Short communication

Direct imaging of the changing polysaccharide network at high levels of co-solute

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Abstract

Aqueous and high sugar gels of agarose and κ-carrageenan were prepared to investigate the changing nature of polymeric matrices. Transmission electron microscopy documented a transformation from an aggregated network in the aqueous environment to a lightly cross-linked structure in high solids. The latter possesses an elastic consistency based on the increasing values of yield strain from high- to low-water systems recorded using compression testing. The work provides further confirmation of the increased amorphicity of polysaccharide networks in high solid preparations.
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The present work constitutes a missing piece of a complex jigsaw that we have been trying to fit together for the last 10 years or so. The 'puzzle' refers to the use of physical science in the characterisation of the structural properties of high sugar/biopolymer mixtures. It all started as a product development exercise in the replacement of gelatin by deacylated gellan in confections (gummy bears, wine gums, etc). Soon, it was realized that polysaccharides at normal levels of industrial use (up to 2%) and in mixture with sucrose and/or glucose syrup (60–90%) possess quite distinct properties from those of the aqueous counterparts. Pictorial rheology as a function of temperature or time of observation exhibits rubber-to-glass consistency, as recorded in the vitrification of amorphous synthetic polymers (Ferry, 1980). Further analysis of results required understanding the evolution of the science of the glassy phenomena by following its development to a highly specialized subject, especially one that cuts across several conventional fields (Ferry, 1991).

Basic information on several high sugar/biopolymer mixtures was obtained and modeled with the synthetic polymer approach, which constitutes the corner-stone of advances in the molecular understanding of rubbers and glasses (Slade & Levine, 1991). A critical discussion of conceptual schemes applied to the vitrification of materials via rheology and differential scanning calorimetry was developed in order to highlight the current state of the art in the field (Kasapis, 1998). In particular, the use of Williams, Landel and Ferry (WLF) equation in conjunction with the free volume theory has been assessed in monomeric dimensions of macromolecules within the glass transition region, and its 'abuse' in collapse phenomena of porous matrices at the macromolecular level (Kasapis, 2005). Further positions characteristic of this framework of analysis include:

(i) the use of the method of reduced variables, which argues for thermorheological simplicity in the time/temperature dependence of relaxation processes (Kasapis, & Mitchell, 2001), and

(ii) the development of the concept of network (mechanical) glass transition temperature (T_g) that reflects the contribution of the biopolymer structure to rheology thus being distinct from the DSC T_g (Kasapis, Al-Marhoobi, & Mitchell, 2003).

Naturally, the morphology of the network responsible for the aforementioned structural properties comes into question. The suggestion that macromolecular assemblies at high levels of sugar remain substantially ordered as in the aqueous environment thus forming 'gel particulates' or 'gel islands' does not agree with the existing body of evidence (Nickerson, Paulson, & Speers, 2004). To start with, the combined WLF/free volume framework is applicable only to the small deformation behaviour of lightly cross-linked synthetic and biopolymer networks, which possess a considerable degree of amorphicity. Deliberate enhancement of helical ordering and aggregation via the addition of counterions (KCl/κ-carrageenan) or increase in the polymer content (high methoxy pectin/sugar) makes it impossible to superpose horizontally mechanical spectra and apply the above approach (Lopes da Silva, Goncalves, & Rao, 1994). Furthermore, there is a considerable drop in the enthalpy (ΔH) of polysaccharide associations at high levels of sugar recorded by DSC, as compared to the enthalpic content of aqueous interactions (Al-Marhoobi, & Kasapis, 2005; Normand et al., 2003). The above argue for reduced cross-linking of polysaccharide networks at saturated levels of sugar.

Networks that contain chain segments in the disordered form should exhibit elastic properties under 'moderate to high loadings', an effect demonstrated during compression testing. Fig. 1 depicts the stress-strain profiles of 2% agarose gels in distilled water and in the presence of 75% glucose syrup (dry solids). Aqueous κ-carrageenan (1.5%) gels with 30 mM KCl were also compressed, and in mixture with 80% glucose syrup. In both cases, materials were dissolved at 90 °C with stirring for 15 min. This was followed by addition of counterions for κ-carrageenan, and temperature reduction

to 80 °C for mixing with glucose syrup in the high solid systems. Excess water was evaporated off to bring the total level of solids to the required concentration. Cylindrical disks of 15 mm height and 60 mm diameter were compressed at refrigeration temperature employing a rate of 0.1 mm/s, and the average of three traces for each preparation is reported.

In Fig. 1, the outcome of compression of aqueous agarose disks is given in the form of stress vs. 'true' units of strain, i.e. $\varepsilon = \ln(L_o/L)$, where L_o is the original height and L is the height of the sample during deformation. A sharp breakdown profile typical of a strong biopolymer gel is recorded for agarose. κ-Carrageenan chains also dissociate rather sharply but, overall, they produce a lower point of yield stress (1.9 and 6.9 kPa, respectively). In both cases, the aggregated nature of the aqueous networks is confirmed via the formation of brittle structures that fracture early between 31 and 35% deformation.

Addition of high amounts of sugar changes dramatically the yield properties of the two materials. A transformation from a brittle to an elastic structure is recorded, with κ-carrageenan remaining the weakest network. Yield strain values for the sugar/κ-carrageenan mixture is around 83% and even higher for sugar/agarose at about 124% deformation. This result strongly supports a common pattern of behaviour, namely the formation of flexible polymeric segments in a high sugar environment that can stretch extensively before relaxing. Values of yield strain in excess of 100% deformation have been reported for the non-aggregated and stretchable (elastic) chains of the gelatin network (Papageorgiou, Kasapis, & Richardson, 1994).

The minor breakage points prior to the network collapse at the yield point in Fig. 1 are a reflection of the heterogeneity of the agarose matrix due to excessive cross-linking and aggregation. The absence of such discontinuities in the high-solid profiles of compression testing further supports the concept of homogeneous sugar/polysaccharide networks of reduced cross-linking. Visual observations offer tangible evidence of the transformation in polysaccharide networks as a function of co-solute content. Thus, agarose and κ-carrageenan form turbid gels that 'snap' readily and exhibit syneresis. Turbidity is dramatically reduced in a high solid environment, which yields the elastic materials of Fig. 1.

The severe disruption of aggregation with addition of sugar is also documented in transmission electron micrographs. Gel specimens were fragmented under a stereo-microscope to small sizes of 2 mm³ using a razor blade, and placed in a fixative of 2% gluteraldehyde in 100% ethanol for 2 h at 4 °C. The gel fragments were washed in three changes of 100% ethanol, fixed once more with 1% osmium tetroxide in 100% ethanol for 1 h at room temperature, and washed. This was followed by soaking in fresh 100% ethanol to ensure complete removal of water. The gel blocks were infiltrated with Melamine resin and polymerized at 50 °C for 18 h. Ultrathin sections, 60–90 nm, of the gels were microtomed and stained with aqueous uranyl acetate for 30 min at room temperature in the dark, and lead citrate for
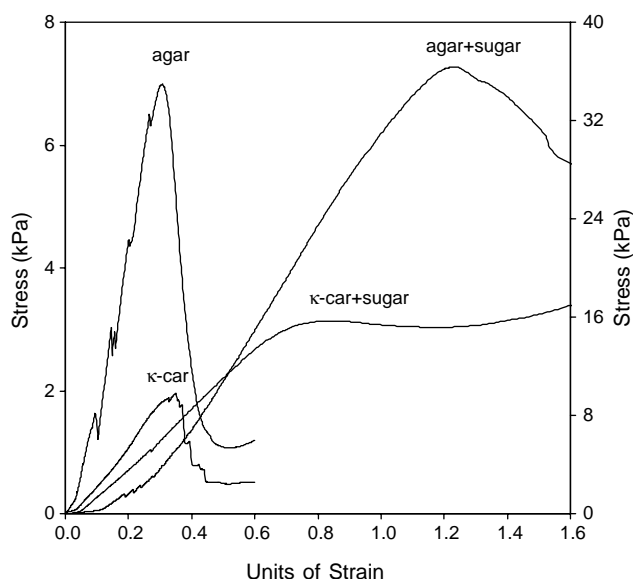

Fig. 1. Stress–strain profiles of 2% agarose and 1.5% κ-carrageenan (30 mM KCl) plotted on the left *y* axis, and 2% agarose/75% glucose syrup and 1.5% κ-carrageenan (30 mM KCl)/80% glucose syrup plotted on the right *y* axis (compression rate: 0.1 mm/s; temperature: 5 °C).

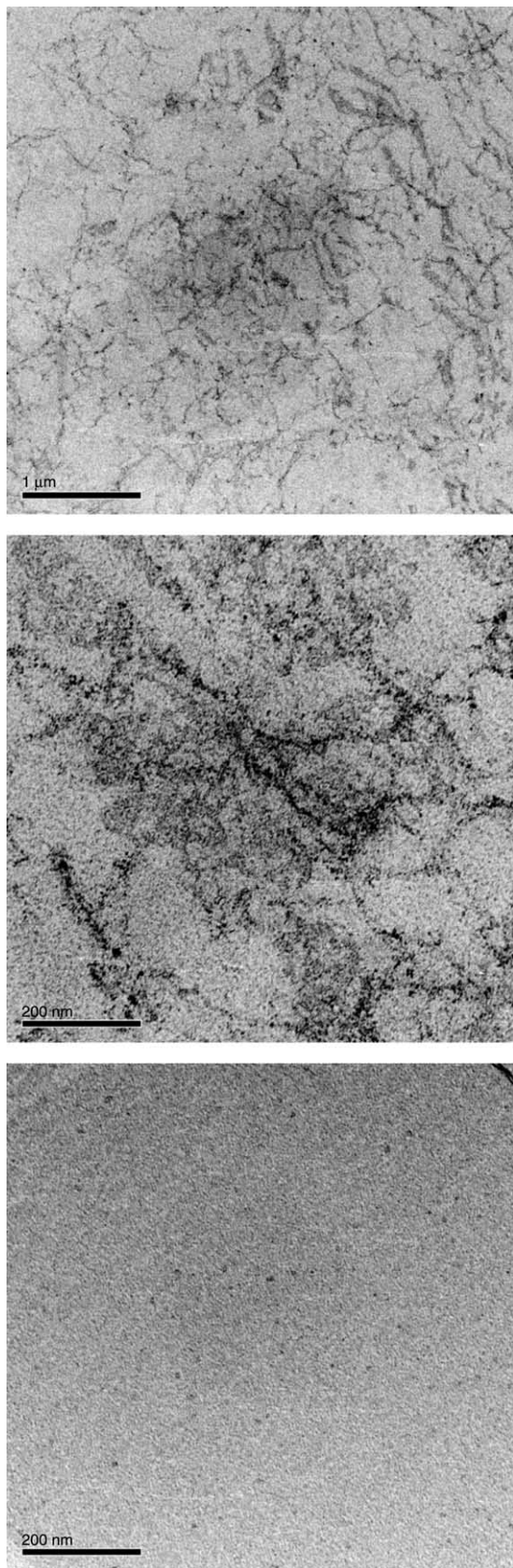Fig. 2. Transmission electron micrographs of 2% agarose gels in an aqueous environment at 30,000× (top) and 150,000× (middle), and in the presence of 75% glucose syrup at 150,000× (bottom).

10 min at room temperature, with the stained sections being examined under a transmission electron microscope (Stokke & Elgsaeter, 1994).

As shown in the top and middle micrographs of Fig. 2, the specimen grid contains well discernible agarose aggregates, which should be interconnected with 'soluble', disordered chain segments. The lateral dimensions of the aggregates range in size between 25 and 40 nm. Their length is much less uniform and extends largely from 0.4 to 1.5 μm. They constitute the structural knots of a dendritic network and should incorporate tens of double helical strands. The TEM image of κ-carrageenan in Fig. 3 (top) is not that densely structured but a few superhelical formations are clearly observed. This is due to the higher concentration of agarose, it's propensity to aggregate, and the relatively low levels of KCl used in this study to structure the κ-carrageenan. The width of the κ-carrageenan filaments appears to vary between 10 and 18 nm.
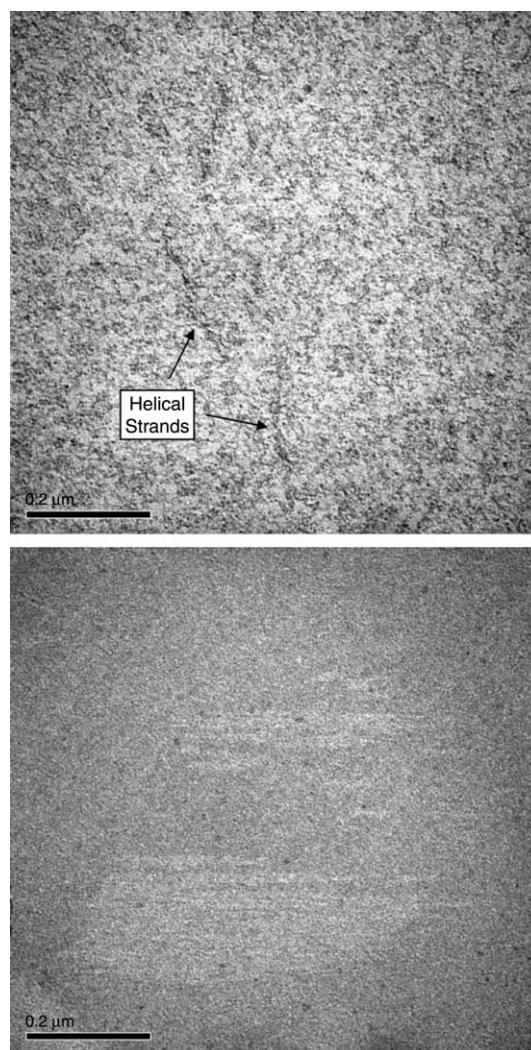

Fig. 3. Transmission electron micrographs of 1.5% κ-carrageenan gels plus 30 mM KCl in an aqueous environment at 150,000× (middle), and in the presence of 80% glucose syrup at 150,000× (bottom).

Addition of co-solute has a pronounced effect on the morphology of both polysaccharide networks, which is congruent with the hypothesis of reduced aggregation. Polymers still form a three-dimensional structure of rubbery consistency but dense agglomerates in agarose or super-helical fibrils in κ-carrageenan are no longer visible (bottom images in Figs. 2 and 3). Similar insights into the transformation from highly aggregated and enthalpic to lightly cross-linked and entropic systems have been reported for deacylated gellan gels (Kasapis, Al-Marhoobi, Deszc-zynski, Mitchell, & Abeysekera, 2003). As mentioned earlier, further verification of the reduced functionality of poly-saccharide chains in a high sugar environment has been recently obtained using DSC, which documented a decline in the enthalpy of coil-to-helix transition (Normand et al., 2003). Thus, there is a considerable drop in the enthalpy (ΔH) of intermolecular associations of polysaccharides in a high solid environment, which can be as low as 30% of the enthalpic content of the aqueous networks (Al-Marhoobi, & Kasapis, 2005).

Finally, certain needs for further theoretical and exper-imental advances are now fairly clear in relation to the effect of high pressure, the interplay of water activity and the glass transition temperature as stability criteria, bridging the theoretical divide between the high and low solid analyses, and the examination of the coupling model as a refined alternative to the free volume theory (Kasapis, & Al-Mar-hoobi, 2005).

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