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Viscometric studies on xanthan and galactomannan systems

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The synergistic effect obtained by mixing xanthan and galactomannans from Brazilian seeds (*Mimosa scabrella* Bentham and *Schizolobium parahybum* (Vell) Blake), with mannose:galactose ratios of 1.1:1 and 3:1 respectively, were examined. Viscosity measurements were performed under different conditions. A strong interaction was observed with xanthan:galactomannan (X:G) from *S. parahybum*, in water at 1 and 2 g/liter. For the mixture of xanthan:galactomannan from *M. scabrella* (X:G 3:1 2 g/liter, in water) despite the latter high galactose content, an increase in viscosity of 32% was observed over that calculated assuming no interaction. The results showed the formation of a gel-like structure with hysteresis between increasing and decreasing shear rate. The interaction occurred mainly when the system was previously subjected to 80°C in water, independent of the conformation of xanthan. The stronger interactions were obtained in aqueous systems. Since the main chain of the *M. scabrella* galactomannan is almost completely substituted, the synergistic effect must have an interaction mechanism different from that previously described by Dea and Morrison (1975), Dea *et al.* (1977), Morris *et al.* (1977), and Lundin and Hermansson (1995) in which xanthan interacts with galactomannan via the unsubstituted region of the latter. © 1997 Elsevier Science Ltd

INTRODUCTION

Synergistic polysaccharide–polysaccharide interactions are attractive commercially and enjoy widespread technological exploitation (Cairns *et al.*, 1987). Expensive polymers may be replaced by a cheaper mixture. When two polysaccharides are mixed, gelation can occur and several types of gel structure may arise, depending on the nature of the components, the rate and extent of polymer demixing, and the gelation mechanism (Morris and Miles, 1986). Xanthan and galactomannans interact in solution to give significant increases in viscosity and also gel formation (Dea and Morrison, 1975, Dea *et al.*, 1977, Morris *et al.*, 1977). Many studies have been carried out to elucidate the behavior of this system.

Galactomannans from native Brazilian species, *Mimosa scabrella* (bracatinga) and *Schizolobium parahybum* (guapuruvu), were investigated (Ganter *et al.*, 1993, 1995). Their seeds furnished high yields of galactomannans, whose mannose to galactose ratios (M:G) are 1.1:1 and 3:1, respectively. Their structures consist of polymeric main chains of (1→4)-β-D-mannopyranosyl residues substituted at O-6 by single-unit side chains of α-D-galactopyranose. Differences in fine structure, namely in the distribution of D-galactosyl units along the mannan chain, exist between galactomannans from different sources and these might be expected to modify interaction properties. Such differences were revealed by the action of mild acid hydrolysis. The oligosaccharides formed were isolated and examined by ^{13}C NMR spectroscopy (Ganter *et al.*, 1995). These galactomannans are non-gelling polysaccharides but give highly viscous aqueous solutions which are relatively stable against variations in pH, salinity, and temperature.

Xanthan gum is the exocellular polysaccharide of *Xanthomonas campestris*, the primary structure consisting of a (1→4)-β-D-glucopyranosyl backbone substituted at O-3 on every second residue with a charged trisaccharide side chain consisting of a glucuronic acid residue situated between two mannosyl units. The terminal mannosyl unit may be substituted at O-4 and O-6 by a pyruvic acid acetal moiety. An *O*-acetyl group is frequently present at O-6 of the internal mannosyl residues (Jansson *et al.*, 1975). Recent studies also suggested a partial acetylation at O-6 on the terminal mannosyl unit as well as a pyruvic acetal (Stankowski *et al.*, 1993). This polysaccharide adopts a helical conformation in aqueous solution. The temperature T_m characterizing the helix-coil transition increases when the ionic concentration increases. The helical conformation gives a stiffer macromolecule and it was shown that the conformation plays a large role in the viscosity of solutions (Milas and Rinaudo, 1984, 1986, Milas *et al.*, 1990). Xanthan solutions have a number of unusual rheological properties that include high viscosity and pseudoplasticity (Millane and Wang, 1990).

Many workers (Dea *et al.*, 1977, Morris *et al.*, 1977, Cairns *et al.*, 1986, Cheetham *et al.*, 1986, Lopes *et al.*, 1992) have proposed that the synergistic interaction between galactomannan and xanthan is based on a cooperative interaction, depending on the fine structure of the galactomannan. This association occurs between the backbone of the xanthan and unsubstituted parts of the galactomannan main chain, forming junction zones (Dea and Morrison, 1975). McCleary (1979) suggested that the striking difference in the degree of interaction with guar (M:G 1.6:1) and carob (M:G 3.4:1) galactomannans appears to be due only to a simple difference in the D-galactose content. However, a strong interaction was shown between xanthan and a galactomannan with a relatively high M:G ratio (*Leucaena leucocephala*, M:G 1.6:1), in which one quarter of the backbone is composed of regions of alternate galactosyl substitution, when compared with guar (M:G 1.6:1). In this case, it was suggested that regions are substituted on one side of the polymer, which is also capable of participating in interactions with xanthan (McCleary, 1979). Millane and Wang (1990) proposed that the specific intermolecular binding between xanthan and galactomannan is formed between mannan blocks and xanthan segments having a cellulose-like conformation.

Another interaction model was proposed by Tako *et al.* (1984) and Tako (1991) in which the charged trisaccharide side chains of xanthan and the unsubstituted backbone of the galactomannans are associated. Mannion *et al.* (1992) and Zhan *et al.* (1993) suggested that xanthan and galactomannan may interact by two distinct mechanisms. One of them requires heating of the polysaccharide mixture, which gives a strong gel which is highly dependent on the side chains of the galactomannan. The second takes place at room temperature and gives weaker, more flexible gels, whose rheological properties have little dependence on the galactose content of the galactomannan. The authors also concluded that the disordering of xanthan, rather than gel melting, is the dominant factor in the mixture. However, this mechanism of interaction remains controversial. Morris and Foster (1994) did not accept the latter proposition, and they suggested that the crucial factor is the thermodynamic stability of the heterotypic mixed junctions formed between xanthan and its cosynergist.

Lundin and Hermansson (1995) proposed a network model in which the larger xanthan superstrands are connected by bridges of smaller carob polymers, represented by their unsubstituted mannan backbones. When the mixture is heated above the xanthan T_m, the interaction between carob and xanthan is competitive since, when aggregates of xanthan helices are formed on cooling, the more substituted galactomannan is excluded from the xanthan aggregates and only binds to the surface of the xanthan superstrands. When mixed at room temperature, the less substituted galactomannan could bind to the surface of xanthan in the same manner as the more substituted.

The present study is to investigate the interaction between xanthan and two galactomannans from Brazilian seeds having different M:G ratios, namely the galactomannan from *M. scabrella* (M:G 1.1:1), a polymer almost totally substituted, and the less substituted galactomannan from *S. parahybum* (M:G 3:1).

EXPERIMENTAL

Xanthan gum, from Sigma Chemical Co., was dissolved in water by stirring (1 g/liter) overnight, at room temperature. The resulting solution was centrifuged at 20 000 *g* for 1 h. To the supernatant was added 20 g/liter of NaCl, stirred until its dissolution. The sodium salt of xanthan was successively precipitated by addition of ethanol (50% v/v) (Rinaudo, 1993), submitted to successive washings in water–ethanol mixtures from 70 to 100% v/v, and dried under vacuum at 30°C. Partial ^{1}H NMR spectra of a native sample were obtained in 5–7 mg/ml D_2O solutions at 85°C (BRUKER AC-300 spectrometer); suppression of the DOH signal showed the percentages of substitution, sodium acetate being used as internal standard. The specific rotation [α] of xanthan was measured in the range of 20 to 90°C, at 300 nm with a Fica Spectropol 1b spectropolarimeter with a 0.5 dm quartz cell thermostated with a Haake thermostat (0.4 g/liter polymer in each experiment). The $[\alpha]_D^{20}$ values of galactomannans were measured at 589 mn in an Automatic Digital Polarimeter ACATEC PDA 8 200 with a 2 dm cell.

Seeds of *M. scabrella* were collected from the metropolitan region of Curitiba, Paraná; seeds of *S. parahybum* were collected on the campus of Universidade do Rio dos Sinos, São Leopoldo, Rio Grande do Sul. The seeds were each bleached for 10 min in boiling water (Ganter *et al.*, 1996). The polysaccharides were extracted from crushed seeds of *M. scabrella* and the isolated endosperm of *S. parahybum* (yield 17% and 25% w/w, respectively) with water at 25°C for 4 h with mechanical stirring and then centrifuged at 2000 *g* for 15 min. The supernatant was precipitated with an equal volume of ethanol, washed, and dried, as described for xanthan.

Galactomannans were successively hydrolysed with 1 M trifluoracetic acid (4 h, 100°C), the hydrolyzates evaporated, and the residues reduced with sodium borohydride and the products acetylated with pyridine–acetic acid (1:1 v/v, 16 h, at room temperature). The resulting alditol acetates were analyzed by GLC using a model 5890 S II HP Gas Chromatograph at 220°C (FID and injector temperature, 250°C) with DB-210 capillary column (0.25 mm i.d. × 30 m), film thickness 0.25 μm, the carrier gas being nitrogen.

^{13}C NMR spectroscopy was performed on solutions of galactomannans that were submitted previously to sonication (Branson B2, 150 W apparatus), with a BRUKER AC-300 spectrometer at 75 MHz in the Fourier transform mode, with complete proton decoupling at 80°C, using D_2O as solvent contained in a tube of 0.5 cm i.d. The spectral width was 200 ppm. Chemical shifts were expressed in δ (ppm) relative to the resonance of DDS (sodium 4,4-dimethyl-4-silopentane-1-sulphonate), used as internal standard ($\delta = 0$).

Size exclusion chromatography (SEC) was carried out with an on-line differential refractometer, a low angle light scattering detector and a viscometer line (Tinland *et al.*, 1988). The eluent was 0.1 M NH_4NO_3 with 0.5 g/liter NaN_3, two Shodex columns, OH-pak 804 and 805, being used in series. The polysaccharide solution (1 g/liter) was filtered through a 0.2 μm membrane. The d*n*/d*c* of xanthan and galactomannan from *M. scabrella* was determined as 0.135.

Viscometric determinations were performed with a Brookfield LV DVIII ($10 < \dot{\gamma} < 350\,s^{-1}$) at 20°C. The solutions were obtained by mixing the pure gum solutions dispersed separately in distilled and deionized water or 0.01 M NaCl and stirred at room temperature for 14 h to a final concentration of 1 or 2 g/liter. The mixture with different proportions of the pure xanthan and galactomanan was prepared at 80°C, stirred for 10 min in a closed system, and the samples were then allowed to cool at room temperature and then at 4°C overnight. The water contents of both products were evaluated gravimetrically and were taken into consideration in all solution preparations. Carbohydrate contents were assayed by the phenol–sulfuric-acid method (Dubois *et al.*, 1956).

RESULTS AND DISCUSSION

Polymer characterization

Some properties of the galactomannans derived from *M. scabrella* and *S. parahybum* are shown in Table 1. The results obtained for xanthan are also included. The major models for intermolecular interaction between xanthan and galactomannan, previously described by Dea *et al.* (1977), Morris *et al.* (1977), McCleary (1979), Cairns *et al.* (1986) and Cheetham *et al.* (1986), show a close correlation with the degree of substitution of the mannan chain. The degree of substitution of the galactomannans from seeds of *M. scabrella* and *S. parahybum* was determined by GLC and ^{13}C NMR spectroscopy and the results are similar to those previously described (Ganter *et al.*, 1993). The specific rotations were, as expected, strongly influenced by the galactose content, as shown by Noble *et al.* (1986).

These galactomannans with different M:G ratios were chosen for the interaction studies. One of them, from the seeds of *M. scabrella*, has a high galactose content (M:G 1.1:1). There are only few examples of galactomannans described in the literature with this degree of substitution, such as lotus, lucerne and red clover (Sharman *et al.*, 1978, McCleary, 1979), However, their synergic interactions have not been studied. The other selected galactomannan was isolated from the endosperm from the seeds of *S. parahybum*,

Table 1. Polysaccharide analyses

Polysaccharide	M:G	$[\eta]_{25}$ [a] (ml/g)	M_w [a]	$[\alpha]^{D}_{20}$
Galactomannan from *M. scabrella*	1.1:1	740	1.4×10^6	+77
Galactomannan from *S. parahybum*	3.0:1	1 280	1.35×10^6	+32
Xanthan	—	3 850	3.5×10^6	−125

[a] Obtained using SEC with LALW, viscosity and refractive index detectors. Eluent (0.1 M NH_4NO_3).

and had an M:G ratio of 3:1. The differences in the chemical structure of these galactomannans are characteristic of the source of the materials.

The previously described structural characterisation of these galactomannans (Ganter *et al.*, 1992, 1993) suggests a random distribution of D-galactosyl groups along the main chain, which was in agreement with the structures of the oligosaccharides obtained by mild acid hydrolysis (Ganter *et al.*, 1995).

The use of steric exclusion chromatography (SEC) with multidetection shows the molecular weight distribution (M_w) and intrinsic viscosity $[\eta]$ for the different samples (Table 1). SEC using a light scattering detector is considered as the only valid method to characterize polysaccharides over a wide range of molecular weights (Rinaudo, 1993). Both galactomannans studied showed high and similar molecular weights (*M. scabrella* M_w 1.4×10^6 and *S. parahybum* M_w 1.3×10^6). The intrinsic viscosities agree with values given in the literature (Ganter *et al.* 1992; Cuvelier and Launay, 1988). The higher intrinsic viscosity for *S. parahybum*, with the same average molecular weight as *M. scabrella*, seems to indicate the presence of small fractions of loosely aggregated macromolecules.

^{1}H NMR spectroscopy was used to determine the ratio of pyruvate/acetate groups in a sample of purified commercial xanthan. Two peaks at δ 2.1 and 1.5 are attributed to acetyl and pyruvate groups, respectively, as previously described by Rinaudo *et al.* (1983) and Smith *et al.* (1981). Quantitative contents were calculated from the integrals of ^{1}H signals, in comparison to sodium acetate (internal reference): yields of pyruvate and acetate were approximately 33% and 35%, respectively, per repeat unit.

In order to establish the characteristic temperature (T_m) corresponding to the conformational change of xanthan, the chiroptical studies were carried out in water and 0.01 M NaCl, at a xanthan concentration of 0.4 g/liter, at increasing temperatures. The values of T_m were obtained at 62°C in 0.01 M NaCl, but no transition was observed in water. This dependence agrees with the usual role of external salt, which screens the intrachain electrostatic repulsions and stabilizes the helical conformation. Following these data, in water at room temperature and in water or 0.01 M NaCl at 80°C, this temperature was chosen for mixing the polymers, such that the xanthan is in the coil conformation.

RHEOLOGICAL PROPERTIES

Flow behavior of polysaccharides

Flow curves were determined over a range of polymer concentrations of 0.1 to 1.5 g/liter in water, and shear rate ($10 < \dot{\gamma} < 350\,\mathrm{s}^{-1}$), for galactomannans of *M. scabrella* and *S. parahybum*, and also for xanthan. No shear rate viscosity dependence was experimentally observed over this range of shear rates for the galactomannans and this confirms the results previously obtained for a larger range of shear rates ($10^{-2} < \gamma < 1000$) (Ganter *et al.*, 1992). For xanthan, Newtonian behavior in the same range of γ was obtained only at concentrations lower than 0.4 g/liter. Then, as would be expected, a transition from Newtonian to non-Newtonian behavior was observed, shifting to lower shear rates when the concentration increases (Milas *et al.*, 1990). The range of $\dot{\gamma}$ (10–$350\,\mathrm{s}^{-1}$) was chosen for experimental convenience, i.e. better stability of the measurements, in the range of $\dot{\gamma}$ covered and for the polymer concentration used, and the negligible effect of shear rate on viscosites.

Interaction of polysaccharides

Although neither xanthan nor galactomannans form gel separately, mixtures of the polysaccharides yield gels. In order to compare the behavior of a solution of xanthan mixed with each one of the two galactomannans, viscosity measurements were performed. The specific viscosity of mixed polysaccharides systems were plotted as a function of the mixing ratio for given shear rate ($\dot{\gamma} = 70\,\mathrm{s}^{-1}$) (Fig. 1).

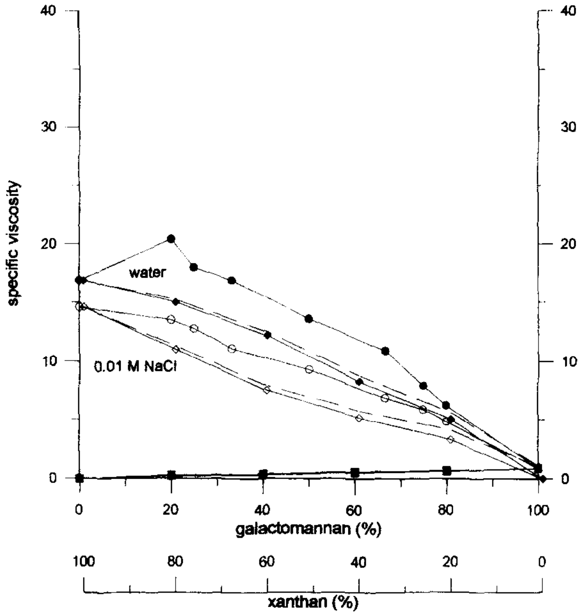

Fig. 1. Specific viscosities of pure polysaccharide solutions and their mixtures, preheated at 80°C, at a total concentration of 1 g/liter in water and 0.01 M NaCl, measured at $\gamma = 70\,\mathrm{s}^{-1}$ at 20°C: galactomannan of *M. scabrella* in water (■) and in salt (□); xanthan in water (◆) and in salt (◇); xanthan–galactomannan mixtures in water (•) and in salt (○); values calculated for mixtures assuming no interaction (- -).

Figure 1 shows the viscosity behavior of xanthan and galactomannan from *M. scabrella* at a total polymer concentration of 1 g/liter, in water and 0.01 M NaCl, respectively. The results show the influence of the conformation of xanthan on the increase of viscosity, which is supposed to reflect the intermolecular interaction. The xanthan is in the disordered conformation in water but in the ordered one in 0.01 M NaCl, as confirmed by optical rotational measurements. When 0.01 M NaCl was used, a smaller synergistic effect, compared with the results obtained in water, was observed. It can be seen that the maximum viscosity corresponds to a xanthan–galactomannan (from *M. scabrella*) (X:G) ratio of 4:1. At a total polymer concentration of 2 g/liter in water (Fig. 2), a greater interaction was observed and the maximum synergy obtained at a ratio of 3:1. These results reflecting interaction between the two polysaccharides were unexpected due to the high galactose content of galactomannan from *M. scabrella*.

In fact, McCleary (1979) proposed two schematic structures for the interaction between galactomannans and xanthan, involving free mannose of the first polysaccharide. One of these mechanisms implies an interaction between the unsubstituted parts of the galactomannan main chain, and the other does not absolutely require long sections of contiguous unsubstituted D-mannosyl residues, but rather sections where all the galactosyl residues are located on one side of the main chain and which may also serve as 'junction zones'. Such a theory better explains the lower but significant interaction of xanthan gum with guar galactomannan (G:M 1.6:1). No considered model predicts the results obtained with the almost completely substituted *M. scabrella* galactomannan (M:G 1.1:1).

Figure 3 shows the synergy of xanthan and the galactomannan from *S. parahybum* (M:G 3:1). A stronger increase of viscosity was observed with this system compared with that of the galactomannan from *M. scabrella*.

These results are in agreement with the previously proposed influence of the degree of substitution of the mannan chain (Dea *et al.*, 1977, Morris *et al.*, 1977, Cairns *et al.*, 1986, Cheetham *et al.*, 1986).

A maximum in synergistic effect was achieved in water when the mixing ratio of xanthan to galactomannan (X:G) was 1:1 but this only varies slightly over a larger range of composition (X:G 4:1 to 1:3). Similar results were also obtained at a total concentration of 2 g/liter; in contrast, a very small effect was observed in the presence of external salt.

From these data, it is clear that much larger interactions exist in water when the xanthan has the coiled conformation. When the polymers are mixed at a temperature (80°C), higher than T_m, in the presence of external salt, fewer interactions exist when the samples are cooled, as was previously shown (Lopes *et al.*, 1992)

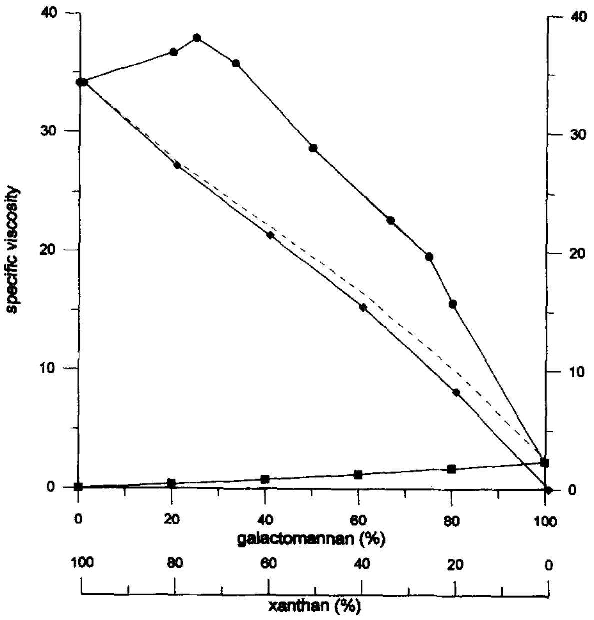

Fig. 2. Specific viscosities of pure polysaccharide solutions and their mixtures, preheated at 80°C, at a total concentration of 2 g/liter, in water, for $\gamma = 70\,s^{-1}$ at 20°C: galactomannan of *M. scabrella* (■); xanthan (◆); xanthan–galactomannan (•); values calculated for mixture assuming no interaction (···).

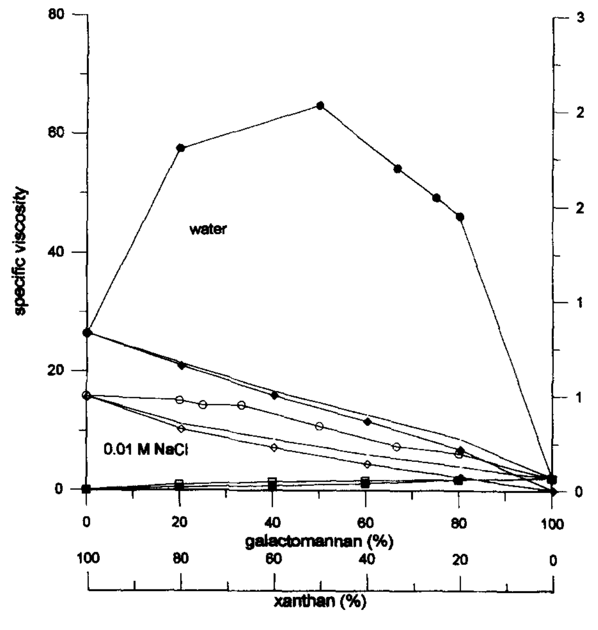

Fig. 3. Specific viscosities of pure polysaccharide solutions and their mixtures, preheated at 80°C, at a total concentration of 1 g/liter in water and 0.01 M NaCl, for $\gamma = 70\,s^{-1}$ at 20°C: galactomannan of *S. parahybum* in water (■) and in salt (□); xanthan in water (◆) and in salt (◇); xanthan–galactomannan mixtures in water (•) and in salt (○); values calculated for mixtures assuming no interaction (···).

Effect of temperature on polysaccharide interactions

In order to obtain information about the role of temperature on the interaction of xanthan and the galactomannan of *M. scabrella*, a new series of experiments was performed by mixing the polysaccharides at room temperature in water (Fig. 4). When the polymer mixture was prepared at 25°C the system showed less synergism than when prepared at 80°C in water (Fig. 2). Under these conditions, the xanthan molecules are in a disordered conformation, as confirmed by chiroptical studies.

The difference observed cannot be explained on the basis of a conformational transition; it is assumed that the decrease in the viscosity of the mixture when heated may explain the greater synergism obtained when the samples are cooled compared with the never heated samples.

Dynamics of polysaccharide interactions

In order to demonstrate the formation of a network between xanthan and the galactomannan of *M. scabrella*, the systems were submitted to viscosity measurements in the shear rate range of 10–100 s^{-1} at a temperature of 20°C. Viscosity measurements were performed with mixtures at total concentrations of 1 g/liter and 2 g/liter, in water. The thixotropic behavior was not observed at 1 g/liter for all X:G ratios tested.

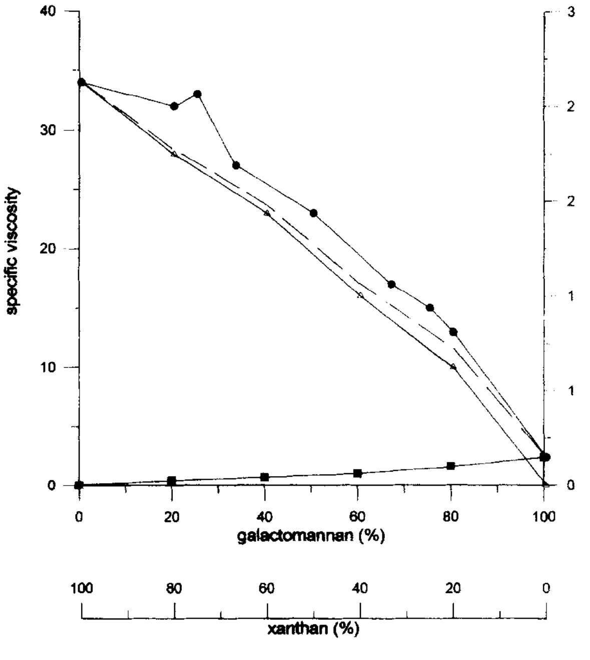

Fig. 4. Specific viscosities of pure polysaccharide solutions and their mixtures (prepared at 25°C), at a total concentration of 2 g/liter, in water, for $\gamma = 70\,s^{-1}$ at 20°C: galactomannan of *M. scabrella* (■); xanthan (Δ); xanthan–galactomannan (•); values calculated for mixture assuming no interaction (- -).

At 2 g/liter, substantial thixotropic behavior was obtained at a X:G ratio of 1:3 for the first cycle of the rheological measurements. Figure 5 shows that during a second cycle, after 120 min at rest, this behavior is less marked, probably due to the breakdown of a weak gel-like structure by shearing during the first passage.

Comparing the shear dependence on the system of galactomannan from *S. parahybum*, a similar thixotropic behavior was observed for all X:G ratios tested. Figure 6 shows that the thixotropic behavior is stronger at 2 g/liter, as compared with 1 g/liter, X:G ratio 1:2. This suggests an increase of the interaction

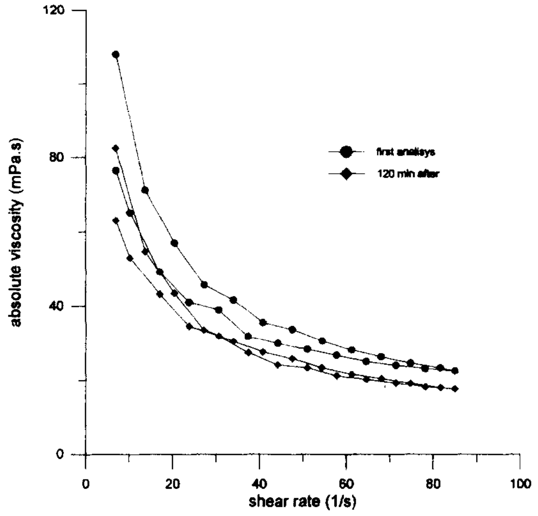

Fig. 5. Absolute viscosity in function of shear rate of analysis of xanthan galactomannan of *M. scabrella* (X:G 1:3), at 20°C. Total concentration 2 g/liter, in water, preheated at 80°C: first cycle (•); second cycle (◆).

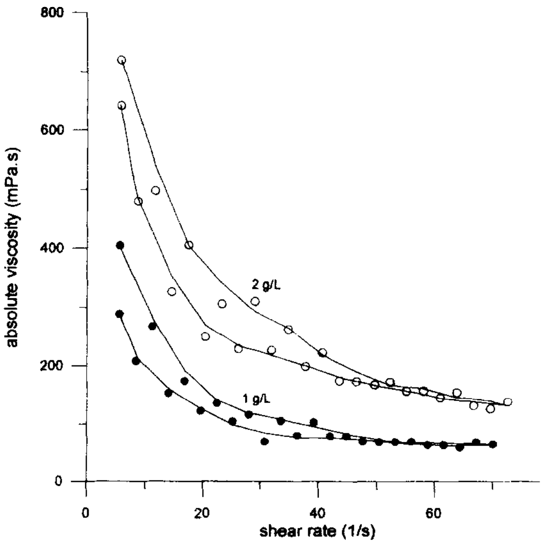

Fig. 6. Absolute viscosity in function of shear rate of xanthan–galactomannan of *S. parahybum* (X:G 1:2), at 20°C, preheated at 80°C, in water: total concentration of 2 g/liter (○) and of 1 g/liter (•).

when the polymer concentration increases and is in agreement with the results discussed previously for *M. scabrella* (Figs 1 and 2). The effect of shear rate is particulary important over the range of lower shear rates ($\gamma < 60\,s^{-1}$).

The experimental results obtained in this work demonstrate the synergism between xanthan and both galactomannans investigated. Taking into consideration the fine structure of the galactomannan from *S. parahybum*, which has unsubstituted regions on the mannan backbone, the mechanism of gelation is in agreement with the former models (Dea *et al.*, 1977. Morris *et al.*, 1977, McCleary, 1979, Cairns *et al.*, 1986, Cheetham *et al.*, 1986). However, it seems that the interaction with the galactomannan of *M. scabrella*, which is highly substituted with galactose on nearly every mannosyl unit, cannot be rationalized using any of the previously proposed models.

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