., et al. (2010). Study on the sol–gel transition of xyloglucan hydrogels. *Carbohydrate Polymers*, 80, 555–562.
- Buckeridge, M. S., Crombie, H. J., Mendes, C. J. M., Reid, J. S. G., Gidley, M. J., & Vieira, C. C. J. (1997). A new family of oligosaccharides from the xyloglucan of *Hymenaea courbaril* (Leguminosae) cotyledons. *Carbohydrate Research*, 303, 233–237.
- Busato, A. P., Reicher, F., Domingues, R., & Silveira, J. L. M. (2009). Rheological properties of thermally xyloglucan gel from the seeds of *Hymenaea courbaril*. *Material Science Engineering C*, 410–414.
- Freitas, R. A., Martin, S., Santos, G. L., Valenga, F., Buckeridge, M. S., Reicher, F., et al. (2005). Physico-chemical properties of seed xyloglucans from different sources. *Carbohydrate Polymers*, 60, 507–514.
- Freitas, R. A., Martin, S., Paula, R. C., Feitosa, J. P. A., & Sierakowski, M. R. (2004). Effect of the oxidation level on the thermogravimetric kinetics of an oxidized galactoxyloglucan from *Hymenaea courbaril* (Jatobá) seeds. *Thermochimica Acta*, 409, 41–47.
- Fry, S. C. (1989). The structure and functions of xyloglucan. *Journal of Experimental Botany*, 40, 1–11.
- Hayashi, T. (1989). Xyloglucan in the primary cell wall. *Annual Review in Plant Physiology and Plant Molecular Biology*, 40, 139–166.
- Khounvilay, K., & Sittikijyothin, W. (2012). Rheological behavior of tamarind seed gum in aqueous solutions. *Food Hydrocolloids*, 26, 334–338.
- Martin, S., Freitas, R. A., Obayashi, E., & Sierakowski, M. R. (2003). Physico-chemical aspects of galactoxyloglucan from the seeds of *Hymenaea courbaril* and its tetraborate complex. *Carbohydrate Polymers*, 54, 287–295.
- Nicolai, T. (2007). Food structure characterization using scattering methods. In D. J. McClements (Ed.), *Understanding and controlling the microstructure of complex foods* (pp. 288–310). Cambridge: Woodhead.
- Nisbet, D. R., Crompton, K. E., Hamilton, S. D., Shirakawa, S., Prankerd, R. J., Finkelstein, D. I., et al. (2006). Morphology and gelation of thermosensitive xyloglucan hydrogels. *Biophysical Chemistry*, 121, 14–20.

- Picout, D. R., Ross-Murphy, S. B., Errington, N., & Harding, S. E. (2003). Pressure cell assisted solubilization of xyloglucans: Tamarind seed polysaccharide and detarium gum. *Biomacromolecules*, 4, 799–807.
- Reid, J. S. G. (1985). Cell wall storage carbohydrates in seeds. Biochemistry of the seed 'gums' and 'hemicelluloses'. *Advance in Botany Research*, 11, 125–155.
- Rubinstein, M., & Colby, R. (2003). *Polymer physics (Chemistry)*. USA: Oxford University Press.
- Shahin, A., Nicolai, T., Benyahia, L., Tassin, J. F., & Chassenieux, C. (2013). Evidence for the coexistence of interpenetrating permanent and transient networks of hydroxypropyl methyl cellulose. *Biomacromolecules*, 15, 311–318.
- Shirakawa, M., Yamatoya, K., & Nishinari, K. (1998). Tailoring of xyloglucan properties using an enzyme. *Food Hydrocolloids*, 12, 25–28.
- Sims, I. M., Gane, A. M., Dunstan, D., Allan, G. C., Boger, D. V., Melton, L. D., et al. (1998). Rheological properties of xyloglucan from different plant species. *Carbohydrate Polymers*, 37, 61–69.
- Tanaka, F., & Edwards, S. F. (1992). Viscoelastic properties of physically crosslinked networks. 1. Transient network theory. *Macromolecules*, 25, 1516–1523.
- Wang, Q., Ellis, P. R., Ross-Murphy, S. B., & Burchard, W. (1997). Solution characteristics of the xyloglucan extracted from *Detarium senegalense* Gmelin. *Carbohydrate Polymers*, 33, 115–124.
- Wientjes, R. H. W., Duits, M. H. G., Jongschaap, R. J. J., & Mellema, J. (2000). Linear rheology of guar gum solutions. *Macromolecules*, 33, 9594–9605.