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Tangible evidence of the tranformation from enthalpic to entropic gellan networks at high levels of co-solute

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Abstract

Transmission electron microscopy was employed to examine the hypothesis, reached earlier using rheological and thermal analysis, of the changing nature of a polysaccharide network with increasing levels of sugar. Mixtures of deacylated gellan (< 1%) with low (0–20%) and high (80–85%) levels of sugar were prepared. Micrographs of the high sugar/gellan gels produced clear evidence of reduced crosslinking in the polysaccharide network, which exhibits a transition from rubber to glass-like consistency upon cooling. © 2002 Elsevier Science Ltd. All rights reserved.

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In the last ten years or so, we have been working on the transition from rubber to glass-like consistency in mixtures of polysaccharides at normal levels of industrial use (up to 2%) and high levels of sucrose and/or glucose syrup (60–85%) (Kasapis, 1998). Properly hydrated polysaccharides are brought in the disordered chain conformation and then mixed with co-solute to form clear homogeneous melts. Mixtures are amenable to the examination of the temperature dependence of viscoelastic parameters that follow molecular processes during sample vitrification (Mitchell, 1998, 2000). We also turned our attention to the details of the time/frequency dependence of the relaxation and retardation distribution functions, as seen in the composite curve of viscoelasticity (Groot & Agterof, 1995). The experimental temperature or time scale of measurement unveils three distinct phenomena: the glass transition region (α relaxation), the secondary mechanical distribution (β relaxation) and the glassy state (Kasapis, Al-Marhobi, & Sworn, 2001). The shapes of the viscoelastic functions in each of the three temperature/time zones are grossly similar for all polysaccharide mixtures and can be related to specific features of the chemical structure. However, each zone represents a separate problem and possesses its own characteristics.

Taking stock from the synthetic polymer approach (Ferry, 1980), a framework of positions characteristic of the composite functions was developed by reducing small-deformation dynamic-oscillation data. Thus, the following was proposed (Kasapis, 2001; Kasapis & Mitchell, 2001):

- Time–temperature superposition of experimental mechanical spectra of complex shear modulus/compliance allows construction of master curves, covering a dozen or more decades of frequency of oscillation, whose fitting with the Williams, Landel and Ferry equation pinpoints the rheological glass transition temperature.
- A theoretical foundation of the superposition of data was given by separating the mechanical properties of linear viscoelasticity into a basic function of time alone (the relaxation or retardation distribution function) and a basic function of temperature alone (temperature dependence of shift factor).
- The shift factor is a fundamental descriptor of change in all relaxation times with changing temperature and determines how much the frequency scale shifts with temperature, whereas the time function determines how much the physical parameters are affected by that shift.
- Determination of the monomeric friction coefficient allows accurate correlation between physical parameters and molecular characteristics thus developing structure–property relationships, for example, through differences in the molecular weight distribution of polymers.
- Iso-free-volume states were engineered that could follow

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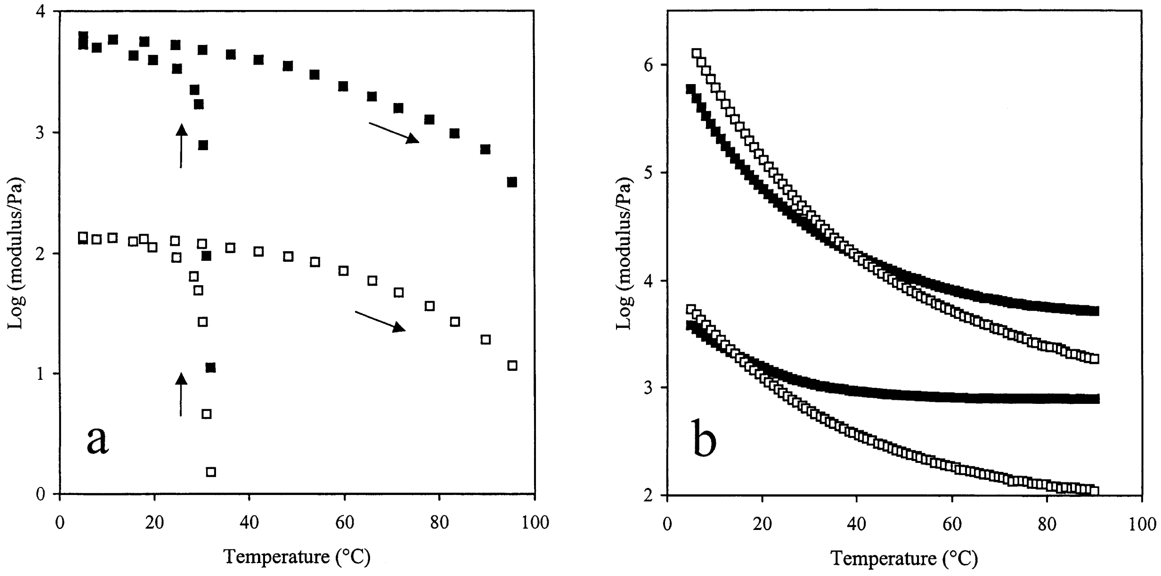

Fig. 1. Cooling and heating profiles at a scan rate of 1 ° min^{-1} of G' (■) and G'' (□) for 0.5% gellan gum with 7 mN Ca^{2+} in the presence of (a) 20% sucrose and (b) 50% sucrose plus 30% glucose syrup (bottom spectrum) and 50% sucrose plus 35% glucose syrup (top spectrum). Arrows indicate the cooling (↑) and heating (→) curves in the gellan sample with 20% sucrose, which develops thermal hysteresis (Papageorgiou, Kasapis & Richardson, 1994).

progress in viscoelasticity thus arguing that, regardless of the physicochemical characteristics of the polysaccharide/co-solute mixture, rate processes in the glass transition region were singularly determined by the mechanism of free volume.

- Attention was called to the secondary relaxation dispersions owing to the rapid, rotational motions of the side chains of polysaccharide chains, which follow the predictions of a constant energy of activation of the reaction rate theory.

The above treatment brings the synthetic polymer approach to the multifaceted behaviour of high-solid biological mixtures during vitrification and furnish, in principle, a formidable body of information based on the ‘unchallengeable’ laws of linear viscoelastic theory. Theoretical computations, however, should be compared directly with observations to see if they work. “If they disagree with experiment they are wrong and does not make a difference how beautiful those computations are”. In that simple statement is the key to science (Feynman, 1992). We have attempted, therefore, to offer tangible evidence of the transformation from enthalpic, aggregated polysaccharide networks in aqueous environment to entropic, lightly cross-linked structures in the presence of high levels of co-solute by using phenomenological rheology and transmission electron microscopy. The polysaccharide employed was deacylated gellan.

Fig. 1a and b reproduce thermal profiles of 0.5% deacylated gellan gum at stoichiometric equivalence of added calcium ions in the presence of low and high levels of co-solute (Papageorgiou, Kasapis & Richardson, 1994). Cooling and heating were carried out at a constant rate of 1 °C min^{-1} and a frequency of 10 rad s^{-1}. At low levels of solids, conventional disorder-to-order transitions were recorded at about 34 °C leading to a sharp increase in the values of shear modulus (Fig. 1a). Within the experimental constraints, modulus traces approach constant levels at 5 °C, with the elastic component of the network being dominant ($\tan \delta = G''/G' = 0.017$). Subsequent heating, unveils a pronounced melting profile, with the gels retaining a cohesive structure at the highest accessible experimental temperature (95 °C). The thermal profiles unveil a sixty degree thermal hysteresis, congruent to the delayed melting of κ-carrageenan and agarose gels, thus arguing for heavily aggregated double-helical strands as the structural knots of the deacylated gellan network (Morris & Norton, 1983).

At high levels of solids, cohesive networks have already been formed at 95 °C, which develop gradually with cooling without exhibiting a sigmoidal transition of structure formation (Fig. 1b). Instead, the out-of-phase element of the mechanical response takes over at low temperatures thus demarcating the onset of the glass transition region. Clearly, higher amounts of co-solute, i.e. from 80 to 85%, act as stronger antiplasticizing agents on the onset of the glass transition region which shifts from 14 to 38 °C seen in the points of intercept between the G' and G'' traces. Heating runs of the high solid samples produce overlapping traces of shear modulus without exhibiting signs of thermal hysteresis. It appears, therefore, that changes in the physical state of the gellan network at high levels of co-solute (>60%) produce a dominant elastic response at 95 °C, whereas at low levels of co-solute dilute-solution behaviour is recorded at the same temperature (Fig. 1a). Furthermore, the consistency of extremely viscous liquids is observed at 5 °C for the high solids mixtures (Fig. 1b), whereas conventional gellan

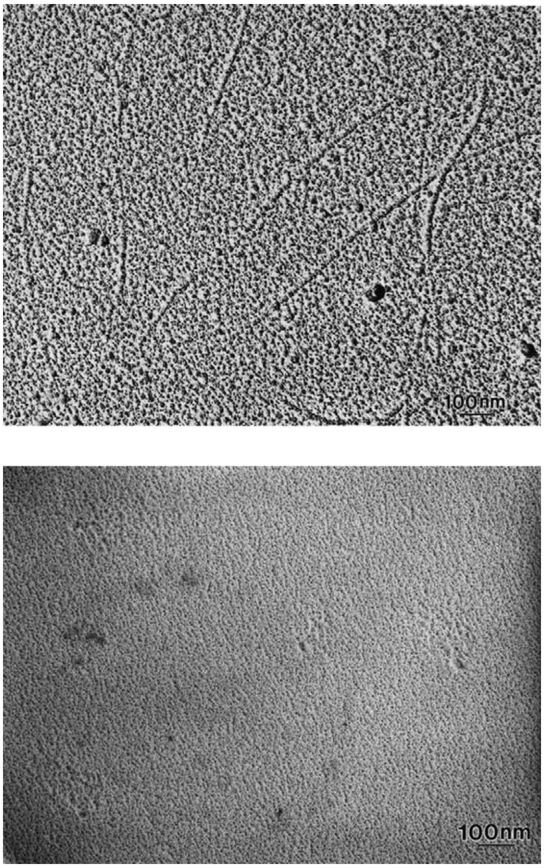

Fig. 2. Transmission electron micrographs of 0.7% gellan with 10 mN Ca^{2+} in aqueous environment (top) and in the presence of 80% glucose syrup (bottom).

gels are obtained at the low end of the working temperature range at 20% sucrose in the system.

The unconventional behaviour of viscoelastic functions with decreasing temperature in Fig. 1b has first been observed for amorphous synthetic polymers, which exhibit the transition zone between rubber and glass-like consistency (Plazek, 1991). Lightly cross-linked or entangled networks are involved and entropic viscoelasticity is conferred by the coordinated motions of the long polymeric sections adjoining the structural knots of networks. Differential scanning calorimetry work argues that there is a dramatic reduction in the structural order of polysaccharide networks at high levels of co-solute, as compared with the aqueous preparations (Evageliou, Kasapis, & Hember, 1998). In addition, aqueous polysaccharide gels exhibit a brittle texture upon compression testing whereas the high solid counterparts transform into rubbery assemblies of flexible chains, which are capable of extensive stretching before relaxing (Sworn, & Kasapis, 1998; Tsoga, Kasapis, & Richardson, 1999).

Transmission electron microscopy was performed in the present communication to examine the validity of the hypothesis arguing for dramatic structural changes in the nature of the polysaccharide network with increasing levels of co-solute. Particular care was taken in the preparation of samples, which were spread evenly on mica squares and frozen rapidly in a cryogen containing 35% isopentane in liquid propane cooled to −198 °C (Atkin, Abeysekera, Kronestedt-Robards & Robards, 2000). Thus formation of air bubbles and crystalline material was avoided. Low and high solids gellan gels were prepared and in both cases macroscopic phase separation, in the sense of prominent pores of a polymer depleted phase excluded by a polymer rich phase, was not visible. The micrograph on the top in Fig. 2 shows an aqueous 0.7% gellan gel at stoichiometric levels of added calcium ions. At the low solids regime, rheological and calorimetric data suggest that the network is formed by heavily aggregated thermally stable junctions (Fig. 1a). Indeed, the micrograph shows a dendritic polymer network of fibrillar thickness up to 40 nm, which should comprise tens of double helical strands per filament.

At the upper range of co-solute (>60%), differential scanning calorimetry argues for reduced functionality of the gellan chains. The chains form a rubbery gel capable of reproducing the vitrification profile of lightly cross-linked synthetic polymers upon cooling (Fig. 1b). Microscopy images of replicates of the high co-solute gellan gels gave a structural appearance congruent with the hypothesis of reduced aggregation in the polysaccharide network; the micrograph on the bottom of Fig. 2 illustrates a typical example. While the polymer still formed a continuous phase that supported the mixture in the form of a cohesive gel, dense fibrillar structures were not visible. In conclusion, we feel that microscopy provided insights and verification of the information gleaned from mechanical and thermal analyses thus documenting the change in the morphology of the gellan network in a high sugar environment.

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