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The effect of pressure on the structural properties of biopolymer/co-solute. Part II: The example of gelling polysaccharides

Stefan Kasapis [a,*], Shyam S. Sablani [b]

[a] Department of Chemistry, National University of Singapore, Block S8, Level 5, Science Drive 3, Singapore 117543
[b] Department of Biological Systems Engineering, Washington State University, P.O. Box 646120, Pullman, WA 99164-6120, USA

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Abstract

The dependence of relaxation processes, as manifest in changes of the glass transition temperature, were examined under pressure (0.1–700 MPa) for the gelatin/co-solute system (part I of this series) and, currently, for preparations of agarose, κ-carrageenan and deacylated gellan in the presence of co-solute (part II). Structural properties were monitored using modulated differential scanning calorimetry and small-deformation dynamic spectroscopy on shear. Response curves as a function of hydrostatic pressure were treated with the combined framework of reduced variables and WLF equation/free volume theory. Shift factors derived from viscoelastic spectra and empirically treated thermograms clearly demonstrate that the effect of increasing pressure is detrimental to the stability of intermolecular polysaccharide associations. Diminishing values of the glass transition temperature with increasing pressure argue that the concept of time–temperature–pressure equivalence applicable to amorphous synthetic polymers is not operational in the structural functions of high-solid polysaccharide gels.

Keywords: Agarose; κ-Carrageenan; Deacylated gellan; Hydrostatic pressure; Mechanical glass transition temperature

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1. Introduction

In his classic work, A.K. Doolittle has shown that the effect of temperature on the viscosity of several organic liquids undergoing vitrification can be described by the concept of free volume (Doolittle, 1951; Ferry & Stratton, 1960). Subsequently, different kinds of rheological data on the relaxation times of macromolecules as a function of concentration, molecular weight distribution and side chain length indicated that free volume was adequate for application in the rubber-to-glass transformation (Dlubek et al., 2005; Ferry, 1991). Recently, the approach has been refined by "the coupling model", which was introduced on the basis that intermolecular interactions in densely packed systems are the ultimate determining factor of molecular dynamics in materials (Ngai, 2000). It is anticipated that attention will be drawn in the future to the area of intermolecular cooperative dynamics in order to overcome possible simplifications associated with the application of the free volume theory to the entirety of the glass transition region (Ngai & Roland, 2002).

The sophisticated "synthetic polymer approach" was extended to the effect of changing pressure on the vitrification of materials (Mpoukouvalas, Floudas, Zhang, & Runt, 2005). At first, limited data on the shear behaviour of polymers, in the comparison of two states at different pressures, argued for the usefulness of free volume as a primary mechanistic variable based on the assumption that its compressibility is independent of pressure (Ferry, 1980). This was in broad agreement with the well-known increase in viscoelasticity and relaxation time of materials with applied pressure. Today, it is known that the above assumption is incorrect, and attainment of the pressure-dependent parameter on compressibility is required to

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[*] Corresponding author. Tel.: +65 6516 4834; fax: +65 6775 7895.
E-mail address: chmsk@nus.edu.sg (S. Kasapis).

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allow satisfactory description of the mechanical response of single and filled elastomers (Fillers & Tschoegl, 1977; Moonan & Tschoegl, 1983). The theoretical value of this type of work lies in the ability to determine molecular parameters during vitrification from a combination of isobaric measurements at atmospheric pressure as a function of temperature and isothermal measurements as a function of pressure (Moonan & Tschoegl, 1984, 1985). Thus, a pressure-time-temperature equation-of-state has been developed in amorphous synthetics to predict their behaviour when subjected to combined pressure and temperature during industrial processing (Ngai & Fytas, 1986; Roland & Casalini, 2003; Tribone & O'Reilly, 1989; Utracki, Simha, & Garcia-Rejon, 2003; Zhang, Casalini, Runt, & Roland, 2003).

In biomaterials, aspects of pressure-induced functionality have been investigated in the solubilisation of galactomannans and xyloglucans (guar, locust bean, tara, tamarind and detarium gum) in order to produce true "molecular" systems with a reduced rate of subsequent aggregation (Picout, Ross-Murphy, Errington, & Harding, 2001, 2003; Picout, Ross-Murphy, Jumel, & Harding, 2002). Reduction in the degree of crystallinity and gelatinization temperature of corn and potato starch with increasing pressure treatment has also been demonstrated (Blaszczak, Fornal, Valverde, & Garrido, 2005a; Blaszczak, Valverde, & Fornal, 2005b). Pressure shift freezing in aqueous and sucrose containing gelatin, agar and deacylated gellan preparations yielded a large number of small ice crystals that helped to retain an acceptable texture in the frozen product (Fuchigami & Teramoto, 2003; Fuchigami, Teramoto, & Jibu, 2006; Zhu, Ramaswamy, & Le Bail, 2005). In addition, alteration of the gelation or flow characteristics of gelatin, xanthan gum, soy and muscle proteins, and reduction in microbial population, digestibility and inactivation of anti-nutritional factors in cereal grains and legumes have been discussed as a function of pressure (Ahmed & Ramaswamy, 2004; Estrada-Giron, Swanson, & Barbosa-Canovas, 2005; Jimenez Colmenero, 2002; Molina, Defaye, & Ledward, 2002; Montero, Fernandez-Diaz, & Gomez-Guillen, 2002).

By comparison, very little fundamental information, if any, is available on the effect of pressure on the vitrification of biomacromolecules. The present paper aims to examine the application of high pressure to gelling polysaccharide/co-solute vitrification and then discuss it, using the synthetic polymer approach, in conjunction with the effect of temperature and recently obtained results on the gelatin/co-solute mixture (part I of this series).

2. Experimental

2.1. Materials

The deacylated gellan sample used in this investigation was a gift from CP Kelco, San Diego, CA, USA (Kelcogel-77109A). Purification and ion-exchanging steps were as follows: Four grams were dissolved in 800 ml of distilled water at 90 °C. A cellulose membrane tube with a pore diameter of 2.4 nm was filled with the solution, sealed and placed in a water bath at 65 °C for 3 days. For two days the water was changed five times a day and then it was replaced with a 7.5 mN NaCl solution which corresponds to the stoichiometric equivalence of the carboxyl groups for this preparation. Finally, the gellan solution was freeze-dried. Previous work showed that this type of solvent had little effect on the measurements of the molecular weight of the polysaccharide, which was found to be around $1.64 \pm 0.2 \times 10^6$ Da (Sworn & Kasapis, 1998).

The agarose sample was supplied by Sigma (product number: A 0576). It is a material of high gel-strength, which achieves values of shear storage modulus of 1.4×10^4 Pa at 5 °C (0.7% gel) (Deszczynski, Kasapis, & Mitchell, 2003). According to the supplier, water, ash and sulfate contents were of less than 7.0%, 0.25% and 0.12%, respectively.

The sample of κ-carrageenan was a gift from Hercules, Lille Skensved, Denmark (batch X6960). ^{1}H NMR analysis showed that κ-carrageenan-like segments (i.e., with a sulfate group at position 2 of the 3,6-anhydride residue) constitute about 8.0% of the polymer. An Amberlite IR-120 exchanging resin from BDH was used to prepare the polysaccharide in the potassium form. κ-Carrageenan in the potassium form was characterized with intrinsic viscosity measurements $[\eta]$ at a constant ionic strength (0.01 M KCl) and at 40 °C yielding a $[\eta]$ value of 10.5 ± 0.2 dl/g (Evageliou, Kasapis, & Hember, 1998).

The glucose syrup was supplied by Cerestar, Trafford Park, Manchester, UK. The dextrose equivalent (d.e. gives the content of reducing end-groups relative to glucose as 100) of the sample is 42, and it contains 18% water. The water content of the glucose syrup was considered in calculating the composition of samples, and the glucose syrup content in this paper refers to dry solids. The material has been thoroughly characterised and details of its composition have been published earlier (Tsoga, Kasapis, & Richardson, 1999).

2.2. Methods

2.2.1. Sample preparation

Polysaccharide solutions for physicochemical studies were prepared by dissolving materials at 90 °C. Following this, the ionic strength was brought to the desired level by mixing with a calcium or potassium chloride solution (deacylated gellan and κ-carrageenan, respectively). High solid materials were made by adding appropriate amounts of glucose syrup to the polysaccharide solutions to produce systems with the required composition. Cylindrical gels of polysaccharide/co-solute (diameter: 30 mm; height: 6 mm) were sealed under vacuum in plastic pouches and left to equilibrate for 7 days at ambient temperature. Following this, the sealed gels were placed in a high pressure single vessel apparatus U3000 (Unipress Equipment, Warsaw, Poland) of 0.5 L volume and 35 mm plunger diameter. The pressure medium was demineralised water, the pressure build up was performed at about 100 MPa per 20 s and the accessible pressure range extended to 700 MPa. At each chosen level, samples were left for 30 min and then the pressurized materials were analysed immediately using thermal and mechanical techniques.

2.2.2. Thermal analysis

MDSC measurements were performed on a TA Instruments Calorimeter *Q*1000 with autosampler (TA Instruments Ltd., Leatherhead, UK). The instrument used a refrigerated cooling system to achieve temperatures of −90 °C and a nitrogen DSC cell purge at 25 ml/min. Hermetic aluminium pans were used. The DSC heat flow was calibrated using a traceable indium standard ($\Delta H_f = 28.3$ J/g) and the heat capacity response using a sapphire standard. Preparations were cooled at rate of 1 °C/min to −90 °C, left there for 30 min, and the glass transition temperature was determined from the midpoint of the heat capacity change observed at the same scan rate upon subsequent heating. Samples of 7–10 mg were analysed at ±0.53 °C temperature amplitude of modulation and 40 s period of modulation. The reference was an empty hermetically sealed aluminium DSC pan. Three runs were generally taken and the average of essentially overlapping traces was considered as the glass transition at subzero temperatures.

2.2.3. Rheological measurements

These were performed with the Advanced Rheometrics Expansion System (ARES), which is a controlled strain rheometer (TA Instruments Ltd., New Castle, DE, USA). Details of the procedure used to establish that any inherent machine compliance was insufficient to significantly offset measured values from the high modulus glass systems, and of the parallel-plate measuring geometry have been reported (Evageliou et al., 1998; Tsoga et al., 1999). Samples were loaded onto the pre-heated platen of the rheometer, their exposed edges were covered with a silicone fluid from BDH (100 cs) to minimize variation in the water content, and they were cooled to subzero temperatures (−50 °C) at a rate of 1 °C/min. This was followed by a heating scan at the same rate to temperatures above 0 °C. Dynamic oscillatory routines on shear provide readings of the shear storage modulus (G') which is the elastic component of the network, shear loss modulus (G''; viscous component) and a measure of the 'phase lag' δ ($\tan\delta = G''/G'$) of the relative liquid-like and solid-like structure of the material (Ferry, 1980; Fillers & Tschoegl, 1977). The applied strain varied from 0.00071% in the glassy state to 1% in the rubbery plateau to accommodate the considerable changes in the measured stiffness of the sample.

3. Results and discussion

3.1. Qualitative profile of the effect of high hydrostatic pressure on the thermal and mechanical properties of gelling polysaccharide/co-solute gels

The scope of both parts of this study is to examine the effect of high hydrostatic pressure on the structural properties of networks with different morphology. In doing so, we considered the non-aggregating associations of gelatin (part I of this series; Kasapis, 2007), and the aggregated order of typical gelling polysaccharides where the charge density ranges from neutral (agarose) to one charge per repeat sequence in deacylated gellan and κ-carrageenan (part II). Materials (polysaccharides, gelatin, co-solute and water) and experimental conditions (slightly acidic preparations, addition of counterions) were designed to emphasize the role played by the intermolecular hydrogen bonds and electrostatic interactions present in these systems (te Nijenhuis, 1997). Modulated differential scanning calorimetry and small deformation mechanical spectroscopy are being used increasingly to elucidate the molecular mechanisms involved in various relaxations (Lopez, Champion, Blond, & Le Meste, 2005; Zhao, Morgan, & Harris, 2005), and they are chosen presently as the main tools of analysis.

Fig. 1 reproduces the shapes of typical MDSC curves obtained for samples of 3% agarose in the presence of 75% glucose syrup solids that have been treated at 0.1 (ambient pressure), 300 and 700 MPa. Samples were cooled at a low scan rate for thermal analysis (1 °C/min) from ambient temperature (24 °C). Then, they were heated at the same scan rate from temperatures well below the glass transition temperature of the mixture, thus exhibiting a "pseudo-equilibrium" relaxation response to the changing

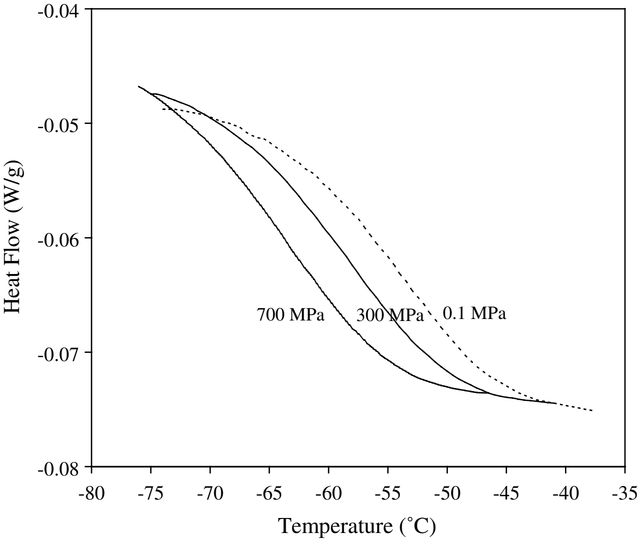

Fig. 1. Heat flow variation as a function of temperature for 3% agarose plus 75% glucose syrup obtained with MDSC at a heating rate of 1 °C/min. Gels were prepared at 0.1 (atmospheric pressure), 300 and 700 MPa.

thermal regime. In the three replicates of each experimental pressure (0.1, 150, 300, 500 and 700 MPa), there is a well-defined change in heat flow (heat capacity) which can be associated with the "endothermic" glass transition region upon heating.

The midpoint of this thermal event is readily detectable and it is considered presently as the T_g. This is known as T_{g2} in the literature, with researchers also reporting values for the beginning (T_{g1}) and completion (T_{g3}) of the sigmoidal curve (Roos, 1987). These are empirical indicators of convenience since, in our view, there is no clear-cut (fundamental) relationship between local segmental motions, which dominate the onset of the glassy consistency thus largely determining the T_g-relaxation phenomena (Ngai, 2000; Ngai & Roland, 2002), and the pictorial thermal event in DSC experiments (Kasapis, Al-Marhoobi, Deszczynski, Mitchell, & Abeysekera, 2003; Kasapis, Al-Marhoobi, & Mitchell, 2003). It will become apparent that the arbitrary choice of an empirical index within the confines of the thermal glass transition region is not critical for the discussion of this work.

Fig. 1 attempted to detect possible variations in the thermal profile of agarose/glucose syrup gels by increasing the applied hydrostatic pressure at intervals of about 300 MPa Table 1 summarizes results at the glass transition temperature obtained between 0.1 and 700 MPa for 3% agarose plus 75% glucose syrup, 2% gellan plus 76% glucose syrup (6.7 mM $CaCl_2$ added) and 1.5% κ-carrageenan plus 76.5% glucose syrup (30 mM KCl added) gels. Variation in polymer concentration reflects the increasing gelling ability of gellan and κ-carrageenan in a high solids environment. Clearly, the progressive increase in the intensity of applied pressure is manifest in irreversible relaxation phenomena which appear to be of comparable magnitude for the three polysaccharide/co-solute preparations. Thus there is a progressive decrease in T_g values from about −55 to −62 °C with increasing the experimental hydrostatic pressure in Table 1. It appears that the treatment of our samples with high pressure resulted in the disruption of the polysaccharide network with vitrification points at 700 MPa approaching those for the single sugar systems. For example, values between −62 and −65 °C have been reported in the glass transition curve of the state diagram of glucose preparations at 78% solids (Roos, 1995).

On the basis of evidence from the calorimetry measurements it could be argued that the microscopic properties of our systems are affected considerably from the pressure-jump between 0.1 and 700 MPa. The MDSC results were augmented by considering the macromolecular characteristics of the network, since typical relaxation processes associated with glass transitions are within timescales that can be accessed rheologically. Fig. 2 reproduces typical polysaccharide/glucose syrup data illustrated for 2% gellan plus

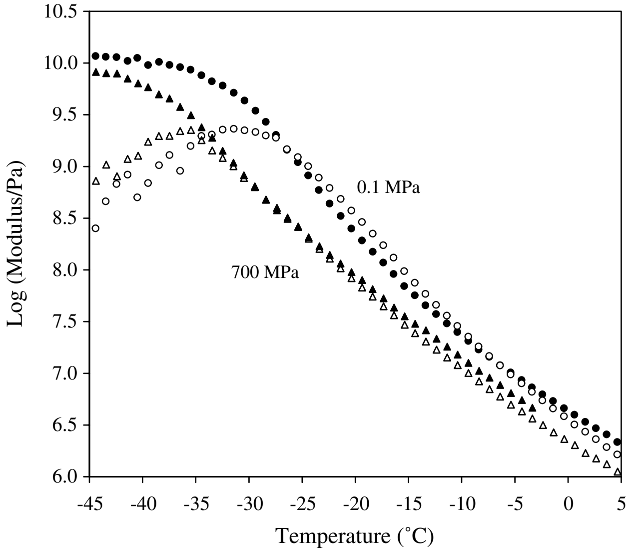

Fig. 2. Temperature variation of shear moduli for 2% gellan plus 76% glucose syrup (6.7 mM $CaCl_2$ added) at a scan rate of 1 °C/min and a strain range of 0.00071% to 1%. Prior to mechanical analysis, gels were pressurized at 0.1 [G' (●); G'' (○)] and 700 [G' (▲); G'' (△)] MPa.

Table 1
Glass transition temperatures (T_g) of polysaccharide/co-solute preparations (78% total level of solids) determined using modulated differential scanning calorimetry

	0.1 MPa	150 MPa	300 MPa	500 MPa	700 MPa
3% Agarose + 75% glucose syrup					
T_g Replications (°C)	−56.3	−57.0	−60.4	−59.4	−61.3
	−56.8	−56.4	−58.1	−63.5	−63.9
	−55.0	−59.4	−60.5	−61.2	−59.9
2% Gellan + 76% glucose syrup (6.7 mM $CaCl_2$)					
T_g Replications (°C)	−56.5	−56.4	−57.3	−57.4	−62.2
	−57.0	−55.5	−56.6	−59.2	−62.3
	−56.2	−53.5	−56.6	−60.6	−61.5
1.5% κ-Carrageenan + 76.5% glucose syrup (30 mM KCl)					
T_g Replications (°C)	−52.9	−54.3	−53.5	−59.5	−60.3
	−53.2	−55.6	−60.6	−62.9	−63.6
	−52.6	−57.4	−57.0	−59.0	−60.2
Average T_g	−55.2 ± 1.8	−56.2 ± 1.2	−57.8 ± 2.0	−60.3 ± 1.5	−61.7 ± 1.9

76% glucose syrup (6.7 mM $CaCl_2$ added) during heating or cooling at as scan rate of 1 °C/min. Furthermore, temperature-dependent changes are depicted for two distinct applications of pressure, namely: 0.1 and 700 MPa.

In both cases, the transition from the rubbery region to the glassy state shows perfect thermal reversibility, i.e., absence of thermal hysteresis. At temperatures above −5 °C, the mechanical response is predominantly solid-like (some G' data are not shown to avoid clutter), with $G' > G''$ at the experimental frequency of 1 rad/s. We also carried out frequency sweeps between 0.1 and 100 rad/s which reproduced the mechanical consistency of typical rubber-like materials (Alves, Mano, Gomez Ribelles, & Gomez Tejedor, 2004). On further reduction in temperature, there is considerable reinforcement in mechanical properties, a behaviour which in the research of amorphous synthetic materials is identified as the glass transition region and reflects the diminishing transverse string-like vibrations of polymeric segments upon cooling (Adhikari & Michler, 2004).

At the lower range of experimental temperatures in Fig. 2 (below −25 and −31 °C at 0.1 and 700 MPa, respectively), there is yet another development. The solid-like character becomes dominant and reaches readings of 10^{10} Pa at −45 °C. This part of the master curve of viscoelasticity is known as the glassy state (Slade & Levine, 1991), where there is little variation of storage modulus with temperature, and an increasing separation between the G' and G'' traces. Clearly, our experimental results confirm that the effect of hydrostatic pressure on DSC patterns is reproducible in the mechanical manifestation of vitrification phenomena. Further elucidation of the pressure dependence of relaxation processes requires development of quantitative relations which include parameters that affect the molecular free volume.

3.2. Quantitative analysis of the dynamic oscillatory modulus for the polysaccharide/co-solute gels subjected to hydrostatic pressure

In the absence of a phase transition as a function of changing temperature or pressure, viscoelastic parameters can be related by implementing horizontal superpositions along the logarithmic axis of time or frequency of oscillation (Jazouli, Luo, Bremand, & Vu-Khanh, 2005). This is known as the method of reduced variables and it necessitates that changes in temperature and pressure affect all relaxation times in the same manner (Heymans, 2003). Following this approach, standard mechanical spectra were taken at regular temperature intervals of three to four degrees centigrade, and a typical sample of the results is illustrated in Fig. 3a and b. Earlier, gels were either treated at atmospheric pressure or pressurized for 30 min between 150 and 700 MPa. Both moduli have relatively low values at high temperatures (e.g., −7 °C), whereas there is a substantial build up of viscoelasticity at the low temperature end (<−40 °C). Data were processed choosing arbitrarily a point within the glass transition region as the reference temperature ($T_o = -17$ °C), and shifting the remaining data along the log frequency axis until a uniform curve was obtained.

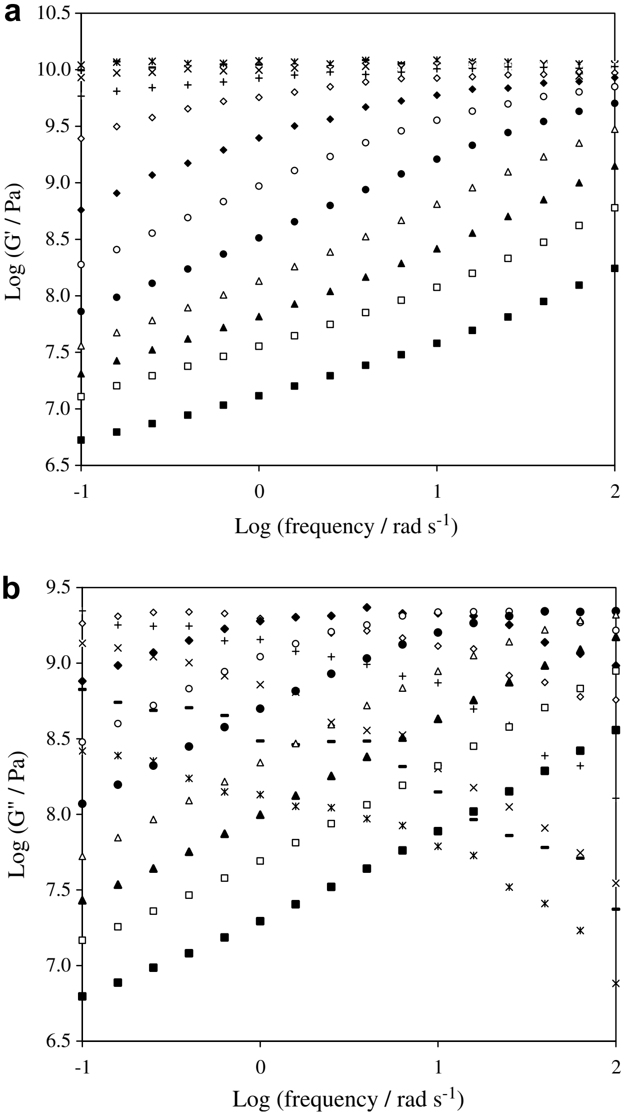

Fig. 3. Real (a) and imaginary (b) parts of the complex shear modulus, plotted logarithmically against frequency for 2% gellan plus 76% glucose syrup (6.7 mM $CaCl_2$ added). Bottom curve is taken at −7 °C (■); other curves successively upward, −11 (□), −14 (▲), −17 (△), −20 (●), −23 (○), −26 (◆), −30 (◇), −34 (+), −38 (x), −42 (–), −46 (*) °C, respectively. The sample was prepared and analysed at ambient temperature.

An example of the master or composite curve obtained in this work for the polysaccharide/co-solute gels is reproduced in Fig. 4, and for both moduli G'_p&G''_p an 11-decade window of reduced frequency has emerged. Gratifyingly, the progress in viscoelasticity in Fig. 4 appears to be the time analogue of the temperature effect in Fig. 2. Thus, there is a cross-over of moduli, with the response at the low end of reduced frequency ($<10^2$ rad/s) being associated with the glass transition region. This corresponds to two-and-a-half orders of magnitude rise in moduli, with G''_p being higher than G'_p, and requires five decades of reduced

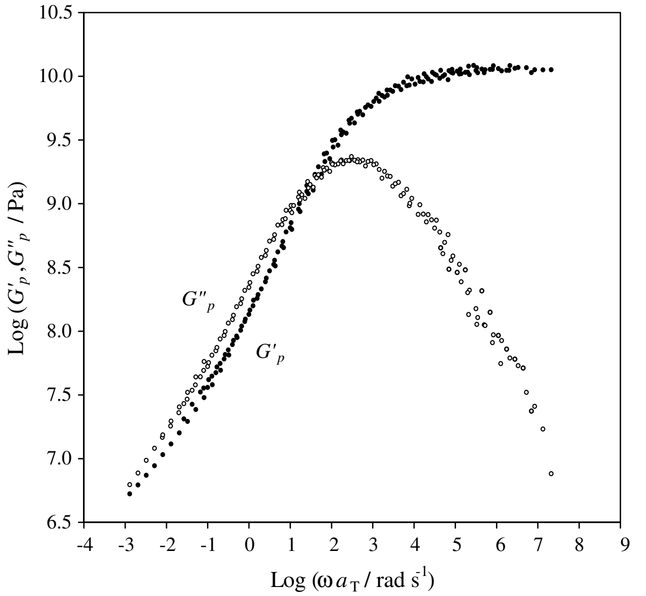

Fig. 4. Composite curve of reduced shear moduli (G'_{p} and G''_{p}) for the sample of Fig. 3 at the reference temperature of −17 °C.

frequency to complete. The crossing of reduced moduli indicates the glass transition frequency (Ω_g), recording a value of 10^2 rad/s at the reference temperature of −17 °C, and introduces the glassy state.

All along, the spectacular development in the frequency profile of storage and loss modulus is accompanied by considerable variation in the values of $\tan\delta$ (the ratio of G'' to G'). A straightforward calculation indicates that the maximum value of $\tan\delta$ (2.06) is achieved at the reduced frequency of 1 rad/s, whereas the progressive change from liquid-like to solid-like response in the glassy state is reflected in considerable reduction in the values of this rheological function (about 0.001 at the reduced frequency of $10^{7.4}$ rad/s). The $\tan\delta$ peak has been considered in the past as an indicator of the centre of the glass transition region, but the empiricism of this approach has been thoroughly discussed (Peleg, 1995). This is mainly due to the appearance of the peak at temperatures/frequencies where must of the stiffness has already been lost or at temperatures/frequencies where different materials have undergone a very different degree of plasticization. The empirical nature of the $\tan\delta$ peak will be contrasted in the following section with a fundamental derivation of the mechanical or network T_g (T_{gn}).

Identical superposition of both G' and G'' traces is a prerequisite for valid application of the method of reduced variables (Shivakumar, Das, Segal, & Narkis, 2005), thus yielding the shift factor (a_{T}) that integrates two sets of temperature data:

$$\log a_{\mathrm{T}} = -\frac{(B/2.303f_{\mathrm{o}})(T - T_{\mathrm{o}})}{(f_{\mathrm{o}}/\alpha_{\mathrm{f}}) + T - T_{\mathrm{o}}}$$

This is the equation proposed by Williams, Landel and Ferry (WLF) and it utilizes the concept of free volume to develop a mechanistic understanding of glass transitions (Ferry, 1991; Ferry & Stratton, 1960). Thus, f_{o} is the fractional increase in free volume at T_{o}, α_{f} is the thermal expansion coefficient, and the value of B is set to be about 1.

The pattern of structural relaxation, as documented in the factor a_{T} for the horizontal superposition of mechanical spectra in Fig. 3a and b, was fitted with the WLF equation. The algorithm follows well the progress in viscoelasticity within the glass transition region thus making free volume the molecular mechanism dictating diffusional mobility (Fig. 5). However, the WLF/free volume approach does not hold for the temperature range of the glassy state (<−25 °C), at which the alternative plot (Levi & Karel, 1995):

$$\log a_{\mathrm{T}} = \frac{E_{\mathrm{a}}}{2.303R}\left(\frac{1}{T} - \frac{1}{T_{\mathrm{o}}}\right)$$

i.e., the modified Arrhenius equation achieves a good linear relationship for the horizontal shift factors (R is the gas constant). The constant activation energy (E_{a}) argues that relaxation processes in the glassy state are heavily controlled by specific chemical features.

The appearance of an Arrhenius-related linear superposition delimits a discontinuity in the development of shift factors at the end of the WLF curvature upon cooling. This change in operational kinetics can be considered to be a measure of the fundamental glass transition temperature at which free volume considerations become secondary to an energetic barrier of molecular rearrangements from one state to another. Mechanical studies were extended to atmospheric pressure and 700 MPa for the three polysaccharide/co-solute gels, with frequency sweeps being reduced to master curves utilising corresponding sets of

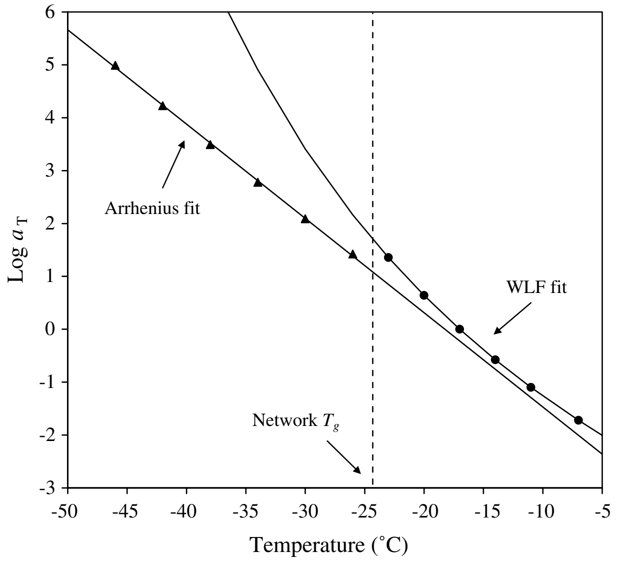

Fig. 5. Temperature variation of the factor a_T for the glass transition region (●) and the glassy state (▲) for the sample in Fig. 3. The solid lines reflect the WLF fit in the glass transition region, and the modified Arrhenius fits in the glassy state. The dashed line pinpoints the prediction of the network glass transition temperature.

Table 2
Network glass transition temperatures (T_{gn}) of 2% gellan + 76% glucose syrup (6.7 mM $CaCl_2$), 1.5% κ-carrageenan + 76.5% glucose syrup (30 mM KCl), and 3% agarose + 75% glucose syrup

	Gellan	κ-Carrageenan	Agarose
T_{gn} (°C) at 0.1 MPa	-24.0 ± 0.4	-15.2 ± 0.5	-32.3 ± 0.5
T_{gn} (°C) at 700 MPa	-30.5 ± 0.5	-19.0 ± 0.2	-36.7 ± 0.4

calculated shift factors, an example of which is illustrated in Figs. 3–5. Results in terms of the value of T_{gn} are summarized in Table 2. This exercise gave a clear confirmation that the temperature-course of vitrification phenomena is altered considerably within the experimentally accessible range of pressure thus creating a differential of about five to six degrees centigrade for the mechanical glass transition temperature.

4. Concluding remarks

This work argues that the thermal and mechanical manifestation of the effect of static confining pressure (hydrostatic in our case) on vitrification phenomena is quite distinct. A fundamental derivation of the network glass transition temperature from mechanical measurements on high-solid polysaccharide preparations produces values that lie well above the empirically derived DSC T_g. It is known that calorimetrically determined glass transition temperatures are affected by the heating rate, whereas there is a frequency dependence on the vitrification parameters obtained with mechanical spectroscopy (Deszczynski, Kasapis, MacNaughton, & Mitchell, 2002). Keeping in mind that the measuring principles of the two techniques may set hurdles for meaningful comparisons, it is apparent from the T_g differences in the three mixtures at pressures of 0.1 and 700 MPa (Tables 1 and 2) that rheological measurements are suited to the characterisation of the behaviour of polymeric networks, whereas calorimetry monitors primarily the diffusional mobility of co-solute.

Quantification of the effect of pressure on the structural properties of individual polysaccharide/co-solute samples documents a break in the time–temperature–pressure superposition, which is operational in the vitrification of amorphous synthetic materials. Thus destabilization of thermal and viscoelastic relaxation processes is monitored under pressure in Tables 1 and 2, as opposed to the positive temperature or pressure effect on the corresponding behaviour of elastomers with hydrophobic interactions (e.g., styrene or butadiene rubbers). The current result is also dissimilar to the response obtained for the molecular interplay between gelatin and co-solute in part I of this series (Kasapis, 2007). That was due to the development of non-aggregated hydrogen-bonding stabilized associations, which exhibit a degree of reversibility once the pressure generating experiment is complete. In the case of gelatin, values of T_g remained constant throughout the range of applied hydrostatic pressure (0.1–700 MPa), a range which is congruent with that reported earlier in the literature of synthetic polymers (Moonan & Tschoegl, 1983, 1984, 1985).

Recent work demonstrated that rubbery polysaccharide gels maintain a degree of intermolecular aggregation in a high co-solute environment (e.g., glucose syrup, sucrose, etc.), albeit diminished in comparison with the extensive volume of enthalpic associations of the aqueous counterparts (Kasapis, Al-Marhoobi, Deszczynski et al., 2003; Kasapis, Al-Marhoobi, Mitchell et al., 2003). In the present investigation, pressure-induced vitrification patterns appear to be largely irreversible due to the destabilization of the remaining aggregated assemblies of the high co-solute polysaccharide networks. Thus disruption and the slow kinetics of recovery of the brittle polysaccharide agglomerates lead to lower values in the micro and macro-examination of the glass transition temperature.

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