Associative phenomena in galactomannan-deacetylated xanthan systems

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Abstract

The interaction between mesquite seed galactomannan (MSG; D-mannose to D-galactose ratio ($M/G \sim 1.1$) and deacetylated xanthan (DX) in 5 mM NaCl leading to synergistic gel formation at 25 °C was investigated and compared with the far more studied system made of xanthan and locust bean gum (LBG; $M/G \sim 3.5$). Rheology and differential scanning calorimetry were used to measure temperatures of gel formation and transition enthalpy as a function of polymer composition, while circular dichroism was used to probe the conformation of DX in the LBG–DX system. MSG and DX associate at 25 °C with a well defined stoichiometry of 0.6:1.0 (w/w) at low ionic strength favouring the disordered coil state of DX. When LBG was used in place of MSG in water or 5 mM NaCl, two types of mechanisms of interpolymeric association are envisaged.

Keywords: Deacetylated-xanthan; Mesquite-seed-galactomannan; Locust-bean-gum; Associative-interactions; Gelation

1. Introduction

Mixtures of xanthan (X), perhaps the most extensively studied bacterial exopolysaccharide [1–3], with galactomannans, plant storage polysaccharides occurring in the endosperm of many legume seeds [4], have the capacity to form elastic gels in aqueous solution, while neither polysaccharide alone forms self-supporting gels [5–8]. This 'synergistic' gelation behavior is rather specific to a few pairs of families of polysaccharides, generally involving $\beta(1 \rightarrow 4)$ plant glycans, namely galactomannans or glucomonannans [9].

The ability of galactomannans to form synergistic gels with X increases with the proportion of unsubstituted mannose residues in the chain backbone. The degree of substitution in these polymers is defined as the molar ratio of D-mannose to D-galactose (M/G). Indeed, gel cohesion increases concomitantly with M/G in mixtures with X [10]. The original proposal to account for this kind of inter-polymer interaction invoked direct binding of barely substituted galactomannans, such as locust bean gum (LBG, $M/G \sim 4.0$), to the surface of ordered xanthan helices [11,12]. This model was later extended to account for the gelation observed in mixtures with galactomannans of greater galactose content than that of LBG obtained from *Leucaena leucocephala* ($M/G \sim 3.1$), rationalizing it as a result of attachment of unsubstituted sides of regularly spaced galactose substitutes along the mannan chain to the xanthan helix [11,13]. In mixtures of guar ($M/G \sim 2.5$) with xanthan, the limited proportion of unsubstituted regions was thought to lead to either only a moderate increase in solution viscosity [14] or gel formation when mixed with DX at 25 °C [15].

Abbreviations: CD, circular dichroism; DSC, differential scanning calorimetry; DX, deacetylated xanthan; ΔG, change in free enthalpy; G', storage modulus; G'', loss modulus; ΔH, change in enthalpy; KM, konjac glucomannan; LBG, locust bean gum; M/G, D-mannose/D-galactose ratio; MSG, mesquite seed galactomannan; M_w, weight average molecular weight; NMR, nuclear magnetic resonance; tan δ, tangent of the phase angle; ΔS, change in entropy; T_{gel}, rheological critical gel point; T_m, mid-point temperature of sol–gel transition enthalpy; T_{DX} and T_X, temperatures of conformational transition of deacetylated and native xanthan, respectively; X, xanthan.

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Experimental evidence shows consistently large increments in viscosity and development of a gel-like response in X–LBG mixtures, even at very low polymer concentration (0.02 g/l) [5–8]. Moreover, dilute solutions of disordered X or DX–guar mixtures (0.6–1 g/l), have also shown large increases in viscosity when the solutions were mixed and cooled below 26 °C [16]. This, and most experimental evidence is consistent with the proposal that the dominant mechanism of interaction in these systems is via direct heterotypic association of the polymers. However, exclusion effects, due to general polymer thermodynamic incompatibility, expected to arise upon mixing two different polymers have also been offered as an explanation to account for phenomena observed in X–guar mixtures at high polymer concentrations (20 g/l) in 0.13 M KCl [17,18].

X–LBG mixtures show broad transitions in differential scanning calorimetry (DSC), with no change in the temperature at the mid-point of the sol–gel transition (T_m 49 °C) both in the presence and absence of electrolyte, and with no displacement of the optimum composition ratio of the mixtures for maximum enhancement of gel strength, which means that LBG interacts with X in both environments [19]. Also, the sol–gel transition process appears to be completed over a wider range of temperature than in mixtures containing konjac glucomannan (KM) [20]. The onset temperature of gel formation and melting in mixtures of X or DX with LBG has been examined extensively by small deformation rheology and found to remain in the range of 43–60 °C in the presence or absence of salt [18,20–22]. Differences in temperature values reported may stem in experimental errors of the different methods and instruments used.

Mixtures of *Schizolobium parahybae* galactomannan ($M/G \sim 3.1$) with native X show both the T_m and T_{gel} of the sol–gel process during cooling centered at 25 °C. In contrast with LBG-containing systems, in *S. parahybae* galactomannan–X mixtures [23], the involvement of the disordered form of X was consistent with data showing that as neutralization of the acidic form of X proceeded in the absence of salt, thus effectively inducing the transition from the ordered (acid form) to the disordered (salt form) [24], the interaction with galactomannan grew progressively.

Evidence to support the hypothesis that the heterotypic association in mixtures of X with galactomannans of M/G lower than 3 is mediated by the disordered X conformer was obtained from the dependence of the circular dichroism (CD) spectra behavior of X in the acid form and at varying degrees of neutralization. The CD spectra revealed that as the X–galactomannan complex forms, it becomes ordered [23]. The role of M/G of the galatomannan has also been addressed on mixtures of xanthan with galactomannans from *S. parahybae* ($M/G \sim 3$) and from *Mimosa scabrella* ($M/G \sim 1.1$) at low ionic strength [25]. In contrast to the *M. scabrella* sample, the cooling traces of the storage modulus (G') of X–*S. parahybae* mixed gels showed a process (a 'bump') at 50 °C, which was related to the X–galactomannnan gelling process at 60 °C [18,20–22], along with a steep increase in G' at $T_{gel} = 24$ °C. This second interaction was close to the $T_{gel} = 20$ °C of *M. scabrella* mixtures. It was suggested that the strength of X–galactomannan interactions obtained in galactomannans of M/G values lower than 3, does not depend greatly on M/G. Two different mechanisms at high and low M/G values were proposed to mediate the interaction with X [25].

Independently, modeling of X-ray diffraction patterns of X–guar stretched fibers, suggests that different molecular arrangements for the X–galactomannan complex can be fitted, including a hybrid-helix assembly [26].

The aim of this study was to increase our understanding of the synergistic gelation of X and galactomannans with regards to the role of galactose substitution. The deacetylated xanthan (DX) used in this study was thought out to provide a model of stronger interaction with galactomannan. We confirm that the observed phenomena are the result of two different modes of interaction involving high and low galactose-substituted galactomannan species and give experimental data to show that the former follows a well defined stoichiometric process. A preliminary account of the present work has been published elsewhere [27].

2. Experimental section

2.1. Purification of polymers

X was purified directly from culture broth (Rhodia, Melle, France; batch No. I8A XE 9701207) by carefully preserving the native structure as described elsewhere [28]. Deacetylation of X was carried out by hydrolysis in 60 mM NaOH for 18 h at 4 °C under N_2 and neutralization with HCl to pH 7.3 before precipitation with ethanol. MSG was purified from a sample of galactomannan flour previously isolated from a mixture of pods from *Prosopis pallida* and *P. juliflora* species extracted by a pilot plant acidic process to remove the seed coat [29] and was kindly supplied by Dr. Gastón Cruz. LBG flour (Meyrpo fleur M-175 from Meyhall, Switzerland) was used in a previous study [20]. Both galactomannan flours were dispersed in water (6 g/l), dissolved by autoclaving for 20 min at 120 °C, left to cool and clarified by centrifugation for 30 min at $15\,000 \times g$. The supernatant was diluted with one volume of water and filtered through 3.0 (MSG and LBG) and 1.2 μm (only MSG) membranes. The polymer was

precipitated by addition of absolute ethanol to the polymer solution up to a final concentration of 36% ethanol (v/v), washed successively with 80, 85, 90 and 100% ethanol, and left to dry under vacuum at room temperature. Dextran was from Pharmacia Fine Chemicals (weight average molecular weight (M_w) 2.0×10^6).

2.2. Preparation of mixed solutions

Stock solutions of DX, MSG, LBG and dextran were prepared either in 5 mM NaCl (MSG–DX and MSG–DX–dextran mixtures) or in water, 5 and 100 mM NaCl (LBG–DX mixtures) by dissolving weighted dry polymers. Mixtures of polymers tested for rheology or DSC were prepared at DX concentrations of 3.0 or 1.0 g/l and adding the necessary amount of MSG, MSG and dextran, or LBG. The solutions were mixed at room temperature and loaded into a rheometer previously equilibrated at 70 °C, unless otherwise stated.

2.3. Characterisation methods

M/G in the galactomannan samples was determined by high-field proton nuclear magnetic resonance (NMR) (300 MHz) spectroscopy in a Bruker AC-300 [30]. M/G for LBG and MSG were 3.4 and 1.1, respectively. The M_w of MSG was assessed in 0.1 M NH_4NO_3 by steric exclusion chromatography using two columns (Shodex OH-Pak 804 and 805) arranged in series, with an 'on-line' multi-detector system: a multi-angle laser-light-scattering detector (Dawn, Wyatt CA, USA), a home-made capillary viscometer and a differential refractometer [31]. MSG had a $M_w \sim 2.1 \times 10^6$. Rheological measurements were conducted using either a Carri-Med CSL 50 rheometer fitted with a Rheo 1000C station or a Thermal Analysis TA-AR 1000 rheometer. Both instruments were stress-controlled fitted with a stainless steel cone and plate tool (cone angle 1°, diameter 60 mm, truncation 27 μm) and temperature control was exerted by Peltier effect. The TA-AR 1000 rheometer was operated with Rheology Navigator® software, which enabled multi-frequency wave measurements (so called 'on-the-flight') during the sol–gel transition. Drying of the samples was avoided by a metal solvent trap filled up with silicon oil. The cooling and heating rates were 1.0 °C/min. Low-amplitude oscillatory measurements were made within the linear viscoelastic strain (γ) region, as verified by strain sweeps on all solutions and gels tested. DSC measurements were made using a Micro DSC III microcalorimeter (Setaram, Caluire, France) equipped with 1 cm^3 batch vessels. The cooling and heating scan rate varied between 0.2 to 0.7 °C/min. CD was measured with a Jobin–Yvon Dichrograph IV over a range of $\lambda = 200$–300 nm with a 1 mm thermostated cell. Temperature was controlled using a Haake F3 circulating water bath fitted with a Haake PG10 programmer.

3. Results

3.1. MSG ($M/G \sim 1.1$)–DX in 5 mM NaCl

The variation in conformational transition temperatures of X (T_X) with ionic strength (comprising the conditions tested for the studied mixtures, namely water, 5 and 100 mM NaCl), has been determined by optical rotation and DSC measurements [33]. From these data and under the assumption that T_X and T_{DX} follow the same variation with ionic strength (with $T_{DX} \approx T_X - 10$ °C [32,33]), the estimated T_{DX} values were 15, 35 and above 100 °C in water, 5 and 100 mM NaCl, respectively.

Fig. 1 shows the temperature course evolution of storage (G') and loss (G'') moduli during cooling from 60 to 10 °C of mixtures of MSG present at two different concentrations (1.5 and 4.0 g/l) with DX (3.0 g/l) in 5 mM NaCl. Under this condition the T_{DX} is expected to be centered at 35 °C. The first rheological process appears for the MSG–DX 1.5:3.0 mixture, when G' exceeds G'' at 37 °C, nearly at the T_{DX}, followed by the onset of the steep rise in G' centered at 25 °C. In the MSG–DX 4.0:3.0 mixture, the crossover of G' and G'' is shifted to higher temperature (47 °C), with a sharp increase in G' also centered at 25 °C, overlapping closely with that of the 1.5:3.0 MSG–DX mixture. Both regions of sharp increase in G' at 25 °C are accompanied by smaller increases in G''. The first rheological process in MSG–DX mixtures occurs at a slightly lower temperature than that reported for LBG–(DX or X) or KM–(DX or X) synergistic gels characterized by gelling temperatures around 50 and 60 °C, respectively, within ionic strengths from 0 to 40 mM [19–21]. In the study by Bresolin et al. [25], the role of ionic impurities (protein) of *M. scabrella* ($M/$

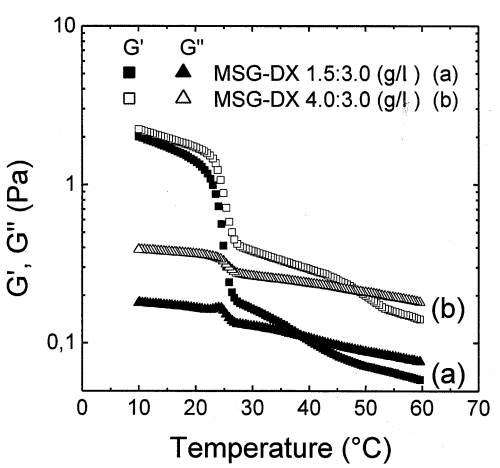

Fig. 1. Temperature dependence of G' and G'' ($\omega = 0.2$ Hz; 10% strain) during cooling (1 °C/min) of mixtures of DX (3.0 g/l) and MSG at: (a) 1.5 g/l or (b) 4.0 g/l in 5 mM NaCl. Measurements were recorded on a TA-AR 1000 rheometer.

$G \sim 1.1$) galactomannan in mixtures with X has been shown to affect the conformational transition temperature (T_m) and transition enthalpy (ΔH) of X. It was shown that in X–*M. scabrella*–galactomannan (3.9% protein) mixtures, increase in the proportion of the galactomannan component, leads to a decrease in ΔH, along with an increase in the T_m, due to the stabilization of the helical conformation.

Clearly, the second rheological event observed in the MSG–DX mixtures occurs within a narrow temperature range (24–27 °C) as a highly cooperative process. In this respect, MSG–DX co-gels differ drastically from those of LBG–DX occurring over a broader range of temperatures, but resemble the cooperative gelation process reported in KM–DX or KM–X co-gels [20]. The storage modulus (G') of MSG–DX gels at 10 °C, is smaller by about two orders of magnitude when compared with that reported for gelling mixtures of KM with DX or X [20,34] and even lower than that of LBG–X mixtures [18,20], having roughly the same range of polymer composition (X concentration 2.4–3.0 g/l) and cooled from $T \geq 60$°C. Interestingly, synergistic gelling mixtures of galactomannan from *Schizolobium parahybae* with a degree of galactose substitution ($M/G \sim 3.1$) slightly lower than that of LBG ($M/G \sim 3.5$) but greater than that of MSG ($M/G \sim 1.1$), had G' modulus at 10 °C, larger only by an order of magnitude than those MSG–DX gels within the similar range of composition (2.0:4.0 g/l) [23]. Therefore, the M/G seems to determine the density of elastically effective junctions (hence G') of the gel for these systems.

We have previously demonstrated [27] that the crossover of G' and G'' in the MSG–DX systems (referred to here as the first process) cannot be taken as a criterion of the critical rheological gel point (T_{gel}), since the contribution of entanglement couplings with long lifetime in the sol to the G' modulus increases as the temperature decreases. The convergence of tan δ at varying frequency has been adopted consistently as a theoretical and experimental criterion of the incipient creation of a permanent gel network. The crossover of G' and G'' can be related to the conformational change of DX (T_{DX}) and the slight displacement of this crossover temperature can be due to ionic impurity in MSG as discussed above in connection to the study by Bresolin et al. [25]. Indeed, for MSG–DX mixtures of polymer concentration (~ 1 g/l of DX), careful analysis of T_{gel}, positions it in the range 24–27 °C, with a slight elevation in T_{gel} as the proportion of galactomannan in the mixture exceeds a MSG/DX weight ratio 0.6.

A detailed comparison of experimental G' and G'' values (Fig. 1), indicates that there is a slight enhancement of the overall gel strength as the concentration of MSG in the mixture increases from 1.5 to 4.0 g/l (compare in Fig. 1 traces a and b). We previously reported [27] that a progressive increase in MSG concentration added to 1 g/l of DX leads to a gradual elevation of G' up to a maximal point, when the MSG–DX mixing ratio is close to 0.6:1.0, and beyond which further increase in MSG concentration does not cause significant evolution of G' [27]. This pattern of behavior seems to persist regardless of the overall polymer concentration.

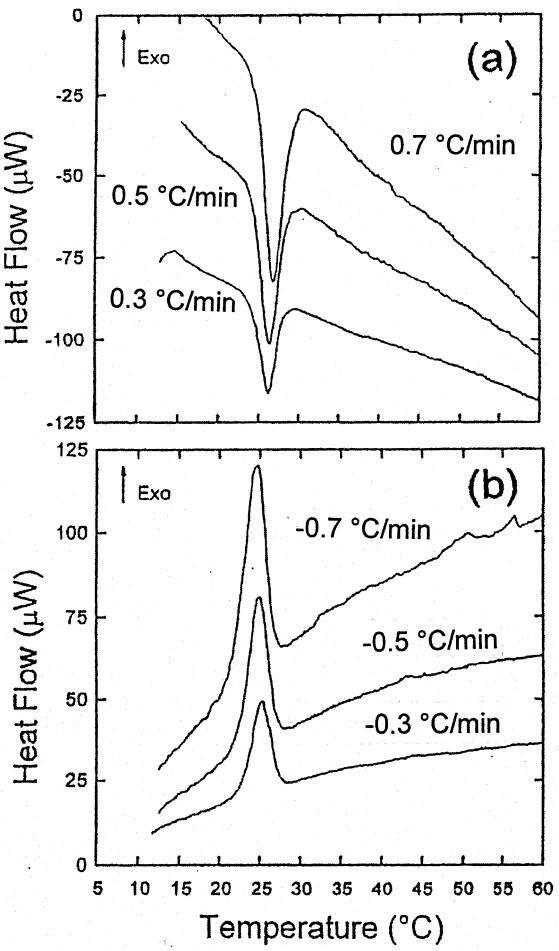

Fig. 2. DSC traces of a DX/MSG mixture (0.9:1 g/l in 5 mM NaCl) during heating (a) or cooling (b) at the scan rates (°C/min) indicated.

DSC thermograms for a MSG-DX mixture of concentration 0.9:1 g/l during cooling or heating at varying scan rate are shown in Fig. 2. The shape of the peaks either during heating (Fig. 2a) or cooling (Fig. 2b) corresponds well to that of highly cooperative processes (2–3 °C) and is similar to that observed in previous studies for mixtures of X and galactomannan of the endosperm of *S. parahybae* ($M/G \sim 3.1$) [23]. Similar cooperative reversible DSC thermograms have been observed in KM–X systems, although the T_m is shifted consistently to 60 °C [19,20]. Inspection of the thermograms in Fig. 2 reveals that the temperature envelope of the exo and endotherms at varying scan rate seems to correspond well with T_{gel} values 25.0 °C [27]. The apparent thermal lag observed between the setting

and melting thermograms was investigated in further detail from the T_m values recorded at different scan rates for heating and cooling mixtures of varying composition. Fig. 3a, shows a 'zero' scan rate extrapolation of T_m values recorded by DSC of MSG–DX mixtures in the domain of MSG concentration 0.1–1.0 g/l. Multiple T_m values at the same scan rate on the heating or cooling lines in Fig. 3a co-respond with MSG–DX mixtures of varying composition (i.e. most scans were recorded at 0.5°C/min). Further close inspection of Fig. 3a, shows that T_m values for the cooling and heating direction, do extrapolate virtually to the same T_m of 25.9 °C at 'zero' scan rate. While in the MSG–DX 4.2:1.0 g/l mixture (Fig. 3b), it can be appreciated that T_m values converge to a somewhat greater temperature (26.5 °C) after extrapolation to 'zero' scan rate, in line with T_{gel} values at the same concentration [27]. The slightly greater T_{gel} and T_m (max 0.6 °C) beyond the 'stoichiometric' point (weight ratio MSG/DX 0.6), is

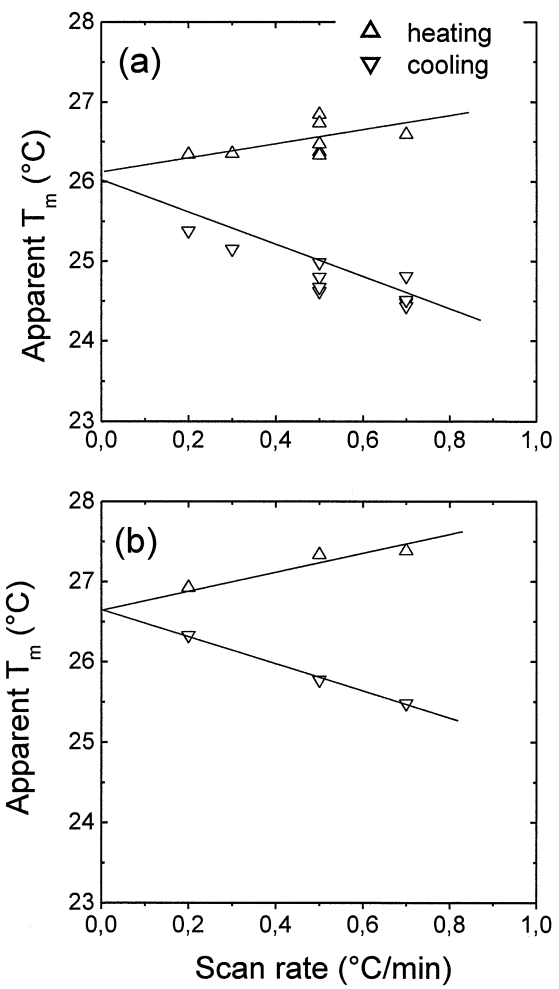

Fig. 3. Scan-rate dependence of T_m for the DSC transition during heating (△) or cooling (▽) for mixtures of DX (1.0 g/l in 5 mM NaCl) and MSG at concentrations: (a) varying from 0.1 to 1.0 g/l or (b) 4.2 g/l.

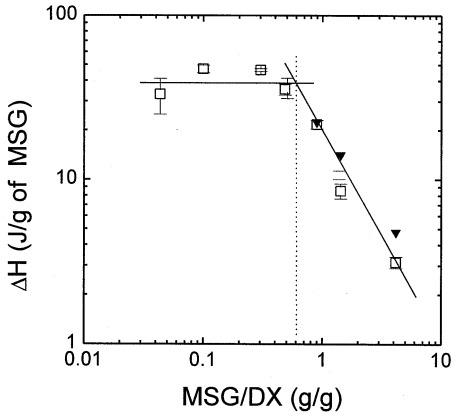

Fig. 4. Experimental (□) and calculated (▼) variation of DSC transition enthalpy (expressed in J/g of MSG) with MSG/DX ratio in 5 mM NaCl. Calculated data were obtained assuming full complex formation at a DX/MSG ratio = 0.5. Error bars represent data collected at various scan rates during heating or cooling traces at fixed composition.

diagnostic of a marginal stabilization effect, perhaps the consequence of a tendency of the system to slow chain dynamics expected for a gel network immersed in a sol dense entangled network, thus effectively providing a slight entropic advantage for the formation of the heterotypic structure at slightly greater temperature, since at T_m $\Delta G = 0$ and hence $T_m = \Delta H/\Delta S$. It is interesting to notice that the T_m value found from these extrapolations of MSG–DX mixtures (25.9 to 26.5 °C), lie above the T_m at 20 °C reported on *M. scabrella* galactomannan–X mixtures [25]. Perhaps this difference in the stability of the mixed gels involving native X or the deacetylated derivative DX, is a consequence of slightly different spatial arrangement in the heterotypic complex related to the acetyl groups position [16].

It has previously been shown that the T_m values of DSC peaks recorded during heating and cooling scans (at 0.5 °C/min) as a function of log[MSG] describe two distinct domains of behavior both during cooling or heating scans. At MSG concentrations of 0.04–0.6 g/l and DX 1 g/l, T_m values seem to show a marginal decrease with MSG concentration. However, at MSG concentration greater than 0.6 g/l T_m values increase with log[MSG], in good agreement with the pattern of behavior observed in T_{gel} values [27]. This well defined MSG/DX weight ratio matches maxima in G' values and minima in phase angle ($\tan\delta$) at the critical gel point [27].

Further experimental evidence, consistent with the proposal that the associative interaction between DX and MSG is governed predominantly by a binding process, came from the analysis of the enthalpy data obtained from DSC peaks. The composition dependence of the transition enthalpy (ΔH) expressed as J/g

of MSG is illustrated in Fig. 4. The plot shows that initially, ΔH values, if expressed in terms of MSG content in the mixtures, remain constant (ΔH around 40 J/g MSG) as the concentration of MSG increases until a MSG/DX ratio 0.6 g/g. Increasing MSG concentration beyond this point results in a monotonic reduction in $\log(\Delta H)$. Included in the plot are calculated data assuming simple additivity of surplus 'unbound' MSG ($\Delta H = 0$) beyond the optimum composition ratio for heterotypic association (0.6:1.0 g/g). The excellent agreement between experimental and calculated values is noteworthy.

It could be argued that polymer exclusion could account for the reduction in enthalpy beyond the optimum binding ratio and the increase in T_m, thus effectively leading to local concentration of NaCl due to unequal solvent partition between the sol and gel phases destabilizing the heterotypic complex. An increase in T_{gel} beyond the optimal mixing ratio [27], as the 'ordered' complex is confined to one phase, may be consistent with this idea. In order to test this hypothesis the following experiment was devised: high M_w (2×10^6) dextran was added to a (1:1) MSG–DX co-gel aiming to drive exclusion of the galactomannan–DX network into one phase. T_{gel} of such a triple system was compared with respect to a control MSG–DX mixtures (no added dextran) of ratios 1:1 and 4:1 (g/l). G' and G'' temperature traces during cooling for the 1.0:1.0 (g/l) MSG–DX mixture were used as controls in the dextran 'exclusion' experiments (Fig. 5). These temperature traces were recorded on the TA-AR 1000 stress-controlled rheometer, making use of the harmonics facility to perform multi-frequency wave determinations which allow generation of multiple stress signals of varying frequency per oscillating cycle, by virtue of which the process of gel formation can be monitored 'on-the-flight', particularly in the vicinity of the sol–gel transition. This approach confirmed that the sol–gel transition is centered around 25 °C. Fig. 6a–c, show rheological critical gel point (T_{gel}) analysis for temperature traces of $\tan\delta$ at varying harmonic frequencies ($\omega = 0.2$–2.0 Hz). It is noteworthy, that the high precision achieved by this means is superior to that observed when individual $\tan\delta$ traces are recorded at different frequency [27]. T_{gel} values for both the 1:1 MSG–DX (25.0 °C) and the 4:1 MSG–DX (26.5 °C) lie within experimental error of the T_{gel} sol–gel curve generated from data obtained by coincidence of individual $\tan\delta(\omega)$ temperature traces on cooling scans [27]. Notice that T_{gel} 25.0 °C for the 1:1:10 (g/l) MSG–DX–dextran mixture does not differ whatsoever from that of the 1:1 MSG–DX mixture. This result argues against the hypothesis that polymer exclusion contributes to the observed increase in T_{gel} when MSG concentration increases, at least under the conditions used.

3.2. LBG ($M/G \sim 3.1$)–DX systems

The role of the conformation of DX on the behaviour of LBG–DX mixtures was re-examined under different electrolyte conditions. As it has previously been discussed, the thermal stability of DX ordered conformation is highly dependent on the ionic strength, hence the DSC and rheology experiments described below for LBG–DX mixtures were conducted under ionic conditions, such that T_{gel} was below, close to and well above T_{DX}. Evolution of G', $G''$and $\tan\delta$ during cooling of LBG–DX (0.3:1.0 g/l) co-gels from 70 to 16 °C in water, 5 or 100 mM NaCl are shown in Fig. 7. The T_{gel} which in this case is marked as the crossover point where $G' = G''$ of the mixture in 100 mM NaCl (Fig. 7a), when $T_{gel} < T_{DX}$, is centered at 56 °C. While in 5 mM NaCl (Fig. 7b), when $T_{gel} \approx T_{DX}$, T_{gel} was shifted to 48 °C. In water (Fig. 7c), when $T_{gel} > T_{DX}$, gels form under a much less cooperative process. Indeed, for temperatures greater than 55 °C, G' and G'' moduli are nearly equivalent and both increase, but G' more rapidly than G'' as temperature decreases. Higher values of G' and G'' in the water traces compared with those shown in 5 and 100 mM NaCl at high tempera-

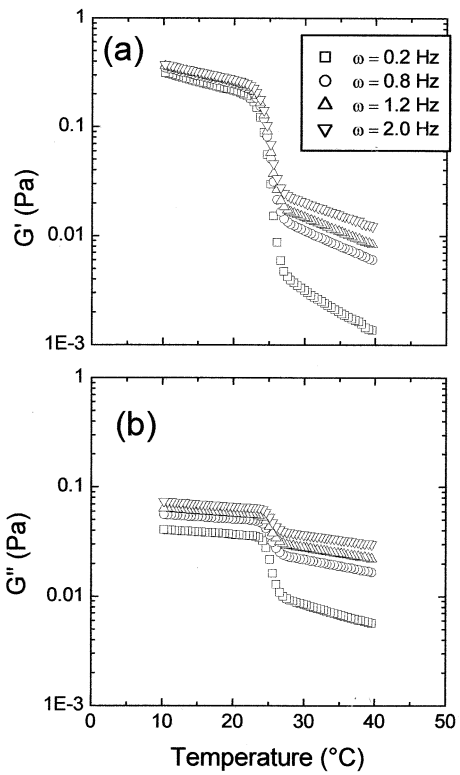

Fig. 5. Variation of (a) G' and (b) G'' (in 5 mM NaCl, 2% strain) for a mixed MSG/DX gel (1:1 g/l) with temperature during cooling (at 1 °C/min). Mechanical moduli were recorded ('on-the-flight') by multi-frequency wave stress at frequencies (Hz): 0.2 (□) fundamental; and (○) 0.8; (△) 1.2 and (▽) 2.0, harmonic. Measurements were performed on a TA-AR 1000 stress-controlled instrument.

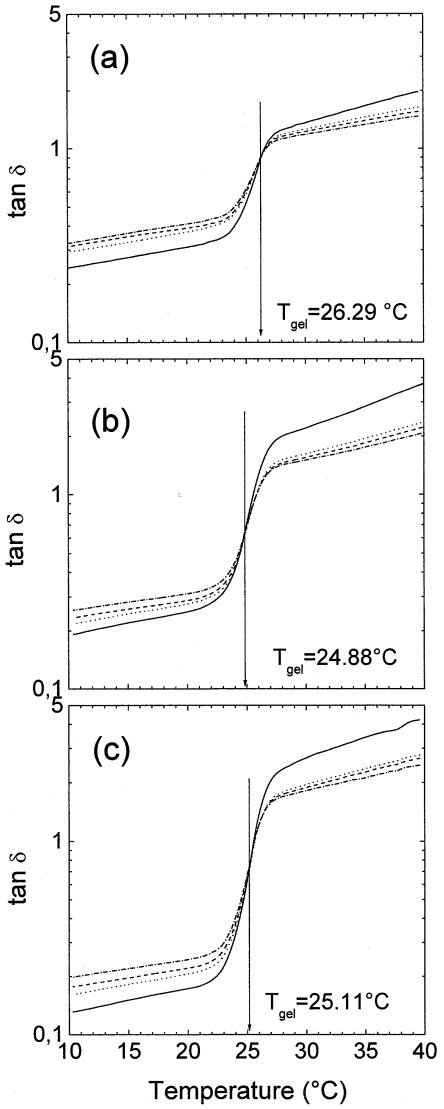

Fig. 6. Temperature dependence of tan δ during cooling (1 °C/min) registered at varying multiwave stress (at frequencies from top to bottom in the high temperature side 2.0, 1.2, 0.8 and 0.2 Hz; strain 2%) for mixed gels of DX (1 g/l in 5 mM NaCl) with (a) 4.2 g/l MSG (b) 1.0 g/l MSG or (c) 1.0 g/l MSG and 10 g/l dextran. Data were acquired as described in Fig. 5.

ture can be attributed to the expansion in macromolecular dimensions expected for DX polyelectrolyte in the absence of added electrolyte. In the G' temperature trace, there is evidence of a second 'step' in the G' and tan δ traces centered at 27 °C. This second process seems to be present also in the G' cooling trace in 5 mM NaCl (Fig. 7b) though it is virtually masked by the network set up at high temperature. Notice that the final values of G' for the LBG–DX co-gels at low temperature in the presence of 5 and 100 mM NaCl are nearly identical. However, in both cases, final G' values are greater than those in water, while final tan δ values are lower in NaCl than in water by an order of magnitude, suggesting major differences in the nature of the cross-linking arrangements in the gel networks.

The existence of a second process occurring at a lower temperature than that of the main sol–gel transition in LBG–DX systems in 5 mM NaCl was confirmed from an experiment using the TA rheometer and the harmonics multi-frequency wave program. Fig. 8 shows a set of temperature traces of G' and G'' moduli during the cooling of an LBG–DX mixture (0.5:1.0 g/l) from 35 to 10 °C, heating to 70 °C and cooled again to 10 °C. Before loading, the mixture had never been heated above room temperature and appeared as a fluid

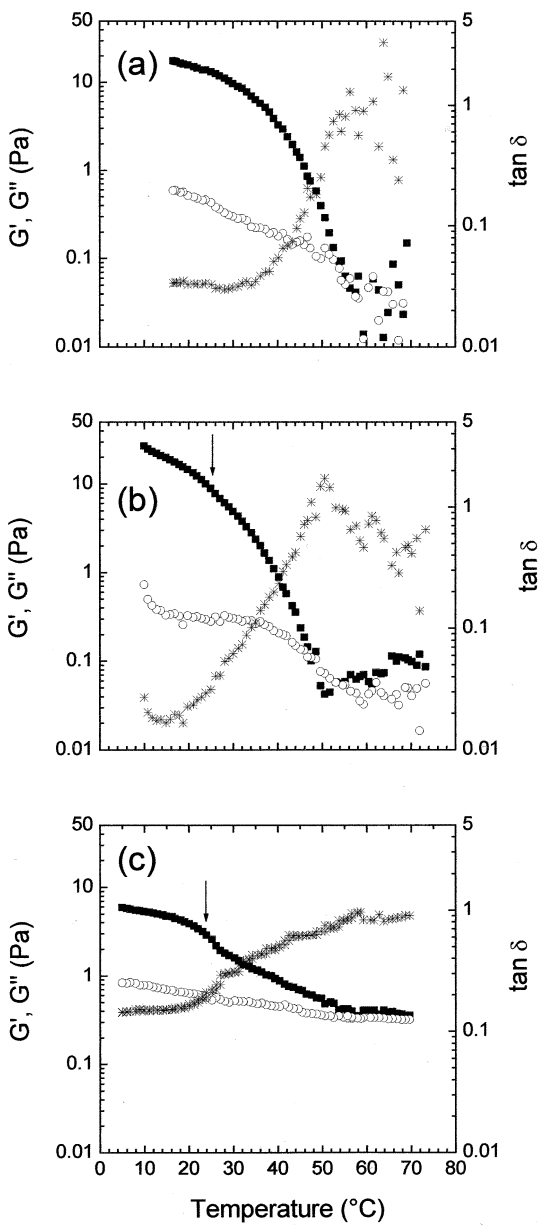

Fig. 7. Temperature dependence of G' (■), G''(○) and tan δ (*) for DX (1 g/l) mixed with LBG (0.3 g/l) during cooling in: (a) 100 mM NaCl; (b) 5 mM NaCl and (c) water. Measurements were recorded on a Carrimed CSL-50 stress-controlled instrument at 20% strain and $\omega = 0.8$ Hz.

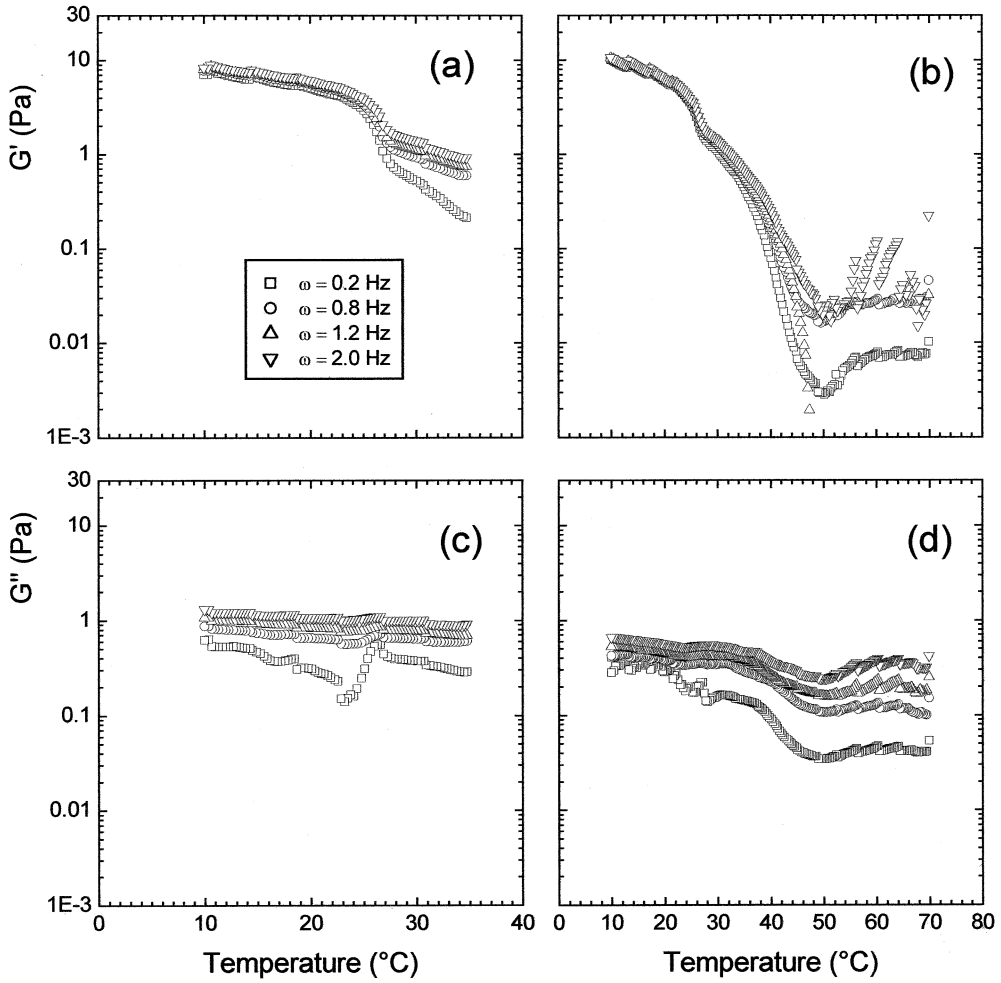

Fig. 8. Temperature dependence of G' (a and b) and G''(c and d) for a mixed solution of DX (1 g/l) and LBG (0.5 g/l) in 5 mM NaCl during cooling (1 °C/min) from 35 °C (a and c) or from 70 °C (b and c) at varying harmonic frequencies (2% strain) as indicated. Multi-frequency wave ('on-the-flight') measurements were recorded as described in Fig. 5.

material. Cooling from 35 to 10 °C, results in the consolidation of a viscoelastic gel, with a clear steep increase in the G' traces recorded at varying ω (Fig. 8a). T_{gel} of this process was 28 °C from the individual tan δ traces (not shown) and as cooling proceeds beyond this temperature, G' traces converge to roughly similar values at low temperature. The corresponding mechanical response in G'' (Fig. 8c) for this thermal sequence, revealed a maximum at 27 °C and a minimum at 23 °C at $\omega = 0.2$ Hz. This phenomenon is likely to reflect the behavior of molecular species with long relaxation times tight in heterotypic elastic junctions, as they start to form. Interestingly, no such anomalous features were observed under similar conditions in MSG–DX systems. When the gel is heated to 70 °C and cooled back to 10 °C, G' and G'' traces describe the behavior shown, respectively, in Fig. 8b and d. The first elevation in G' values starts at 48 °C while the second 'step' in the trace occurs at 27°C, coinciding with the process recorded when cooling from 35 °C. Indeed G' values within the second thermal transition, are nearly identical when cooling from 35 or from 70 °C. The maximum/minimum observed in G'' on cooling from 35 °C, is replaced by a 'bump' in the traces recorded at low ω. At 30 °C the interaction corresponding to the first process takes place without need of further heating.

A small exothermic peak centered at about 27 °C was observed in LBG–DX mixtures in 10 mM KCl when the DX concentration (1 g/l) exceeded 3-fold that of LBG (0.3 g/l) [20]. The original explanation for this peak was that it corresponded to the disorder–order transition of surplus DX. The T_m of this process was lower than that of DX alone under identical conditions, presumably due to short sequences of uncomplexed DX. An alternative explanation [23] suggested that the origin of this peak be related to the association between xanthan and galactomannans of any degree of galactose substitution. However, no further systematic studies had been conducted to understand the dependence of this phenomenon on the specific conditions used.

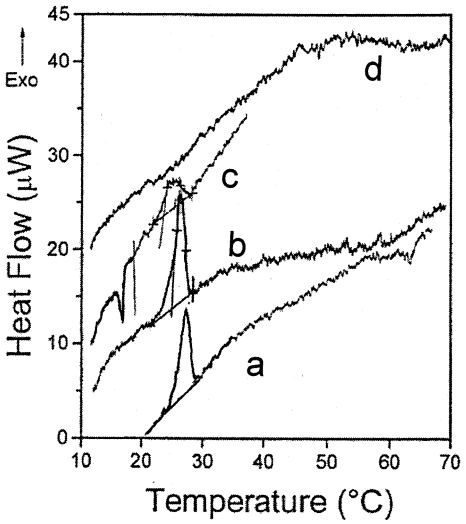

Fig. 9. DSC traces of DX (1 g/l) mixed with LBG (0.3 g/l) during cooling (0.5 °C/min) in: water (trace a); 5 mM NaCl (trace b) or 100 mM NaCl (trace d); or with LBG (0.5 g/l) in 5 mM NaCl (trace c).

A set of representative thermograms for LBG–DX mixtures is shown in Fig. 9 and data for the series of studied systems are summarized in Table 1. Well-defined exothermic processes were observed on cooling mixtures of DX (1 g/l) with 0.3 g/l of LBG in water (trace a) or in 5 mM NaCl (trace b) and with 0.5 g/l of LBG also in 5 mM NaCl (trace c). The T_m of the observed peaks in NaCl (traces a–c) were between 25.0 and 27.4 °C. In 100 mM NaCl (trace d), no discernible peaks were registered. Further close inspection of individual DSC traces reveals unequivocally that no changes in heat capacity can be resolved from the baseline at the onset temperature for gel formation (43–56 °C) in these systems, while the thermal events observed in water and 5 mM NaCl, correspond well with those registered as a 'step' rise in G' (Fig. 7b and c). When the concentration of LBG in the mixture increases from 0.3 to 0.5 g/l in 5 mM NaCl (trace c), the peak is notably smaller and broader than the former one with T_m of 25.4 °C. DSC results recorded in the LBG–DX mixtures studied in water and 5 mM NaCl are compiled in Table 1. Note that under both conditions as the polymer concentration increases in favor of the galactomannan component, ΔH decreases until it eventually vanishes when LBG concentration is 2 g/l. In general, for mixtures of equivalent composition, ΔH and T_m values for the low temperature process in water consistently exceed those in 5 mM NaCl, while in 100 mM NaCl this event is not discernible (Fig. 9d). This fact is consistent with the proposal that the proportion of disordered DX determines the strength of the interaction at 25 °C.

The experimental evidence presented here in LBG–DX systems seems to agree well with the proposal that the amount of 'disordered' DX that survives at low temperature after it has interacted with all LBG available chains, is what drives further association with galactomannan at low temperature. Accordingly, as long as the proportion of disordered X which 'survives' to the association with low-galactose galactomannan is sufficient to interact with remaining high galactose species, at lower temperature, then the low temperature process is observed. In line with this proposal, for low-galactose galactomannans, the microheterogeneity of the galactomannan fine structure controls the balance between the two processes. The low cooperativity and density of associative interactions of the high temperature process could explain the failure of micro-DSC to resolve a thermal transition around the onset tem-

Table 1
DSC of DX at 1 g/l mixed with LBG at varying concentration in water and 5 mM NaCl

Concentration of LBG (g/l)	Solvent	Scan rate (°C/min)	T_m (°C)	ΔH (mJ/g)[a]
0.3	Water	0.5	28.27	9.3
0.5	Water	0.5	27.76	9.2
1.0	Water	0.5	27.28	7.0
2.0	Water	0.5	26.46	3.0
0.3	Water	−0.5	27.00	7.8
0.5	Water	−0.5	26.44	10.6
1.0	Water	−0.5	25.75	5.3
2.0	Water	−0.5	No peak	No peak
0.3	5 mM NaCl	0.5	27.60	5.9
0.5	5 mM NaCl	0.5	26.71	3.1
1.0	5 mM NaCl	0.5	26.51	2.8
2.0	5 mM NaCl	0.5	No peak	No peak
0.3	5 mM NaCl	−0.5	26.38	6.0
0.5	5 mM NaCl	−0.5	25.13	3.2
1.0	5 mM NaCl	−0.5	24.90	2.7
2.0	5 mM NaCl	−0.5	No peak	No peak

[a] Expressed in mJ/g of solution.

perature of gel formation. This effect is probably also related to the absence of a X conformational transition. At temperatures lower than 30 °C, further association of the polymers depends directly on the conformation of X (therefore, on electrolyte concentration), and on the weight ratio of each. The junctions formed at lower temperature are thought to be the same as those formed between galactomannans with a wide range of galactose content ranging from nearly fully substituted to $M/G \sim 3.0$ and native X or DX [23,25,27,35].

Perhaps two interpenetrated networks co-exist involving heterotypic junctions between galactomannans of low and high galactose content with DX. Under this interpretation, if the X component is 'limiting' with respect to a galactomannan bearing enough barely substituted regions, it will get fully engaged in the single high temperature network of greater thermodynamic stability.

In order to gain further support for this interpretation, CD spectroscopy was used to probe DX conformation in the two 'putative' forms of interaction with LBG. The CD spectra of an LBG–DX (0.3–1.0) mixture was measured in 5 and 100 mM NaCl as shown in Fig. 10 and was compared with DX alone under identical conditions. The CD spectra in 5 mM NaCl at 20 °C (Fig. 10a) shows a shift for LBG–DX mixture compared with DX, which is partially disordered under these conditions. This displacement confirms the complex formation between galactomannan and partially disordered X documented in previous studies [23,25]. At 35 °C (Fig. 10b) in 5 mM NaCl, after melting of the low temperature gel, the two CD spectra become similar and are diagnostic of a decrease in the degree of X helicity. In 0.1 M NaCl at 20 °C (Fig. 10c; i.e. in absence of a step in G' trace and DSC peak on cooling down), virtually no difference in CD spectra is observed between the fully ordered DX and LBG–DX. These results suggest the existence of a second form of interaction between DX and LBG which appears at lower temperatures than those at which the gels form (40–50 °C) and only under conditions that destabilize the ordered conformation of the xanthan polymer.

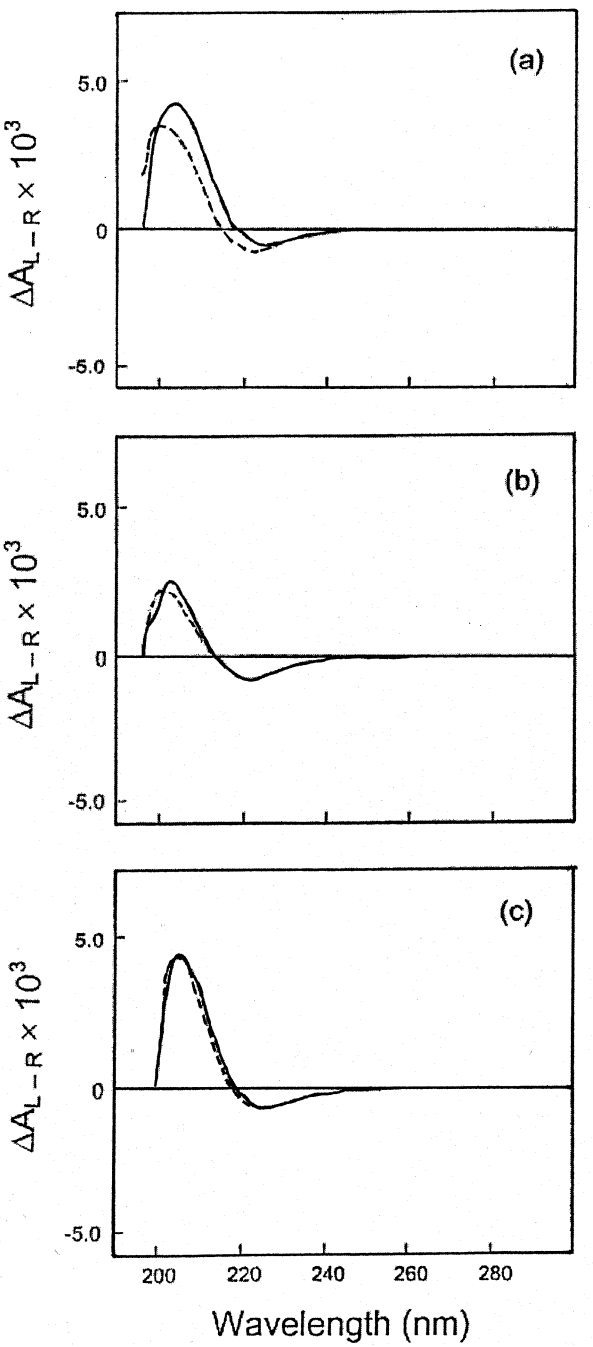

Fig. 10. CD spectra of DX alone (1 g/l, dashed lines) and mixed at the same concentration (continuous lines) with LBG (0.3 g/l) in 5 mM NaCl at (a) 20 °C and (b) 35 °C and (c) in 100 mM NaCl at 20 °C.

4. Discussion

The interaction between MSG and DX reported in this investigation occurs at room temperature (25 °C) and corresponds exactly to that of galactomannan–X systems previously characterized [23,25,35]. It appears to take place between galactomannans irrespective of galactose content and X, under conditions that favor its disordered conformation (namely low ionic strength and high degree of neutralization) [23]. Chandrasekaran and Radha [26] based on X-ray diffraction patterns registered on guar–X stretched oriented fibers, recently visualized molecular models of heterotypic structures of X and galactomannans, which can account for the phenomena addressed and interpretations offered in this investigation. At least four sterically feasible molecular models for the X–galactomannan complex have been proposed, which are appropriate for a galactomannan of any general side-chain composition. One of these predicts a segment of double heterotypic helical structure, involving one chain of X and one of galac-

tomannan. Indeed, in the MSG–DX binary mixed system, if the ratio of mass per unit length (M_L) of each polysaccharide species is considered, namely MSG $M_L = 60$ g/Å and DX $M_L = 90$ g/Å, hence a MSG/DX concentration (w/w) ratio of 0.66 is obtained for a 1:1 contour length ratio of the two polymers.

The present investigation confirms the interaction between X or DX with galactomannans of low M/G as previously proposed [22,23,25], under a mechanism that has the following hallmarks: (1) a transition with a lower thermal stability close to room temperature (T_m and T_{gel} 25–27 °C); (2) high cooperativity, large enthalpy (40 J/g of galactomannan); and (3) an optimum stoichiometry (w/w) at 0.6 to 1.0 galactomannan–xanthan ratio. The stoichiometric optimum of mixing in the case of a fully substituted galactomannan chain corresponds almost perfectly to a 1:1 heterotypic complex. Gel formation T_{gel} and micro-DSC T_m are independent of ionic strength, but appear only in a very narrow range of ionic strengths where the conformational preference of X is the disordered one. This association results in the formation of a viscoelastic gel network.

The evidence presented here, along with other studies conducted in parallel, indicates that this interaction involves high-galactose galactomannan species and disordered X, which adopt an ordered state when complexed. In many respects, this heterotypic interaction resembles that which has been more studied between X and KM (large cooperativity, sharp stoichiometry, and large changes in enthalpy associated).

In the low galactose substitution galactomannan mixed systems, two different kinds of heterotypic junctions can be formed between X and either barely substituted mannan blocks of the galactomannan chain (as it was proposed before by Mannion et al.[22] in LBG), or else fully substituted ones (i.e. as in MSG and *M. scabrella* galactomannan) [23,25]. As shown in Table 1 and Fig. 9, two different permanent gel networks are formed between LBG and DX in either water or in the presence of low salt concentration. Indeed, in water, only a very loose interaction between X and low-galactose LBG can explain the observed values of G' and G'' that are lower than those in 5 or 100 mM NaCl. This suggestion is in favor of the hypothesis for the interaction between ordered X and mannan blocks (i.e. the high temperature process). In the case of water, in which the high temperature interaction is not favored and if sufficient remaining X is available in coil form, the lower temperature association is clearly enhanced at the expense of the former one. In 5 mM NaCl, it is demonstrated that the two mechanisms co-exist (two thermal transitions are observed by sensitive rheology); but in 100 mM NaCl where the xanthan molecule is ordered throughout the range of temperature covered (70–10 °C), the high temperature process dominates.

It was also shown that the overall degree of complex formation decreases when the proportion of LBG increases progressively in the mixture, thus effectively 'using up' all the X (1 g/l), until it vanishes completely at LBG concentration of 2 g/l. We, therefore, conclude that for a very narrow salt concentration, the formation of complexes between LBG and DX seems to follow two different competing mechanisms of different thermodynamic stability. Indeed, only in 5 mM NaCl are the two mechanisms of gelation seen clearly to coexist.

For many years it was accepted that only galactomannan-unsubstituted regions attached to X chains. However, this particular view is now challenged in order to account for the results documented here and in related studies.

Acknowledgements

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