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Rheological investigation of the structural properties and aging effects in the agarose/co-solute mixture

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Abstract

Small deformation dynamic oscillation, large deformation compression and creep testing were carried out to examine the structural properties of agarose in the presence of 50:50 preparations of sucrose and glucose syrup. The content of co-solute varied from 10 to 93% in the mixture. Increasing additions of sugar transform gradually the enthalpic, brittle networks of the polysaccharide to 'rubbery' structures of enhanced thermal stability. At saturated levels of solids there is a phase inversion from the gel-like characteristics of the agarose network to the liquid-like response of the continuous phase of co-solute. The latter undergoes vitrification upon cooling thus exhibiting the mechanical glass transition region and the glassy state. Given time, vestigial development and eventual three-dimensional linkage of agarose helices reverts the high viscosity liquids to rubbery gels exhibiting considerable yield strain (140% deformation). The work is compared to sugar/polysaccharide mixtures in the literature with the view to rationalizing underlying molecular processes and defining universal effects.

Keywords: Agarose; Sucrose/glucose syrup; Aging effects

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1. Introduction

The market value of the sugar confectionery industry in UK is worth around £1.4 billion and this figure can be doubled if chocolate items containing sugar confectionery are included (Strak & Morgan, 1995). In general, the confectionery industry is regarded as one of the successes of the food industry worldwide, with products having a long shelf life, low water content and therefore a relatively low transport cost. New manufacturers from countries where labour is cheap, e.g. Eastern Europe, are able to enter the market and sell products on the same terms as existing sellers. Thus the industry of developed countries is potentially vulnerable to loss of manufacturing capacity, but appreciates that innovative science is one factor that can contribute to the retaining of market share. The food industry has undoubtedly benefited from the understanding of the functional behaviour of polysaccharides that developed over the last three decades (Rees, 1970).

The proliferation of research in the functional properties of low solids proteins and polysaccharides led to well established relationships between their conformational characteristics and structural behaviour in single and mixed solutions and gels (Dea, 1993; Whistler, 1993). For example, a topic of interest is the phase behaviour and segregative or associative interactions between biopolymer chains in aqueous environment, where conventional gelation properties are observed. Food applications relate particularly to the development of low fat spreads, processed soft cheeses and low protein yoghurts (Chronakis & Kasapis, 1995). The importance of the glassy state and the glass transition region for the understanding of the behaviour of high solids foods also became widely appreciated (Kedward, MacNaughton, Blanshard, & Mitchell, 1998; Mitchell, 1998). Besides gelatin, a traditionally popular hydrocolloid, the confectionery industry is increasingly prepared to consider non-starch polysaccharides as gelling agents with multi-functional characteristics (Oakenfull, 2000).

Agarose is one of the biopolymers considered by major food ingredient and product manufacturers for the development of high sugar/low biopolymer formulations, which are central to many confectionery products. It is a neutral polysaccharide whose primary structure approximates to

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the alternating repeating sequence (Matsuhashi, 1990):

$\rightarrow$3)-β-D-Gal*p*-(1 $\rightarrow$ 4)-3, 6-anhydro-α-L-Gal*p*-(1$\rightarrow$

In common with other polysaccharides, gelation of agarose follows a cold setting mechanism that involves interchain association of co-axial double helices into extended junction zones (Arnott et al., 1974). Between junction zones there are interconnecting chain sequences. These remain disordered and mobile thus ensuring solubilisation of the network (Rees, Morris, Thom, & Madden, 1982). A generally accepted model of structure formation argues that concentrated agarose solutions (>2%) demix into polymer-rich and polymer-depleted regions, with double helix formation and subsequent aggregation occurring mainly in the polymer-rich regions (San Biagio, Bulone, Emanuele, Palma-Vittorelli, & Palma, 1996). However, a recent study achieved superposition of the experimental gelation/cure curves on a universal master curve thus suggesting self-similarity of network formation regardless of agarose concentration (Normand, Lootens, Amici, Plucknett, & Aymard, 2000). Instead, the molecular weight of the polysaccharide was found to affect strongly the gelation temperature and the critical concentration for gel formation.

Simple salts are common co-solutes in aqueous polysaccharide preparations and a study by Piculell and Nilsson (1989) showed increasing destabilization of the agarose gel in the order of the lyotropic series suggesting that anions bind to different extent to agarose coils. Watase, Nishinari, Williams, and Phillips (1990) looked at the effect of low concentrations of sucrose and glucose (up to 35%) on the mechanical properties of agarose gels subjected to small deformation longitudinal vibrations. Observations documented an initial reinforcement followed by a decrease in the values of dynamic Young's modulus with increasing levels of co-solute. Recently, the small and large deformation properties of agarose gels in the presence of high levels of sugar (85%) were investigated (Tsoga, Kasapis, & Richardson, 1999). Phenomenological rheology reproduced the rubber-to-glass transition recorded for amorphous synthetic polymers, which was rationalized with the combined framework of Williams, Landel and Ferry—free volume theory (Ferry, 1980; Plazek, 1991). This allowed estimation of the rheological glass transition temperature (T_g), which for 0.7% agarose with 50% sucrose and 35% glucose syrup was found to be −41 °C (Kasapis & Al-Marhoobi, 2000).

The present work is presented in two parts. Thus we undertake a systematic study of the effect of co-solute on the mechanical properties of agarose gels covering a range of solids from 0.7 to 93% in the formulation. A series of ideas is developed to discuss changes in viscoelasticity with increasing levels of co-solute from the dilute to the condensed state. In the second part, we implement a time-resolved study of the relative stability of the enthalpic/low-solid and entropic/high-solid networks of agarose/co-solute mixtures.

2. Experimental

2.1. Materials

The agarose sample was supplied by Sigma (product number: A 0576). It is a material of high gel-strength, which achieves storage modulus values of 1.4×10^4 Pa at 5 °C (0.7% gel in Fig. 1(a)). According to the supplier, water, ash and sulfate contents were of less than 7, 0.25 and 0.12%, respectively. Sucrose was of AnalaR grade from Sigma. The glucose syrup used was a Cerestar product (Batch NX4). The dextrose equivalent of the sample is ≈42. The total level of solids is 81% and glucose syrup compositions in this work refer to dry solids. Gel permeation chromatography established the polydisperse nature of glucose syrup, which upon cooling transforms from a viscous solution to a clear glass (Ong, Whitehouse, Abeysekera, Al-Ruqaie, & Kasapis, 1998).

2.2. Methods

2.2.1. Sample preparation

Agarose was dissolved in distilled water at 90 °C with constant stirring for 20 min. The temperature was reduced to 70 °C and appropriate amounts of sucrose and glucose syrup were added. Excess water was evaporated at 70 °C until formulations of the required solid content were obtained. Moisture contents were verified by vacuum oven drying at 80 °C. Samples with a solid content in excess of 85% were prepared by boiling under vacuum in order to reduce the temperature and time of experimental operation. This approach reduces the risk of chemical changes such as caramelisation and polysaccharide degradation. Good overlap of experimental data was achieved for samples of 85% solids prepared both by boiling down and by boiling under vacuum.

2.2.2. Small deformation measurements

These were carried out for most of the samples on the Control Stress Bohlin CS 10 rheometer. Parallel plate geometry of 40 mm diameter and 1 mm gap was employed. As with κ-carrageenan and deacylated gellan, aqueous or low co-solute agarose samples undergo sharp disorder-to-order and aggregation processes which produce contracted gels with a notable degree of slippage (Richardson & Goycoolea, 1994; Whittaker, Al-Ruqaie, Kasapis, & Richardson, 1997). However, this slippage was avoided by using filter papers adhered to the customary smoothed parallel plate geometry. Slippage is eliminated when the co-solute level exceeds 40% since the high levels of sugar and as we shall argue, the changing nature of the agarose network contribute to the adhesiveness and pliability of the system. Beyond this level of co-solute a smooth-surface geometry can be employed with equal validity, in this case, parallel plates.

The high rigidity of the concentrated agarose/sugar mixture (>80% solids) lies beyond the limits of Bohlin CS

10, which can not be trusted at shear modulus values above 10^6 Pa. To circumvent this problem, a controlled strain rheometer was used which employs an actuator to apply a deforming strain to the sample and a separate transducer to measure the resultant stress developed within the sample (Advanced Rheometric Expansion System of Rheometric Scientific, Piscataway, NJ, USA). The high resolution actuator has a low end dynamic strain of 5 microradians. The transducer is essentially non-compliant and covers a torque range from 0.02 to 2000 g cm. During experimentation the torque varied accordingly to maintain a constant strain, which allowed recording of modulus values in excess of 10^{10} Pa.

Samples were loaded on the preheated measuring geometry of the rheometer and exposed edges were covered with silicone fluid to minimize evaporation. Temperature ramps were then implemented from 80 to 0 °C at a scan rate of 1 °C/min while taking readings at 1 Hz (about 6.28 rad/s). At the end of the cooling run, mechanical spectra of storage modulus (G′), loss modulus (G″) and complex dynamic viscosity (η^*) were recorded in the frequency range of 0.1–100 rad/s (Richardson & Kasapis, 1998). This was followed by a heating regime to 90 °C at the same experimental conditions as for the cooling run. Particular care was taken during preparation, loading and analysis of the samples within the linear viscoelastic region to ensure reproducibility of results within a 3% margin.

2.2.3. Large deformation measurements

Key parameters have been derived by compression testing using a TA-XT2 Texture Analyser from Stable Microsystems. In doing so, hot solutions were poured into ring moulds of 12.5 mm height and 38 mm internal diameter. Solutions were allowed to stand for 24 h at ambient temperature (24 °C) thus forming coherent gels. Compression testing of the cylindrical gels was performed on the parallel plate geometry of TA-XT2 at ambient temperature and an experimentally convenient rate of 0.2 mm/s. Samples were compressed to 10% of their initial height, i.e. 90% compression. No attempt was made to control the conditions at the plate surfaces by lubrication or the use of sandpaper. Five samples were tested for each concentration of co-solute and average values are reported here.

Creep compliance experiments were carried out on the Bohlin CS 10 at 5 °C. Briefly, a constant shear stress was applied to a sample and the resultant strain was recorded as a function of time. The imposed stress was then withdrawn and the degree of recovery of the sample was recorded again as a function of time. The instantaneous elastic deformation occurring within 1 s of the stress application, the retardation pattern and the relaxation properties of the mixtures were found to vary widely with co-solute content for the same amount of applied stress. All experiments were carried out in triplicate and mean results are reported.

3. Results and discussion

3.1. Polymer dominated structural properties in Agarose/Co-solute mixtures

In the present series of experiments, agarose was used at a fixed concentration of 0.7% and the co-solute content was varied from 10 to 92.3% in the mixture (50:50 sucrose: glucose syrup). Fig. 1(a) illustrates the changes in G′ and G″ observed during controlled cooling of the polysaccharide in the presence of 10% co-solute. Gelation is marked by a sharp rise in shear modulus over a narrow temperature range (~31 °C). At the end of the thermal run, modulus traces level off with the solid-like component of the network being

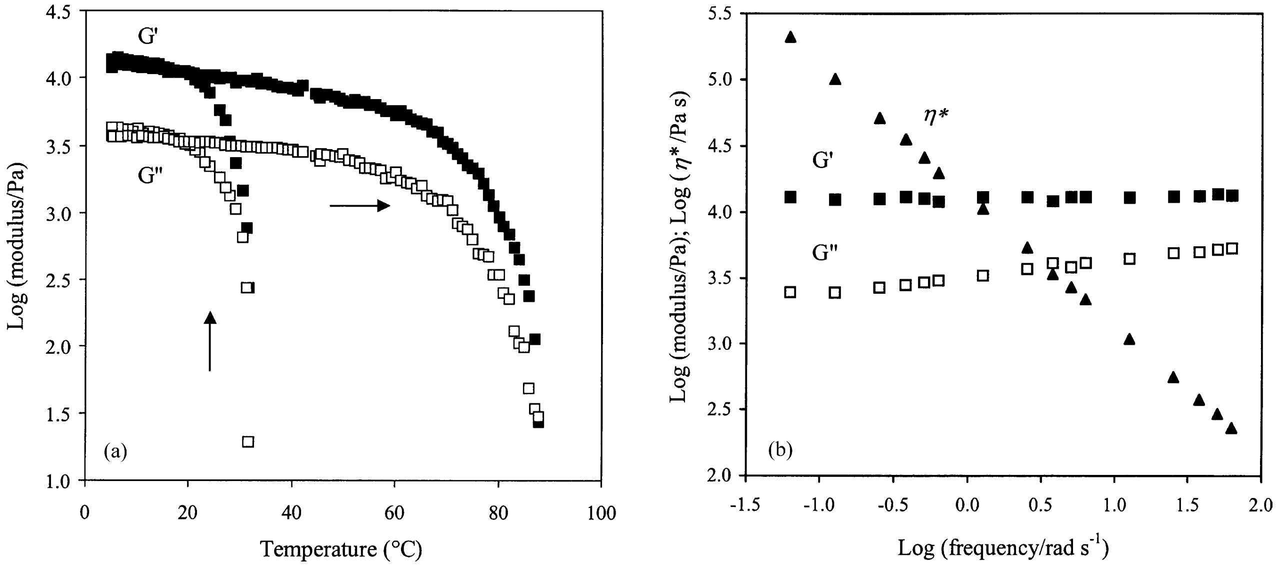

Fig. 1. Temperature (a) and frequency (b) variation of G′, G″ and η^* for a sample of 0.7% agarose plus 10% co-solute (50:50 sucrose/glucose syrup). Scar rate: 1 °C/min; frequency: 1 Hz; temperature: 5 °C (arrows indicate the cooling and heating profiles).

dominant. Thus, solutions essentially indistinguishable from water convert into stiff gels with a G″/G′ ratio (tan δ) of 0.29 at 5 °C (frequency of 6.28 rad/s). There is no real effect of isothermal runs at 5 °C on the rigidity of agarose gels, as shown by the overlapping cooling/heating traces below 20 °C. Further heating, however, unveils substantial thermal hysteresis of almost 60°, with the gels melting at about 90 °C.

The type of behaviour observed here for agarose has also been reported for deacylated gellan and κ-carrageenan and relates to the additional energetic requirement for melting of highly aggregated networks (Morris & Norton, 1983). Samples at 5 °C produce mechanical spectra shown in Fig. 1(b). These are characteristic of strong gels (Ross-Murphy, 1984), with values of G′ dominating those of G″, little frequency dependence in either modulus and a linear decrease in log η^* with increasing log of the dynamic frequency of oscillation (ω).

Increasing additions of co-solute to the agarose preparations transform gradually the thermal profile of structure formation (Fig. 2). For example, 75% co-solute has a stabilizing effect on the ordered conformation of the polysaccharide with vestigial structure formation occurring at about 40 °C. Furthermore, the temperature band of gelation becomes broader, as compared to that in Fig. 1(a), indicating a less co-operative process of coil-to-helix formation at a relatively high co-solute content. When the samples are held at 5 °C, there is a substantial time-dependent change in the values of shear modulus which double during an isothermal run of two hours. The progressive increase in the mechanical strength of high sugar/agarose mixtures gives rise to thermally stable structures up to the late stages of heating. In contrast, low sugar/agarose preparations become fluid at the upper range of experimentally accessible temperatures in Fig. 1(a). A leap in thermal stability at high concentrations of co-solute has been reported for networks of deacylated gellan and κ-carrageenan (Evageliou, Kasapis, & Hember, 1998; Papageorgiou, Kasapis, & Richardson, 1994), and it will be discussed below in conjunction with the present results.

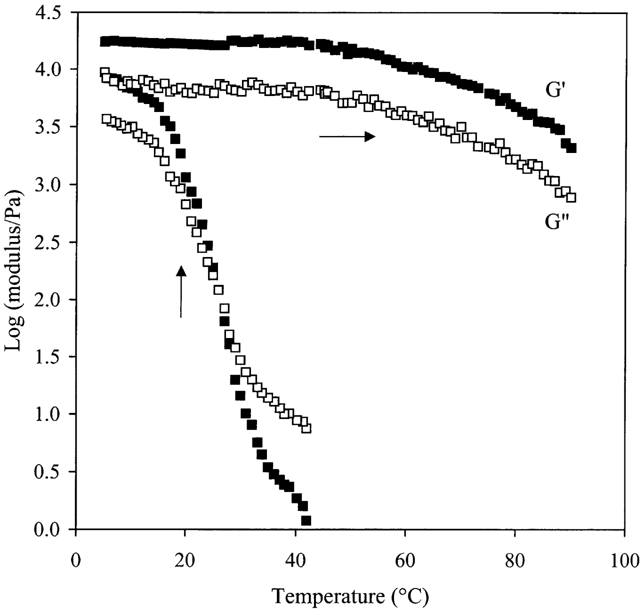

Fig. 2. Cooling and heating profiles of G′ and G″ for a sample of 0.7% agarose plus 75% co-solute (50:50 sucrose/glucose syrup). Scar rate: 1°C/min; frequency: 1 Hz (arrows indicate the cooling and heating profiles).

3.2. Co-solute dominated structural properties in high solids agarose mixtures

The general form of modulus development during our experimental routine changes dramatically when 5% co-solute is added to the sample in Fig. 2 thus bringing the total concentration of co-solute to 80% in Fig. 3. The foregoing discussion addressed polymer-dominated phenomena, albeit of changing functionality with increasing levels of sugar in the mixture. It appears, however, that there is a phase inversion from the solid-like consistency observed so far to the liquid-like phenomena of 80% co-solute in Fig. 3(a). The viscous solution response obtained at the beginning of the cooling run is retained at low temperatures, with the samples achieving tan δ values of 21 at 5 °C (frequency of 6.28 rad/s). This, of course, is in direct contrast with the viscoelastic ratio of the agarose gel in the presence of 75% sugar in Fig. 2 (tan $\delta \approx 0.53$ at 5 °C). Mechanical spectra at the end of the isothermal run at 5 °C show a liquid-like response with well-separated modulus traces of a dominant viscous element and a relatively flat frequency dependence of complex dynamic viscosity (Fig. 3(b)).

In Fig. 3(a), the liquid-like response accelerates abruptly at temperatures below 20 °C thus contributing to the aforementioned high values of the G″/G′ ratio. This phenomenon is captured at its full magnitude in Fig. 4, which reproduces overlapping cooling and heating profiles of 0.7% agarose in the presence of 92.3% sugar solids. The baseline of structural behaviour was also identified by running separately a single sugar sample of 93% solids. At high temperatures, both samples are pourable solutions which become increasingly viscous with cooling. For most of the temperature range, the viscous element of the material remains dominant with the samples passing from the state of flow to a region known as the ‘glass transition’. In terms of overall shape, magnitude and temperature band, the latter process is congruent with the viscoelastic profiles of amorphous synthetic polymers and diluted systems during vitrification (Peleg, 1992; Plazek, 1996).

Clearly, at saturated levels of co-solute (≳ 80%) there is no parentage in the gel-like viscoelasticity of agarose networks, with single sugar systems or polysaccharide/co-solute mixtures showing similar patterns of vitrification within experimental error. Finally, the modulus traces cross over at about 25.5 °C producing a network with a dominant elastic component (G′ > G″). This is the glassy state at which the storage modulus achieves values in excess of 10^{10} Pa and in addition to stretching and bending of chemical

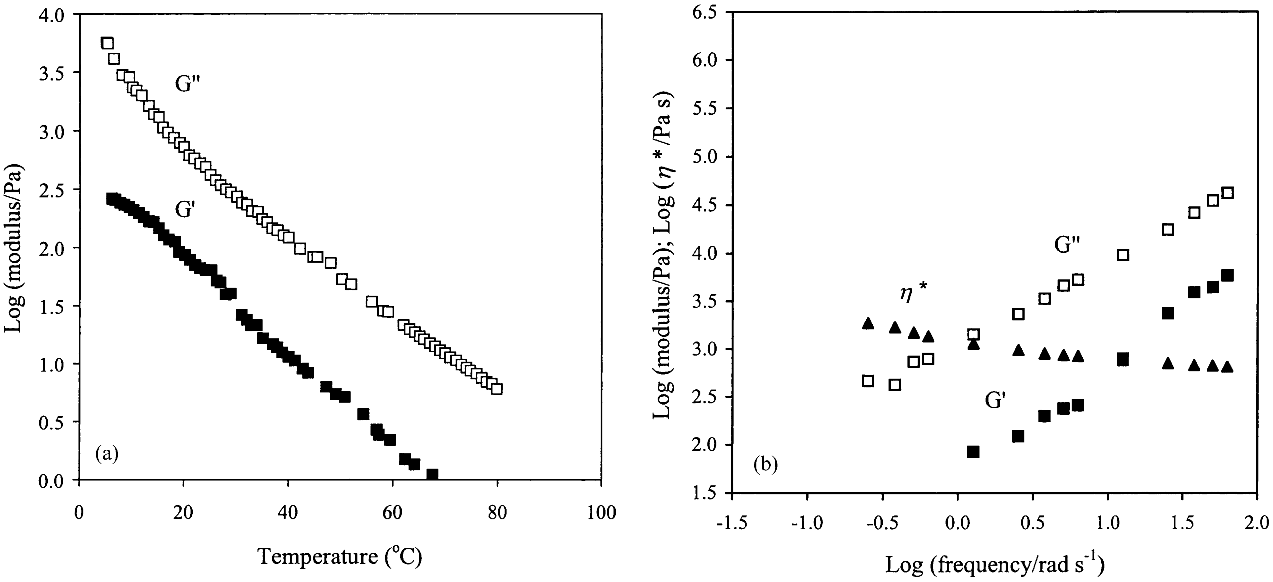

Fig. 3. Temperature (a) and frequency (b) variation of G′, G″ and η^* for a sample of 0.7% agarose plus 80% co-solute (50:50 sucrose/glucose syrup). Conditions as in Fig. 1.

bonds, β mechanical relaxations are ubiquitous (Kasapis, Al-Marhobi, & Sworn, 2001; Shen & Eisenberg, 1967).

3.3. Descriptive account of molecular processes occurring in the agarose/sugar mixture

Once the two distinct profiles of viscoelasticity in the temperature and frequency domains were identified, the overall variation of shear modulus at the end of the cooling run was followed and then plotted as a function of increasing concentration of co-solute in Fig. 5. The aim of this exercise was to piece together qualitatively the spectacular differences in the structural properties observed in the agarose/sugar mixture. This section of the paper also alludes to the universal effect of sugar on the network functionality of gelling polysaccharides.

Addition of co-solute up to 40% leads to a systematic increase in the values of storage modulus, which is characterized by a relatively flat dependence on the experimental frequency of oscillation at 5 °C. This process was observed in κ-carrageenan and deacylated gellan gum, which also form double helical strands stabilized in large aggregates (Grasdalen & Smidsrød, 1987; Smidsrød & Grasdalen, 1982). It is possible that the bulky polyhydric molecules of sucrose and glucose syrup are effectively excluded from the polysaccharide surfaces thus increasing the surface tension of the mixture relative to the surface tension of the polysaccharide/water system. This event should replace partially the polymer-water hydrogen bonds with gel-stabilising, polymer–polymer interactions in the agarose/water/sugar mixture.

Small deformation measurements of agarose samples with a co-solute content between 40 and 75% unveil a small but reproducible drop in the values of storage modulus at 5 °C. Deacylated gellan and κ-carrageenan exhibited a sharp drop at intermediate levels of co-solute (Kasapis, 1998). It is considered that structure formation in a polysaccharide network is stabilized thermodynamically by hydrogen bonding with water molecules attached directly to the double helices or in the form of a hydration layer around the polymeric agglomerates (Gekko & Kasuya, 1985; Gekko, Mugishima, & Koga,

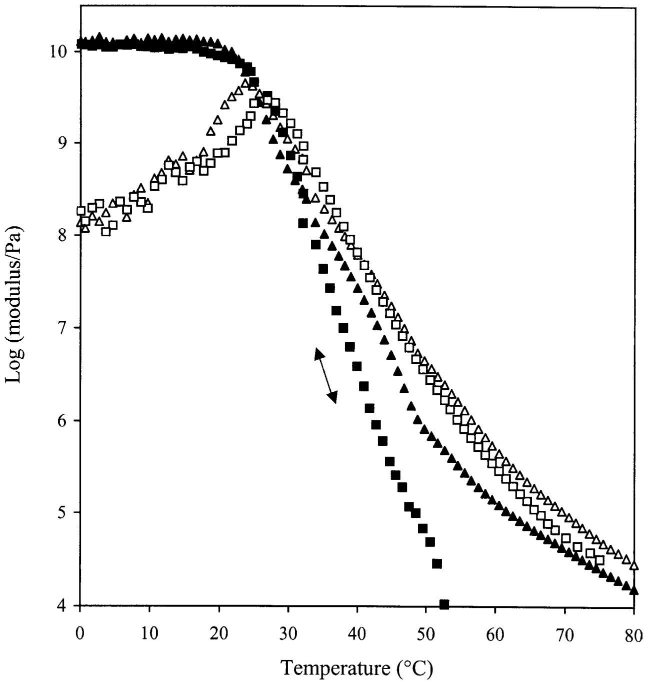

Fig. 4. Thermal profile of storage (G′) and loss (G″) modulus for 0.7% agarose with 46.2% sucrose + 46.2% glucose syrup (G′ ▲; G″ △), and a sample of 46.5% sucrose + 46.5% glucose syrup (G′ ■; G″ □). Experimental conditions as in Fig. 2 (arrows indicate the overlapping cooling and heating profiles).

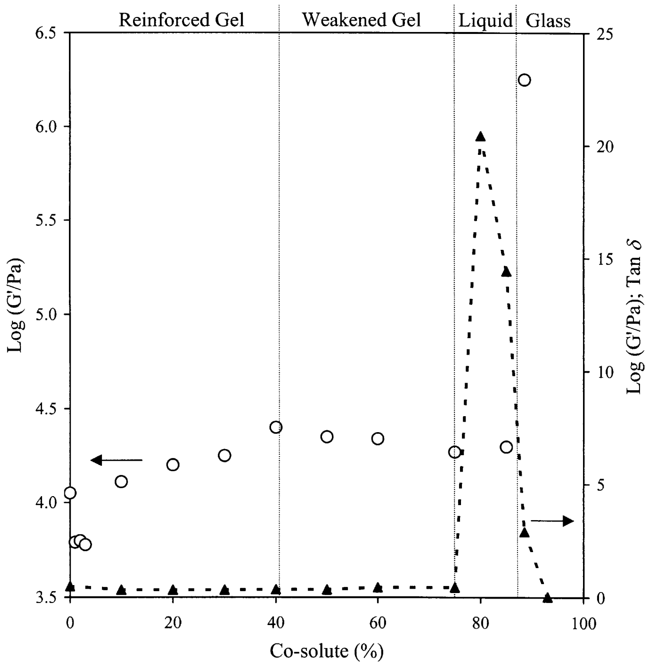

Fig. 5. Effect of co-solute on the G′ (○; left *y* axis) and tan δ (▲; right *y* axis) for 0.7% agarose preparations. The value of G′ at 80% co-solute is on the right *y* axis. Temperature: 5 °C, frequency: 1 Hz.

1985). Therefore, it is reasonable to assume that the increasing shortage of water molecules and their preferential hydrogen bonding with polyhydric sugars destabilize partially the rigid structural knots of the aqueous agarose gel at intermediate levels of co-solute.

At 80% co-solute, phase inversion occurs from the agarose network to a continuous sugar phase with the polysaccharide being confined to discontinuous inclusions. In Fig. 5, the values of storage modulus collapse almost hundredfold from about 18.6 to 0.2 kPa at 75 and 80% co-solute, respectively (frequency of 6.28 rad/s). Changes in the topology of our mixture are also illustrated in the values of the tangent of phase angle δ, which soar from well below 1 for the agarose gel to 20.4 at 80% co-solute. Sucrose/glucose syrup concentrated ($\geq$78%) blends of gelatin showed a similar transformation from rubbery protein structures to viscous solutions where sugar formed the continuous phase and the gelatin inclusions could undergo a conformational transition upon cooling (Al-Ruqaie, Kasapis, & Abeysekera, 1997).

The agarose double helix appears to have an interior cavity with a diameter of 0.45 nm (measured at the narrowest points between atomic centres). Oxygen atoms, namely O(2) of galactose and O(5) of 3,6-anhydrogalactose which are engaged in hydrogen bonds can be found in the interior of the helix. According to this, it was suggested that the agarose cavity is occupied by water molecules, which participate in a hydrogen bond that contributes to the stability of the double helix (Arnott et al., 1974). This comes in agreement with the proposal that water is the single solvent in which agarose forms gels and that it is necessary part in the aggregation of the helices and the formation of the junction zones (Watase, Nishinari, & Hatakeyama, 1988). Thus, both helix and aggregate formation of agarose may be destabilized upon addition of 80% co-solute to the system.

At the top of the co-solute range, the main point at issue is the dramatic drop in the values of tan δ at 5 °C, which demarcate an index of reference in the changing structural properties of the mixture. Thus, there is a gradual reversal to a disproportionate increase in G′ at 85 and 88.5% sugar, as systems move deep into the glass transition region with the values of tan δ falling to 14.4 and 2.9, respectively. As depicted in Fig. 4, systems have advanced well towards the glassy state at 93% solids yielding extremely rigid networks of $G' = 1.19 \times 10^{10}$ Pa at 5 °C and viscoelastic ratios well below 1 ($\tan \delta = 0.019$ in Fig. 5).

The diminishing functionality of agarose chains at levels of co-solute above 40% should also be seen in the large deformation properties of the polymeric network, which can be readily identified on compression testing. Fig. 6 reproduces the force-deformation profiles of agarose/sugar gels following a 24 h curing at 24 °C. To break the self-supporting structure and follow the subsequent flow properties, compression continued until the height of the sample, L, was ~10% of the original height, L_o. At such large deformations, the simple definition of strain (ε) as $(L_o - L)/L_o$, is no longer valid because the fractional change in height relative to the absolute change increases rapidly as $L \rightarrow 0$. Instead, the 'true' strain is given by $\varepsilon = \ln (L_o/L)$ in Ross-Murphy (1984).

As anticipated, the aggregated agarose chains in the absence of co-solute form a brittle gel that fractures at about

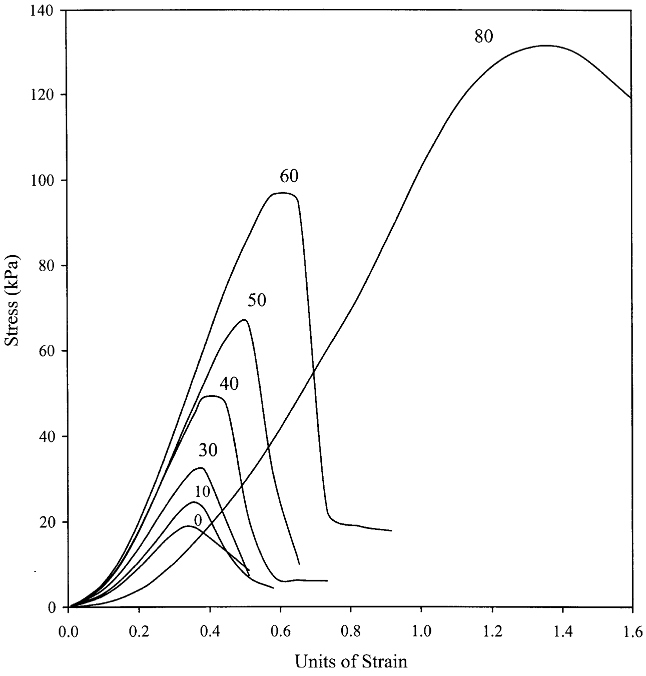

Fig. 6. Stress-strain profiles of 0.7% agarose samples with increasing levels of co-solute (50:50 sucrose/glucose syrup) indicated by the experimental traces. Compression rate: 0.2 mm/s; temperature: 24 °C.

0.34 units of strain. Similarly, aqueous gels of deacylated gellan fracture early under compression at 0.3 units of deformation whereas the yield strain of the rubbery gelatin gels remains close to 100% deformation (Papageorgiou, Kasapis, & Richardson, 1994b). Addition of up to 40% co-solute has little effect on the yield strain of agarose networks, which extends to 0.38 units in Fig. 6. The effect of high-solid addition is accelerated at 50 and 60% co-solute, with gels fracturing at 0.51 and 0.65 units of deformation, respectively. Finally, elastic structures exhibiting an ultimate deformation of 143% are obtained at saturated levels of solids (~81%), a result which argues for diminishing brittle associations in the polymeric material.

Similar phenomena have been reported for the mechanical properties of deacylated gellan gum (Sworn & Kasapis, 1998), suggesting a common pattern of behaviour in the high sugar/polysaccharide mixtures. The above can be rationalized by considering a transformation of the aqueous polysaccharide network to a structure of reduced cross-linking where substantial parts of the chains are flexible entities capable of extensive stretching before relaxing. This assertion is supported by the observation that at high levels of co-solute κ-carrageenan networks show a reduced enthalpic content per gram of the polymeric material (DSC work), with gels acquiring elastic large deformation properties (Evageliou et al., 1998).

3.4. Aging of agarose/co-solute preparations

The effect of co-solute concentration on the overall rheology of the mixed systems was summarized in Fig. 5, which showed an interesting feature of phase inversion from polymer to solvent-driven characteristics in the temperature function. The level of solids in the region of structural transformation is, of course, relevant to the ambient and frozen confectionery manufacture, which requires knowledge of product stability upon storage. To explore this issue, we carried out small deformation measurements for up to 70 h, i.e. much longer than previous studies on high sugar/biopolymer systems.

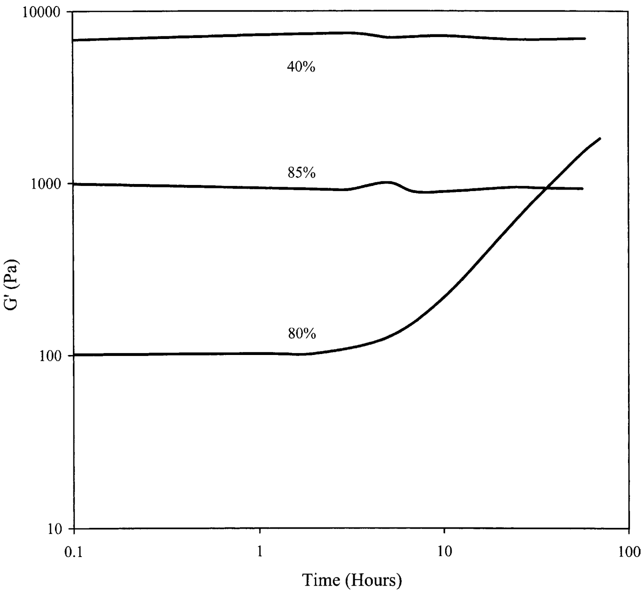

Fig. 7. Effect of aging on the storage modulus of 0.7% agarose samples at various levels of co-solute (50:50 sucrose/glucose syrup) indicated by the experimental traces. Temperature: 5 °C, frequency: 1 Hz.

Fig. 7 illustrates changes in the values of storage modulus recorded for mixtures of agarose and co-solute in the range of 40–85%. This covers the areas of enthalpic associations of strong gels, the sharp drop in viscoelasticity at the phase inversion point and the liquid-like response of sugar-saturated mixtures. On cooling to ambient temperature, a three-dimensional agarose structure develops at 40% co-solute. However, the subsequent isothermal run shows no variation in the network rigidity with time, a result which further argues for the formation of a 'true' gel made of 'permanent' intermolecular associations within the experimental constraints. Throughout the experimental run, the elastic component of the network dominates over the viscous response, as shown in Fig. 1(b).

A flat mechanical response is also observed at the upper range of co-solute (85%), where suppression of enthalpic ordering prevents formation of a continuous agarose network. Thus the overall viscoelasticity is predominantly liquid-like ($G'' > G'$), as shown in Fig. 3(b). Both types of phase topology can be seen in the agarose sample of 80% co-solute, which at the onset of the isothermal run produces a flat liquid-like behaviour. It appears, however, that vestigial development of intermolecular associations is possible due to increased mobility and the presence of additional water molecules, as compared to the mixture of 85% co-solute. Given time, there is a spectacular increase in the values of storage modulus in Fig. 7, which results in the transformation of the high-solid solution to a rubbery agarose gel.

The disparate response of the two contributing molecular mechanisms emanating from the liquid and gel-like behaviour of our mixtures can also been seen in the relationship between stress and strain, as reflected in a conventional creep testing experiment. Fig. 8(a) reproduces the retardation curve of 0.7% agarose plus 40% sugar gel carried out under stress-controlled conditions of 100 Pa. This was preceded by aging of the gel for up to 25 h at 5 °C. Clearly, the instantaneous deformation, occurring within 1 s, remains unaltered for all samples at about 0.025% strain. The generated sample displacement levels off early in the retardation cycle and remains within the linear viscoelastic region at about 0.16% strain. Upon removal of the imposed stress, the network responds instantaneously in the form of a sharp relaxation curve. This is an elastic mechanism that effectively recovers the entirety of the original shape of the sample.

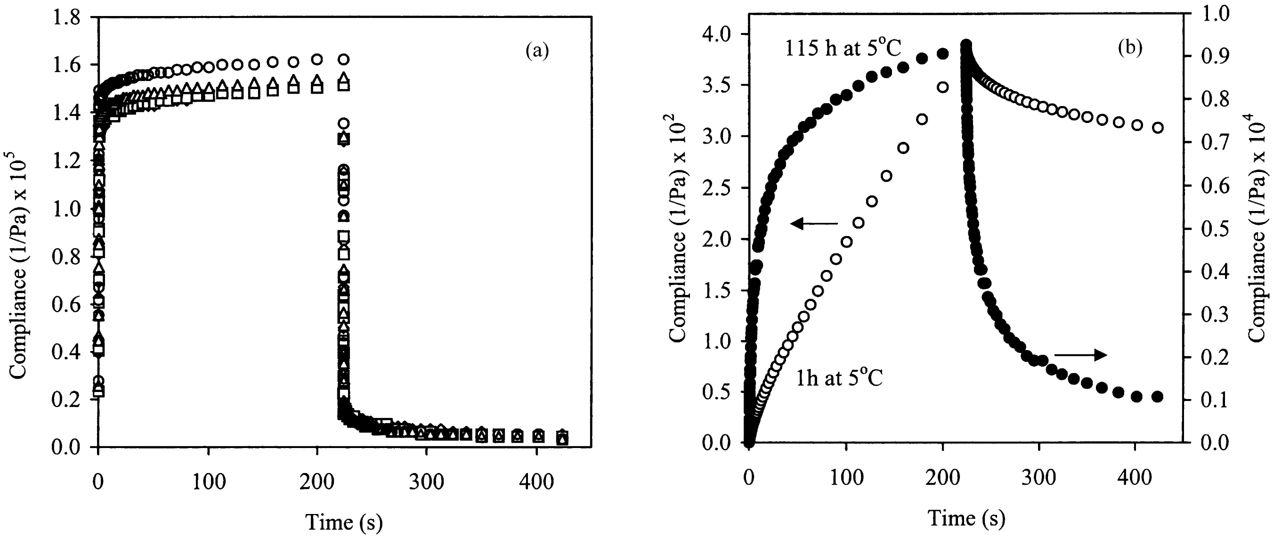

Fig. 8. Creep compliance testing for samples of (a) 0.7% agarose plus 40% co-solute (50:50 sucrose/glucose syrup) aged for 1 h (▽), 3 h (□), 7 h (△) and 25 h (○), and (b) 0.7% agarose plus 80% co-solute (50:50 sucrose/glucose syrup) aged for 1 h (○; left *y* axis) and 115 h (●; right *y* axis).

In contrast to the preceding paragraph, stress-controlled tests unveil substantial aging effects in agarose samples with high levels of co-solute (80% in Fig. 8(b)). Following curing for 1 h at 5 °C, mixtures largely abolish their structural integrity and recover only 22% of the final deformation at the end of the relaxation period. An entirely different response is obtained for the same sample aged 115 h, which exhibits relatively low compliance values in the retardation curve and almost 90% recovery in the relaxation curve.

4. Conclusions

The aim of the work was to combine various rheological techniques in order to rationalise changes in the morphology of agarose networks with increasing levels of sucrose/glucose syrup in the mixture. This understanding can be used by the food industry to innovate, since preparations are related to high sugar gum-type confectioneries. Network transformation at high co-solute content creates systems that behave rheologically like ‘viscoelastic materials’. Depending on co-solute concentration and sample aging, the four states of viscoelasticity, i.e. terminal flow, rubbery zone, glass transition region and the glassy state are identified. This gives rise to a model where the overall structure of the mixture is governed by the mobility of small amounts of the polymeric component once its intermolecular associations have been modified by the high levels of sugar. In developing such a thinking, we find support from similar patterns of behaviour in high sugar/κ-carrageenan and /deacylated gellan mixtures whose mechanical response is amenable to analysis in terms of the ‘synthetic polymer approach’ (Kasapis & Mitchell, 2001).

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