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The glass transition zone in high solids pectin and gellan preparations

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Dynamic oscillatory data on deacylated gellan and high methoxy pectin, in the presence of sucrose and glucose syrup, are presented over a wide temperature range. High levels of the co-solute induce a transition from rubberlike to glasslike consistency in the two biopolymers. The time–temperature superposition method is employed to construct composite curves, around the glass transition region, covering a nine-decade frequency range. The shift factors thus derived are fitted against the experimental temperatures using the Williams, Landel and Ferry equation. These observations are interpreted on the basis of the free volume theory which allows calculation of thermal expansion coefficient and fractional free volume parameters, at the glass transition temperatures of the high solids gellan and pectin systems. Values are in excellent agreement with those for synthetic polymers, a result which emphasizes the universal utility of the above approach (experiment and theory) in studying glasses of synthetic or biological origin. © 1997 Elsevier Science Ltd.

(Keywords: high methoxy pectin; gellan; high-solids)

INTRODUCTION

Pectin is the collective name for a group of polysaccharides extracted mainly from citrus peel or apple pomace. Found in the middle lamellae of plant tissues and also in the primary cell wall, it is a structural carbohydrate and is to a large extent responsible for firmness and form-retention of the tissues as well as softening during ripening[1]. The pectin molecule exists in nature as a branched, partially methyl-esterified chain where the backbone is based on a repeat sequence of 1,4-linked α-D-galactopyranosyluronic residues. Pectins with a degree of esterification (DE) above 55% are known as high methoxy pectins whereas those with a lower DE are low methoxy pectins[2]. The chain is interrupted periodically by the insertion of 1,2-linked rhamnose, which cause the chain to kink, and side chains (short 'hairy' regions) thus preventing propagation of the ordered structures of the network. Between the kinks, gelation in low methoxy pectins occurs by means of dimerized galacturonan sequences which incorporate an array of chelated divalent cations (commonly calcium) to offset electrostatic repulsions between the carboxyl groups ('egg box' binding)[3]. High methoxy pectins require a minimum content of soluble solids (above 55%) and an acid pH (around 3.0) in order to form gels where the binding forces between the chains are hydrogen-bond bridges and hyrophobic forces between methoxy groups[4].

Gellan is the extracellular polysaccharide produced by aerobic fermentation of the micro-organism *Sphingomonas elodea* (formerly known as *Pseudomonas elodea*). The polymer is recovered in its deacylated form which is a linear polyanion with a tetrasaccharide repeating unit containing two β-D-glucose and one β-D-glucuronic acid residues $(1 \rightarrow 4)$ glycosidically linked. The fourth sugar, α-L-rhamnose, forms a $(1 \rightarrow 3)$ link with the next tetrassacharide repeat unit, creating a twist in the chain[5]. X-ray diffraction studies show that the ordered structure involves two left-handed 3-fold helical chains organized in a parallel fashion in an intertwined duplex[6]. The helix is stabilized by ions of various valencies with the strength and stability of the resulting gels being profoundly influenced by the concentration and type of counter ions present[7]. On 15 December 1994, the European directive on miscellaneous additives for food and drug use, in which gellan is listed as E418, was approved at a European Council meeting in Brussels[8].

In general, gelation of polysaccharides during cooling involves conversion from a disordered coil conformation at a high temperature to a rigid, ordered structure at a lower temperature. In the case of deacylated gellan (0.5% w/w), increasing levels of salt promote the disorder to order transition (e.g. the gelation temperature rises from 31.1°C to 53.5°C at 0.05 and 0.26 M NaCl respectively) and increase the 'pseudoequilibrium' storage modulus of the network (G' is 0.14 and 9.7 kPa at the above levels of salt; 5°C)[9]. This type of transformation is associated with some form of barrier to rotation either of a segment of the main chain or of a side chain and a

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certain activation energy E_a is required for reaction to ensue. Stopped-flow polarimetry on xanthan[10] and d.s.c. on methylcellulose[11] have shown that the temperature course of the transition enthalpy follows the linear Arrhenius relation with constant values for E_a, and obeys a first-order kinetic scheme of a single helix structure for these two biopolymers.

A linear relationship between the natural logarithm of viscoelastic parameters and E_a/RT is also seen for amorphous synthetic polymers [e.g. an atactic polystyrene or a poly(*n*-octyl methacrylate)] at temperatures below the glass transition temperature (T_g) and at the high temperatures of the low-viscosity polymer melts[12]. By contrast, the transition zone from rubberlike to glasslike behaviour corresponds to a non-linear temperature dependence of viscoelastic functions and it is better fitted by the Williams, Landel and Ferry kinetic scheme[13]. The WLF approach has proved to be widely applicable and three decades after its introduction, Sperling acknowledged that 'for a generation of synthetic polymer scientists and rheologists, the WLF equation has provided a mainstay both in utility and theory'[14]. Regrettably, the application of WLF kinetics to aqueous preparations of biopolymer gels has failed due to development of crystallinity or intermolecular enthalpic interactions as a function of temperature, resulting in a change in the distribution of relaxation times during the coil to helix transition[15]. The recent paper by Lopes da Silva and co-workers on pectin dispersions only highlights the problem with the authors admitting: 'but a smooth master curve could not be obtained for both moduli simultaneously or for each one individually ... satisfactory reduction of the data to a single curve was not obtained, irrespective of the frequency shift factor used, for each modulus individually or with vertical shift factors higher than those calculated by the experimental temperature-density factor'[16].

Work in this laboratory has now documented the glass transition zone for high solids gellan and pectin dispersions using the WLF principle, a result which might be useful in the study of theory and applications of glass transitions in biopolymer systems.

EXPERIMENTAL

Materials

The citrus peel pectin was supplied by Hercules. It is a rapid set sample with a galacturonate content (esterified and non-esterified) of 83% and a high degree of esterification (70%) on a dry basis. Preparations were extensively dialysed against water (four changes) and freeze dried to give a pure pectinic substance. Intrinsic viscosity measurements on the pure polysaccharide at 0.1 M NaCl gave a value of $3.42 \pm 0.22\,\mathrm{dl\,g^{-1}}$ (25°C). The concentration dependence of storage modulus (G'), treated with the cascade formalism, produced a minimum critical gelling concentration of 0.32% (pH 3.0; 60% sucrose plus 5% glucose syrup; $10\,\mathrm{rad\,s^{-1}}$, 0.5% strain). Samples were made by dissolving the freeze dried material at 95°C using mechanical agitation and then by adding sucrose (up to 50% w/w) and appropriate amounts of glucose syrup (from 4 to 36%). Finally, the pH value was adjusted to 3 using a 2 M solution of HCl. The glucose syrup was supplied by Cerestar. 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 20% water. The water content of the glucose syrup was considered in calculating the composition of samples. The purpose of the glucose syrup was to inhibit crystallization of sucrose on cooling. Material composition in this work is given on a dry basis.

The deacylated gellan was supplied by the NutraSweet Kelco Company (Kelcogel; batch 77109a). Atomic absorption analysis produced the following average ionic composition: Na^+: 6330 ppm; K^+: 46 800 ppm; Ca^{2+}: 3660 ppm; and Mg^{2+}: 1140 ppm. This corresponds to a conversion of 115%, which is typical of the gellan polysaccharide containing a slight excess of ions over and above the amount needed for stoichiometric equivalence. Synthetic tap water (a solution comprising 1000 ppm NaCl and 143 ppm $CaCl_2{\cdot}2H_2O$ in deionized water) was used to bring the cation normality of each gellan sample during preparation to roughly twice the stoichiometric equivalent of the polymer. High solids systems were made by dissolving gellan at 90°C (stirring for 15 min) and adding appropriate amounts of sucrose up to 50% w/w. At higher levels of co-solute glucose syrup was added (up to 35%) bringing the maximum solids content to 85%. As for the pectin samples, gellan gels were acidified using 2 M HCl to a pH value of 3.8. Aqueous gellan solutions with the polymer in the disordered conformation were dialysed extensively against a 25 mM NaCl solution at 70°C and analysed at 50°C yielding an intrinsic viscosity value of $4.49 \pm 0.2\,\mathrm{dl\,g^{-1}}$.

The gellan sample was further characterized by the estimation of its molecular weight. To ensure a disordered coil conformation at the operational temperature, the g.p.c. column temperature was 75°C, gellan was converted to Me_4N^+ and Na^+ salt forms by cation exchange on Amberlite IR 120 (Me_4NCl and NaCl were of AnalaR grade from BDH). Solutions were dialysed extensively against deionized water and freeze dried. The chromatography was performed on Shodex columns KS806, KS804, and KS802 in series. These are packed with divinyl benzene crosslinked polystyrene, sulfonated, and in the sodium form. From the freeze dried gellan samples a solution (0.5% w/w) was made with water alone, and with a 90/10 mixture of dimethyl sulfoxide (DMSO) and water. The system was calibrated with a set of pullulans, P-82, from Shodex, which gave a range up to 1.6×10^6 Da, combined with a narrow distribution. Elution was with water at 75°C and a flow rate of $1\,\mathrm{ml\,min^{-1}}$. A differential refractometer was used for detection. Results showed that the solvent for the gellan had little effect on the average molecular weight measurements (M_w). M_w figures for the Me_4N^+ form were 1.73×10^6 and 1.76×10^6 Da in water and DMSO/water respectively, and M_w values for the Na^+ form were 1.53×10^6 and 1.55×10^6 Da in water and DMSO/water respectively.

Rheological methods

Dynamic oscillation routines have been used to demonstrate the formation of structures with different viscoelastic properties in low and high solids pectin and gellan systems by following the gelation or vitrification of samples occurring with decreasing temperature. Samples were loaded onto the pre-heated plate of the rheometer and maintained at high temperature (90°C) for a short time to reach thermal equilibrium. Then they were subjected to an oscillation of set frequency (1.6 Hz which corresponds to an angular frequency of 10 rad s^{-1}) and strain (0.5% for gellan and 1% for pectin) whilst cooling at a rate of 1°C min^{-1} to 5°C (gels) or −20°C (glasses). This was followed by a frequency sweep at the lowest temperature and a heating regime to 95°C. Readings of the rigidity modulus (G'), viscous modulus (G'') and dynamic viscosity (η^*) variation with temperature and frequency were obtained, and a measure of the 'phase lag', δ ($\tan \delta = G''/G'$), giving an indication of the relative liquid-like and solid-like character of the sample. Samples which showed the transition from the 'rubbery' to the glassy state were scanned down again and mechanical spectra were monitored at regular temperature intervals. Measurements were performed on a commercial controlled stress Carri-Med CSL 500 rheometer using parallel plate geometry and 1 mm gap, or on a sensitive prototype designed and developed in house by one of us (R. K. Richardson), using cone and plate geometry of cone angle 0.05 rad and 50 mm diameter.

To circumvent problems of thermal expansion/contraction during heating and cooling scans, the cone was truncated over 45% of its diameter, giving a gap of 0.5 mm between the flat surfaces of the two elements, but keeping strain constant at a fixed, maximum, value across the outer portion (which constitutes 80% of the total area). The combined working range of the two rheometers was further extended by the use of parallel plates with diameter from 40 to 8 mm thus achieving time–temperature superpositions of shear moduli ranging from 10^{-1} to 10^{8} Pa. Frequency and strain sweeps of silicone oil have shown that at the experimental settings of our temperature runs (strain $\geq$ 0.5%; frequency of 1.6 Hz) the commercial rheometer can be trusted at modulus values down to 10 Pa. The same calibration demonstrated a sensitivity of 0.1 Pa for the measuring transducer of the prototype, with the stretched torsion wire (elastic element) being designed to monitor structures of up to 10^{3} Pa. As a result, cooling of a 50% sucrose plus 36% glucose syrup blend from 90°C to −20°C in *Figure 6* creates a common two-orders-of-magnitude range of operation with overlapping moduli values.

Particular care was taken to establish that any inherent machine compliance in the commercial rheometer was insufficient to significantly offset measured values from the high modulus glass systems. This was achieved by progressive adjustment of geometry settings whilst measuring samples of high known modulus (ice), and maximum plate diameter and minimum gap consistent with accurate results could then be determined. In doing so, rubber to glass transitions were reproduced within a 3% to 5% margin. A special silicone fluid (Dow Corning 100 cs) of sufficiently high viscosity to prevent evaporation at high temperatures, and of sufficiently low molecular weight to remain liquid-like at sub-zero temperatures was used to cover the exposed edges of our samples.

RESULTS AND DISCUSSION

A brief resumé of the utility of the TTS principle on the WLF equation

During the first half of this century empirical time–temperature shifts of dielectric[17] and mechanical[18] data on synthetic materials, monitored at different temperatures, had already been used to construct 'master' curves along an abscissa of logarithmic time or frequency. The time–temperature superposition (TTS) approach was then used by Ferry and co-workers to describe the transition zone of amorphous synthetic polymers from rubber-like to glass-like consistency, where the shear moduli increase by several orders of magnitude as a function of time or frequency[13]. They also introduced an empirical expression (the WLF equation) to fit their data

$$\log a_T = -C_1^{o}(T - T_o)/(C_2^{o} + T - T_o) \qquad (1)$$

where C_1^{o} and C_2^{o} are constants, and T_o is an arbitrary chosen reference temperature. For the storage modulus, the term a_T is equivalent to G'_T/G'_{T_o} and is known as the shift factor since it implies that a value of G' measured at temperature T and experimental frequency ω is equivalent to the storage modulus at temperature T_o and frequency ωa_T. It follows that measurements at a variety of temperatures, if plotted as G' against ωa_T, will all correspond to measurements at T_o and form a single composite curve. Since the development of shear modulus in a macromolecular system with substantial

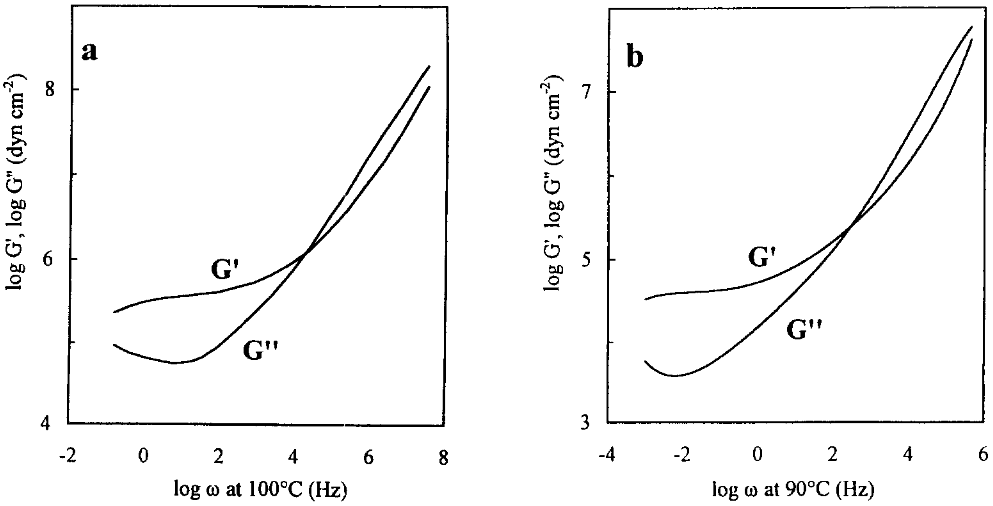

Figure 1 Time–temperature superposition for the mechanical spectra of (a) poly(*n*-octyl methacrylate) at 100°C, and (b) 0.5% gellan plus 85% co-solute sample at 90°C, covering parts of the rubbery and the glass transition zones (from refs 35 and 23, with permission)

Table 1 Small deformation viscoelastic functions taken for the 1% high methoxy pectin sample in the presence of increasing amounts of co-solute (from 54 to 86%). The glucose syrup concentration refers to dry solids

			Pseudoequilibrium modulus (Pa)		Tan δ at 1 Hz			t_{gel} (°C)	t_{mel} (°C)	
Sucrose (%)	Glucose syr (%)	Total solute (%)	90°C	5°C	90°C	5°C	95°C	1°C min^{-1}	1°C min^{-1}	Onset of glass transition (°C)
50	4	54	—	315	>1	0.576	>1	18	28	—
50	8	58	—	485	>1	0.698	>1	34	86	—
50	12	62	—	1646	>1	0.294	0.450	74	>95	—
50	16	66	—	1774	>1	0.293	0.287	70	>95	—
50	24	74	—	2813	>1	0.541	0.363	71	>95	—
50	28	78	2865	—	0.168	>1	0.201	>95	>95	15
50	36	86	12 820	—	0.514	>1	0.416	>95	>95	24

entropic character should be proportional to ρT the values of G' at temperature T are usually multiplied by $T_0\rho_0/T\rho$ before data are processed (ρ_0 and ρ are the densities of the material at T_0 and T, respectively)[19]. However, the values of vertical temperature–density shift factors are small and for glass transitions one should be able to match adjacent curves without vertical shifts. *Figure 1a* shows the application of the reduced variables method at the onset of the transition zone for poly(*n*-octyl methacrylate) taken at 100°C. Clearly, with increasing frequency the viscous component comes to dominate, and the intersection of G' and G'' traces can be used to define an operational boundary between the timescales of the plateau and transition zones.

Reduction of dynamic data for the high solids gellan system

With hindsight, it is remarkable to see that the WLF work on biopolymers started as a technological project to address the problem of gelatin-based sweets sticking together when stored at high temperatures; perhaps Prof. W. I. B. Beveridge was right in arguing for the important rôle of chance in research![20] In that project, the immediate aim was to explore the possibility of using gellan to create a continuous heat-stable matrix, with gelatin dispersed through it as a discontinuous phase. As described below, however, soon we looked for fundamental knowledge by characterizing the effect of high concentrations of dissolved solids on the gel properties of gellan alone.

In the absence of co-solutes, gellan displays a sharp sol–gel transition on cooling, due to the formation and cation-mediated aggregation of intermolecular double helices[9]. Addition of co-solutes up to a concentration of 50% w/w had the anticipated effect of raising the gelation temperature and the mechanical strength of the network, as seen for other biopolymers (agarose, κ-carrageenan, gelatin)[21,22]. At higher concentrations ($\geq$60%), however, an entirely different pattern of response was observed[23]. Mechanical spectra recorded at high temperature were qualitatively similar to those of gels ($G' > G''$, with little frequency dependence of either modulus). On cooling, the spectra became more like those of biopolymer solutions ($G'' > G'$, with both values increasing steeply over several decades with increasing frequency of oscillation).

Although this pattern of modulus response in the time–temperature domain has not previously been reported for polysaccharides, as mentioned in the preceding section such behaviour is well known in the glass transition zone of polymer melts. Following the synthetic polymer approach, therefore, we carried out frequency sweeps at 90°C, 70°C, 50°C, 30°C, and 5°C for several high solids gellan blends. *Figure 1b* shows successfully superimposed data for the 0.5% gellan plus 85% co-solute sample at the reference temperature of 90°C, yielding log a_T values of 0, 0.60, 1.51, 2.59, and 4.65 at the five aforementioned temperatures[23]. The resulting composite is very similar to the curve of its synthetic counterpart with a plateau zone occurring at 90°C, a four-decade enhancement of viscoelasticity over the temperature range from 90°C to 5°C, and a cross over of moduli (G'' becomes higher than G') at about 250 Hz. Continuous cooling from 90°C to 5°C produced a similar cross over from solid-like to liquid-like response, with increasing amounts of co-solute shifting the interception point at higher temperatures, e.g. at about 14°C and 38°C for the 80 and 85% co-solute samples, respectively (0.5% gellan; scan rate 1°C min^{-1}; frequency 10 rad s^{-1}). Although the above constitutes extremely encouraging evidence, we felt obliged to repeat the same exercise with a second polysaccharide before delving deeper into the application of the WLF/free volume framework to biopolymers, which is the main contribution of this work. After all, the strength of the WLF principle lies in its universal application to all systems that undergo a true rubber to glass transformation.

Gelation of high methoxy pectins in the presence of sucrose and glucose syrup

As described in the preceding section for the gellan polysaccharide, the small deformation properties of pectin–sucrose–glucose syrup systems fall into two distinct patterns of behaviour. *Table 1* delimits the two molecular mechanisms in terms of the amount of co-solute in the blend. Lowering the pH to 3 and addition of 54% solids allows gelation of the polymeric material. *Figure 2a* demonstrates a typical structural progression of gelation occurring as a result of a temperature–time sweep. The sharp rise in G' was taken as a marker of the onset of gelation (t_{gel}), which for the 1% pectin plus 62% co-solute sample was found to be $\approx$74°C. Upon cooling, gelation is characterized by a sharp increase in 'solid-like' character which remains unaltered during the subsequent isothermal run (5°C), with the traces being parallel and G' always above G'' ($G' = 1.65$ kPa; tan $\delta = 0.294$ at 1 Hz). A three-decade

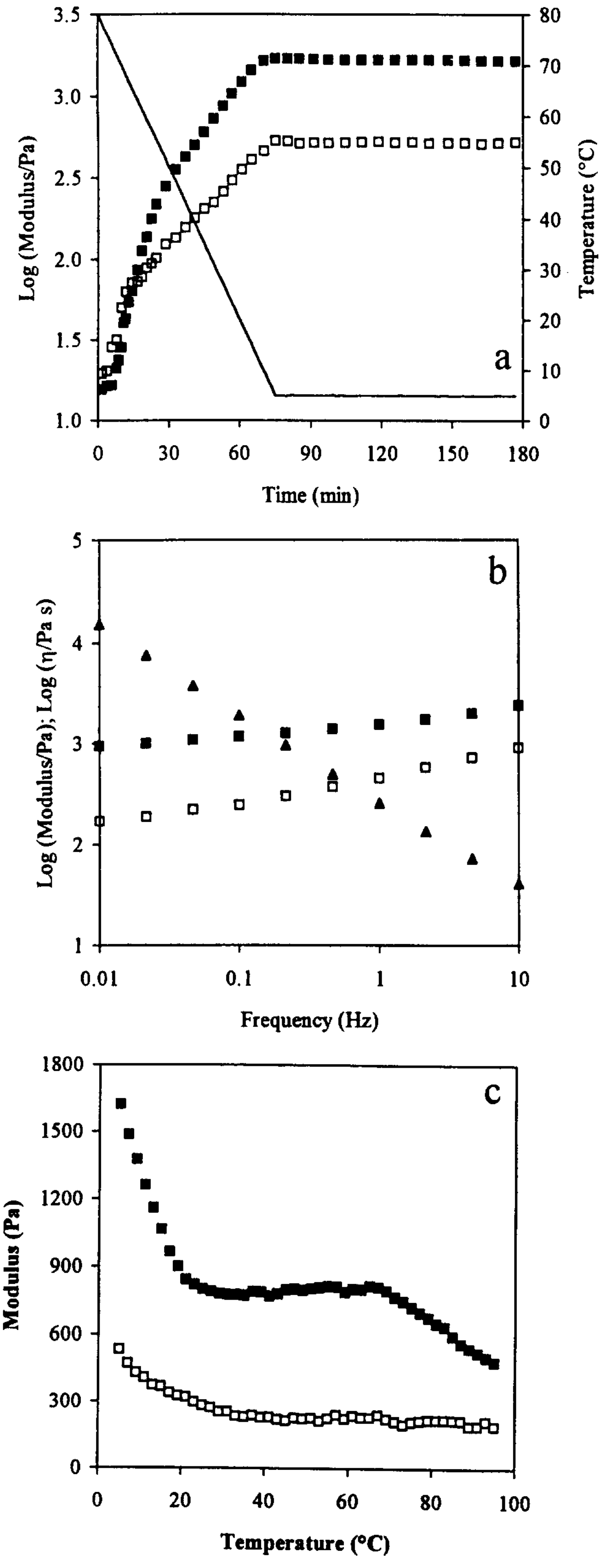

Figure 2 The development of storage modulus (■), loss modulus (□), and dynamic viscosity (▲) for 1% pectin plus 62% co-solute monitored during (a) a temperature–time run (1.6 Hz; 1°C min^{-1}; 100 min at 5°C), (b) a frequency sweep at 5°C, and (c) the subsequent heating scan (1.6 Hz; 1°C min^{-1})

mechanical spectrum is shown in *Figure 2b* (5°C). Within the experimental constraints the elastic component dominates and remains relatively flat up to a frequency of 1 Hz ('pseudoequilibrium' modulus). At higher frequencies, a stronger modulus–frequency dependence is observed since the relaxation processes in the system become more comparable to the timescale of the experimental measurements. Frequency sweeps were followed by a heating regime to 95°C at a scan rate of 1°C min^{-1} (*Figure 2c*). Clearly, the reduction in storage modulus is seen in two discrete 'waves' with the first one ending at about 30°C. The second step is still incomplete at the highest experimental temperature (95°C) thus creating a thermally irreversible network within the accessible temperature range. The result is a large degree of thermal hysteresis occurring between gel formation and dissociation in the high methoxy pectin structure (contrast with *Figure 2a*).

The effects of increasing co-solute concentration from 54 to 74% (*Table 1*) are a more thermally stable sol to gel transition (values of t_{gel} jump from around 20°C to 70°C), a stronger pectin network at 5°C (values of G' rise from 0.3 to 2.8 kPa), and eventually the formation of thermally irreversible junction zones (t_{mel} is >95°C at solid levels beyond 62%). The evolution of order in high methoxy pectin systems has been followed by Walkinshaw and Arnott using X-ray diffraction studies on dried fibres of pectin where the polymer exists as a three-fold right handed helix[24]. Computer modelling indicates a triangular pattern of polymer chains held together by hydrogen bonding in one set of channels and hydrophobic attractions between methyl groups in a second set of channels running through the centre of the crystalline aggregate. Recently, Chronakis and co-workers investigated the gelation of pectin in the presence of ethylene glycol (EG), a chemical which is a poor solvent for the hydrophilic parts of the pectin molecule[25]. Initial addition of ethylene glycol (30–60%) encourages the formation of stronger pectin gels with the network strength peaking at 60% co-solute, followed by a subsequent reduction in storage moduli at conditions of low water activity (60–80% EG). On the basis of the above model for gelation involving a two element mechanism, it was proposed that the first range of ethylene glycol concentration (30–60%) promotes mainly the ordered structure of hydrophilic pectin segments (polymer–polymer interactions), and results in stronger networks. However, higher levels of co-solute (60–80%) 'dissolve' the hydrophobic clusterings of methyl groups thus causing the eventual demise of the elastic network.

In the present investigation, the hydrophilic nature of sucrose and the small molecular weight glucose syrup (d.e. = 42) should maintain a calyx of hydrogen bonds around the clusterings of methyl groups which are a requisite of gel strength. Furthermore, increasing amounts of co-solute will increase the proportion of polysaccharide segments involved via hydrogen bonds in the formation of junction zones. The positive effect of sugar-glucose syrup blend on network development is reproduced in *Table 1* where the values of pseudoequilibrium modulus at 5°C increase steadily as a function of co-solute concentration. Since the cohesion of hydrogen bonds is reduced with temperature, one cannot fail to notice that the bimodal 'melting' profile of pectin gels (*Figure 2c*) might be due to the disintegration of hydrophilic interactions at low temperatures (first step), followed by the second trace of heat stable hydrophobic associations (see also the recent work on hydrophobic thermogelation of methylcellulose by Haque and Morris)[26].

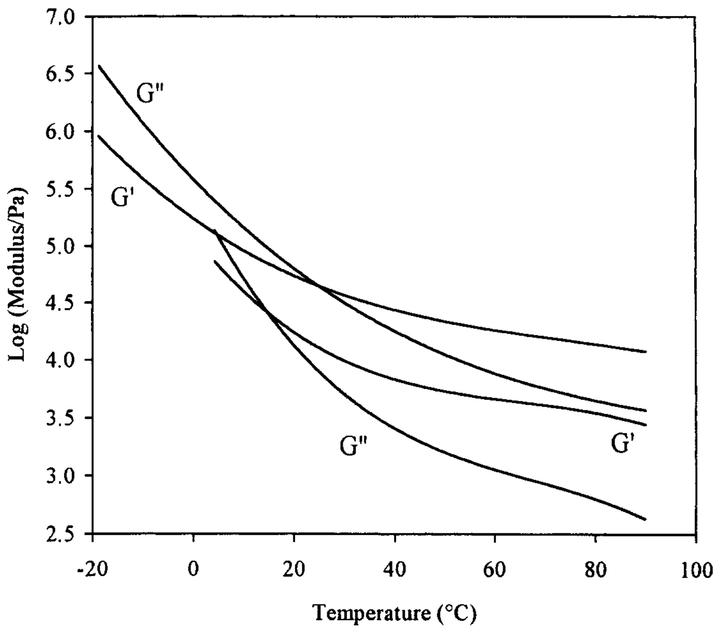

Figure 3 Cooling spectra at a scan rate of 1°C min^{-1} for 1% pectin samples at 78% and 86% co-solute (bottom and top runs, respectively)

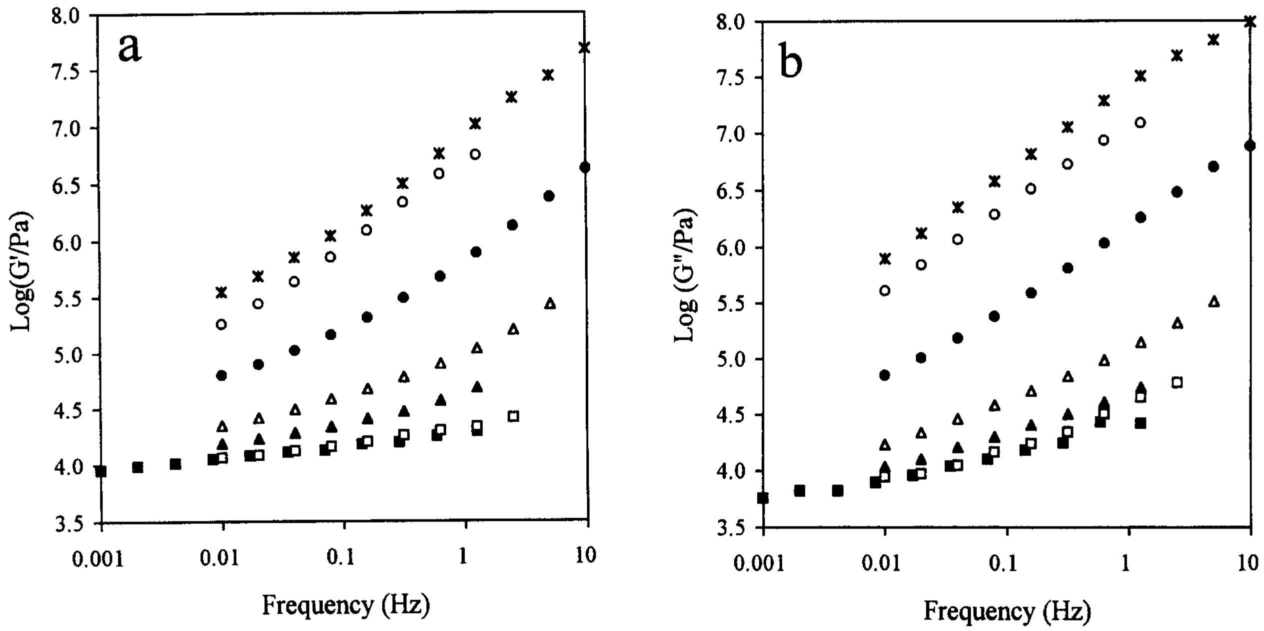

Figure 4 Variation of (a) storage modulus, and (b) loss modulus with frequency for 1% pectin plus 86% co-solute sample at 90°C (■), 70°C (□), 50°C (▲), 30°C (△), 10°C (●), −5°C (○), and −20°C (*)

Application of the time–temperature superposition approach to high solids pectin preparations

At levels of co-solute ≥78% (*Table 1*), a spectacular break from the viscoelastic behaviour of the previously described pectin preparations occurs. At the same experimental settings, samples exhibit a dominant elastic response at 90°C. During controlled cooling (1°C min^{-1}) at a fixed frequency (1.6 Hz) the viscoelastic functions change in the way seen for synthetic macromolecules so that at low temperatures the loss modulus overtakes the values of G' (*Figure 3*). Qualitatively, the creation of high modulus on cooling will depend on chain rigidity and the energy of intermolecular interactions. At high temperatures the configurational entropy will be high and there will be many ways for molecules to oscillate resulting in a relatively low 'rubbery' modulus. As the temperature falls low energy configurations start to predominate and the volume of voids between molecules decreases producing the effect of a glass transition zone. Clearly, higher amounts of co-solute (from 78 to 86%) act as stronger antiplasticizing agents on the onset of glass transition, with the point of interception between the G' and G'' traces shifting from 15°C to 24°C.

In addition to temperature, a striking change in viscoelastic parameters in the transition zone occurs as a function of time or frequency. In *Figures 4a* and *b* the storage and loss moduli of 1% pectin plus 86% co-solute are plotted logarithmically against frequency for the temperature range from 90°C to −20°C. It can be seen that there is a steady increase in the gradient of experimental traces as the temperature is reduced. At 70°C or 90°C and frequencies below 0.1 Hz, there is an approximately constant low modulus which signifies the 'rubbery' region. At lower temperatures there are the

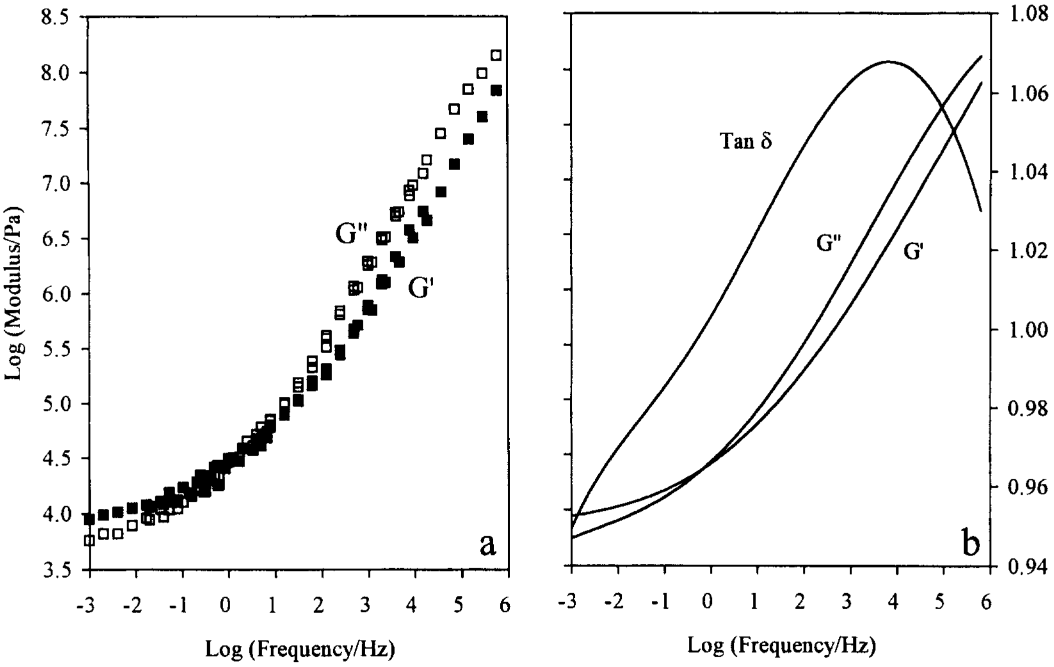

Figure 5 Application of the method of reduced variables to storage modulus (G'), loss modulus (G''), and tan δ for the 1% pectin with 86% co-solute preparation (the reference temperature is 90°C)

frequency-dependent readings of the glass transition modulus.

The data of *Figure 4* were treated with the method of reduced variables. To open a short parenthesis, the main criterion for the applicability of this approach requires that the temperature dependence of shift factor is identical for long and short relaxation functions or in other words both the G' and G'' curves, for a considerable overlapping between experimental shapes at different temperatures, match at the same set of a_T values. First, a reference temperature of 90°C was chosen so that the total temperature range of experiments could be reduced at the T_0. Second, logarithmic plots of $G'T_0\rho_0/T\rho$ and $G''T_0\rho_0/T\rho$ against frequency of oscillation were constructed with the densities ρ_0 and ρ being experimentally determined. Vertical shifts due to factor $T_0\rho_0/T\rho$ ranged from about 1 to 20% of the original modulus values at 90°C and −20°C, respectively. Third, a spreadsheet (Microsoft Excel 5) was used to shift horizontally and thus to overlap adjacent mechanical spectra. The horizontal distances were recorded as Δ log a_T and were then added progressively from T_0 to obtain the log a_T at each temperature.

Figure 5a illustrates the time–temperature superposition of moduli for 1% pectin plus 86% co-solute which produced a nine-decade frequency window. The logarithmic shift factors (log a_T) used for the horizontal alignment of the mechanical spectra taken at 90°C, 70°C, 50°C, 30°C, 10°C, −5°C and −20°C were respectively 0, 0.30, 0.72, 1.40, 2.90, 4.10 and 4.78. *Figure 5b* reproduces the two viscoelastic functions with the parameter derived from their ratio (tan $\delta = G''/G'$). At low frequencies (between 10^{-3} and 10^{-1} Hz), a part of the 'rubbery' zone is captured where there is no apparent rearrangement of long intermolecular associations and the elastic component dominates (tan $\delta < 1$). At higher frequencies, the short configurational rearrangements, which contribute to the loss modulus, take place increasingly within the frequency of oscillation and the glass transition commences at about 1 Hz. The tan δ values become 1 and continue to rise thus underlining the disproportionate development of the viscous element in this region. Eventually the system will enter the glassy state (frequencies higher than 10^6 Hz) where chain movement can no longer occur within the available time. Then the only residual movement is of a solid-like character attributed to stretching and bending of chemical bonds, and as a result the G' and G'' traces cross over yet again. Judging from the levelling off and subsequent descent of the tan δ magnitude (*Figure 5b*), the experimental observations extend well into the glass transition region, the end point of which would be reached when tan δ passes again through the value of 1, at the glass transition frequency (Ω_g)*.

The behaviour of pectin–sugar–glucose syrup mixtures contrasts strongly with the viscoelastic properties of the co-solute on its own. *Figure 6* illustrates an isochrone (frequency of 1.6 Hz) for a 50% sucrose plus 36% glucose syrup sample cooled down at 1°C $\min^{-1}$. Whereas in *Figure 3* solid-like characteristics were observed at the beginning of cooling, the co-solute is clearly liquid-like ($G'' \gg G'$) whose response to a frequency sweep is that of a Newtonian solution with a dynamic viscosity of 0.6 Pa s (90°C). Remarkably, the frequency sweep of 1% pectin plus 86% co-solute at 90°C (*Figures 4* and *5*) produces a 'rubbery' spectrum with a shear thinning dynamic viscosity of 1.6 MPa s at

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*The onset of the glass transition zone can also be taken from the intercept of the extrapolation of the relatively frequency independent G' trace at the plateau zone and a tangent to the experimental G'' curve with a slope of 1/2, which is expected by the modified Rouse theory at the vitrification of lightly cross-linked polymers. Regarding the end point of glass transition, the maximum value of tan δ in *Figure 5b* or in a corresponding cooling run has been used as the glass transition frequency or temperature. However, we feel that the third cross-over of moduli in the master curve of viscoelastic functions defines accurately the onset of the glassy state, since the only residual contraction there is of a solid like character ($G' \gg G''$)

10^{-3} Hz. The antithesis persists at the other end of the temperature range, where a glassy transformation is observed due to a predominant viscous component in the polymer–solute system (*Figures 3* and *5*). Instead, the physical state of co-solute turns solid-like as a result of partial crystallization of sucrose which contributes substantially to the elastic component of the sample ($G' \gg G''$ below -15°C in *Figure 6*). Similar temperature profiles have been obtained for solutions of sucrose/glucose syrup with or without thinned oxidized starch where additional work on d.s.c. shows a broad endothermic spectrum at sub-zero temperatures which corresponds to crystal melting[27].

Clearly, the presence of pectin not only alters radically the viscoelastic properties of sucrose/glucose syrup mixtures but also prevents co-solute crystallization. As a matter of fact, we have noticed that addition of pectic substances (or gelatin) to a sugar solution allows dissolution of higher amounts of the oligosaccharide before crystals are removed from a sugar solution. This phenomenon might be traced back to hydrophilic interactions between the polymer segments and sugars, and certainly merits further research. In terms of our investigation, however, we have documented that the blending of pectin and high levels of co-solute leads to a time/temperature dependence unlike that of conventional gels, viscous syrups and crystalline lattices expected by the two components.

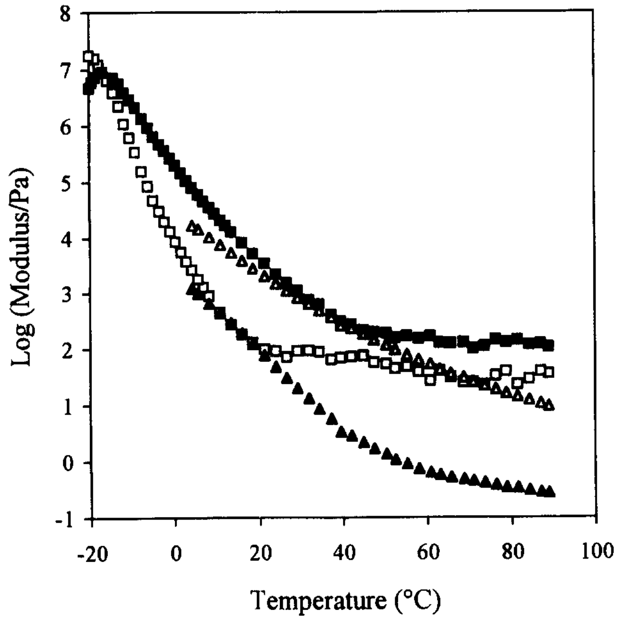

Figure 6 Controlled cooling runs (1°C $\min^{-1}$) of a 50% sucrose plus 36% glucose syrup mixture, as monitored on a sensitive in-house prototype [G' (▲); G'' (△)] and a commercial high torque rheometer [G' (□); G'' (■)]

Treatment of the glass transition of high solids gellan and pectin preparations with the free volume approach

The free volume approach is based on the concept of holes or voids between molecules, or packing irregularities which collapse with a lowering of temperature thus controlling the mobility of molecules and making the free volume (u_f) rather than temperature the independent variable for calculation, for example, of shear deformations[28]. The total volume per unit mass (u) of a polymer is the sum of u_f and the occupied volume (u_o) which includes the short van der Waals distances and the space taken up by thermal fluctuations. The dimensionless number u_f/u defines the useful parameter of fractional free volume, f.

Using the above concept, Doolittle[29] showed that the viscosity of alkanes over a wide range of temperatures could be described by the mathematical expression

$$\ln \eta = \ln A + B(u - u_f)/u_f \tag{2}$$

where A and B are empirical constants, the value of the latter was found to be ≈ 1[30]. The fit thus obtained was much better than for the well known equation of Arrhenius

$$\ln \eta = \ln C + D/RT \tag{3}$$

where C and D are empirical constants, with the latter being used as the activation energy (E_a) of a particular process, and R is the gas constant. In the high solids gellan/pectin work, an Arrhenius temperature dependence of shear moduli, presented in the form of shift factors, should comply with the following equation

$$\log a_T = \frac{E_a}{2.303R}\left(\frac{1}{T} - \frac{1}{T_o}\right) \tag{4}$$

In *Figure 7* the set of shift factors, derived at the reference temperature of 90°C, is plotted against the experimental

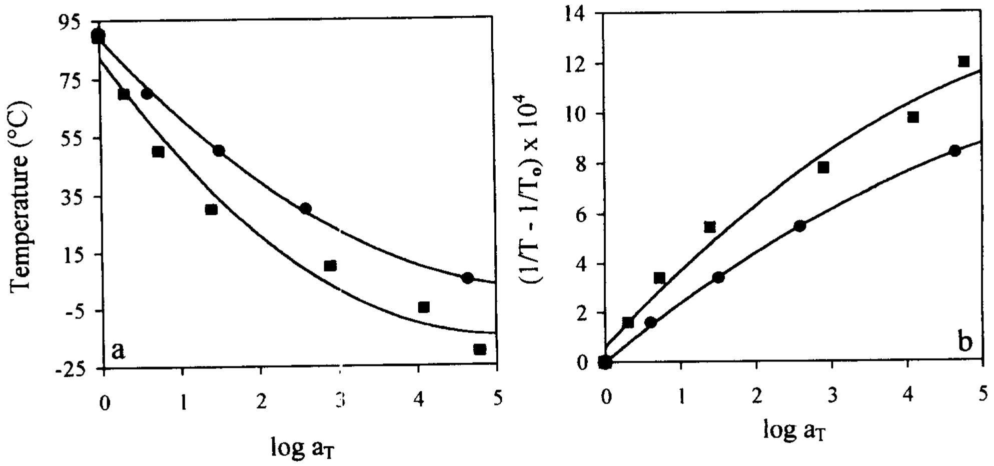

Figure 7 Exponential fitting of shift factors (a_T) against temperature in (a) degrees Celsius and (b) in the Arrhenius form for the (■) 1% pectin plus 86% co-solute, and (●) 0.5% gellan with 85% co-solute samples (reference temperature of 90°C)

temperatures in degrees centigrade and in the form given in equation (4). Now if the temperature dependence of shear modulus had followed an Arrhenius-type behaviour, we should have obtained straight lines and a constant activation energy would have been determined for the transformation from the 'rubbery' to the glassy state. Instead, polynomial fits are shown which yield an increasing energy term with decreasing temperature, as observed in analogous plots for synthetic materials[31].

Going back to the idea that the contraction of free volume is an appropriate parameter for the estimation of viscoelastic functions, a similar formula to equation (4) can be produced using the fractional free volume $f = u_f/u$ instead of temperature and working from equation (2)

$$\log a_T = \frac{B}{2.303}\left(\frac{1}{f} - \frac{1}{f_o}\right) \tag{5}$$

Based on the observation by Kovacs[32] that the temperature dependence of specific volume has two distinct linear regions, with a discontinuous change in the expansion coefficient α_f defining the position of the glass transition temperature, we reach the following relation

$$f = f_o + \alpha_f(T - T_o) \tag{6}$$

Substitution for the fractional free volume in equation (5) gives

$$\log a_T = -\frac{(B/2.303f_o)(T - T_o)}{(f_o/\alpha_f) + T - T_o} \tag{7}$$

which is, of course, the WLF equation for $C_1^o = B/2.303f_o$ and $C_2^o = f_o/\alpha_f$. To estimate the parameters C_1^o and C_2^o for the high solids gellan and pectin samples, we used the WLF equation in the following form[33]

$$\log a_T = C_1^o(T - T_o)/(T_\infty - T) \tag{8}$$

where T_∞ is known as the Vogel[34] temperature and in this context is equal to $T_o - C_2^o$. Calculated shift factors were fitted to the experimental temperatures using a least-squares fit (Microsoft Excel 5 'Solver' package) which set the intercept to zero and varied T_∞ until the best straight lines were produced. For the quality of fits shown in *Figure 8*, the Vogel temperatures for 0.5% gellan plus 85% co-solute and 1% pectin with 86% co-solute were 197 K and 170 K, respectively. Considering the observation that T_∞ is about 50°C below the glass transition temperature[35], T_g values for the above mixtures were found to be respectively −26°C and −53°C. For the same samples the magnitudes of C_1^o were estimated, from the gradients of the best-fit lines, to be 4.55 and 3.83 (reference temperature of 90°C), and the corresponding C_2^o values were 166 and 193 degrees [from equation (8)].

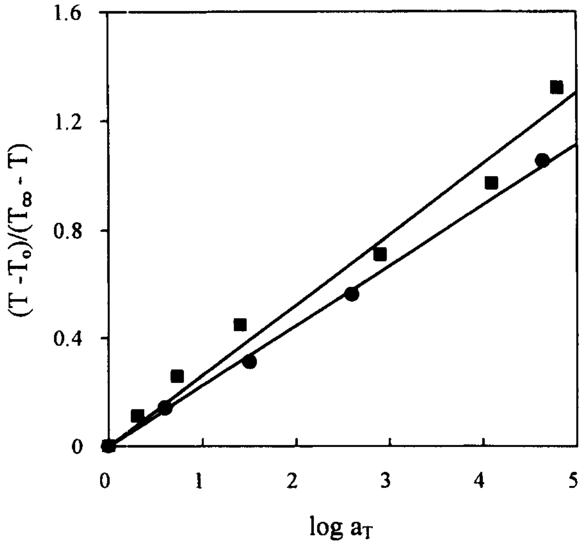

Figure 8 Linear fitting of shift factors (a_T) against temperature in the WLF form for the (■) 1% pectin plus 86% co-solute, and (●) 0.5% gellan with 85% co-solute samples (reference temperature of 90°C)

The last criterion for the applicability of WLF analysis to our systems is the evaluation of fractional free volume at the glass transition temperatures (f_g). In doing so, the coefficients C_1^o and C_2^o have to be transformed to the values appropriate to T_g as the reference temperature. Since equation (1) should hold for any reference temperature T_o somewhere within the range of experiment, the data obtained at 90°C can be used for calculation of the corresponding values at T_g by the relations:

$$C_1^o = C_1^g C_2^g/(C_2^g + T_o - T_g)$$
$$C_2^o = C_2^g + T_o - T_g \tag{9}$$

Gratifyingly, a straightforward calculation produced very similar glass transition parameters for both biopolymers, as also seen in previous studies of synthetic polymers. These were, for 0.5% gellan with 85% co-solute: $C_1^g = 15.1$; $C_2^g = 50$ deg, and for 1% pectin plus 86% co-solute: $C_1^g = 14.8$; $C_2^g = 50$ deg. Finally, equation (7) was used for derivation of the thermal expansion coefficient and the fractional free volume at the glass transition temperatures. Again, the values of α_f were in accord with experience, being 5.7×10^{-4} and 5.9×10^{-4} deg^{-1} for the high solids gellan and pectin, respectively. Values of α_f for various synthetic polymers were found to be in the range of $2.5–12.5 \times 10^{-4}$ deg^{-1} (35). This calculation is done on the basis that the difference ($\Delta\alpha$) between the expansion coefficients in the liquid (α_L) and the glassy (α_G) state is equivalent to α_f, assuming that, on a total specific volume vs. absolute temperature graph, the lines of glass volume and occupied volume are parallel.

For both preparations, the magnitude of f_g was found to be ≈0.029. Similarly, this result is well within the range of fractional free volumes estimated for the great majority of diluted/undiluted synthetic polymers ($f_g = 0.026 \pm 0.005$)[35]. That the values of f_g agree well with the proportion of free volume at the glass transition temperature for organic liquids of low molecular weight[13] and inorganic glasses[36] (found to be ≈0.03), allowed Ferry and his colleagues to propose that the temperature dependence of relaxation processes at the glassy state is independent of chemical structure. The present work on biological materials further reinforces the universality of the free volume approach as applied by the WLF equation. The consistency is particularly interesting if it is considered that our samples are multi-component systems, where the biopolymers have been 'diluted' with sucrose, glucose syrup and water.

CONCLUSIONS

This area provides an opportunity to glean and apply information from the realm of synthetic polymer science to the specialized problems of biopolymers. In terms of the application of free volume theory to biopolymer glasses, we feel that a start has been made but there is tremendous scope for further development. Besides the basic necessity of extending the work to other biopolymers, eventually a detailed programme should explore kinetic aspects of biopolymer glass formation, the effects of mechanical shear, and the influence of biopolymer conformation on the composite properties. The chemical natures of polymers, solvents, and co-solutes will also provide a fertile area for exploring the factors which relate intermolecular interactions to macroscopic phenomena in the transition region between 'rubbery' to glassy states. On the commercial front, determining to what extent the systems are metastable and how the WLF kinetics of glass transition affected by the time–temperature path which the product follows will allow us to manipulate product formulations and processing to generate and control innovation functions and textures.

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REFERENCES

1. Rolin, C., in *Industrial Gums*, ed. R. L. Whistler and J. N. BeMiller. Academic Press, London, 1993, pp. 257–293.
2. Christensen, S. H., in *Food Hydrocolloids*, Vol. III, ed. M. Glicksman. CRC Press, Florida, 1986, pp. 205–230.
3. Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C. and Thom, D., *FEBS Lett.*, 1973, **32**, 195.
4. Mitchell, J. R. and Blanshard, J. M. V., *Journal of Texture Studies*, 1976, **7**, 341.
5. Jansson, P-E., Lindberg, B. and Sandford, P. A., *Carbohydrate Research*, 1983, **124**, 135.
6. Chandrasekaran, R., Millane, R. P., Arnott, S. and Atkins, E. D. T., *Carbohydrate Research*, 1988, **175**, 1.
7. Grasdalen, H. and Smidsrød, O., *Carbohydrate Polymers*, 1987, **7**, 371.
8. Anonymous, *Food Ingredients International*, 1995, 2 Mar/8 Apr.
9. Papageorgiou, M., Kasapis, S. and Richardson, R. K., *Food Hydrocolloids*, 1994, **8**, 97.
10. Norton, I. T., Goodall, D. M., Frangou, S. A., Morris, E. R. and Rees, D. A., *Journal of Molecular Biology*, 1984, **175**, 371.
11. Haque, A., Polysaccharide substitutes for gluten: molecular processes and structures. Ph.D. thesis, Cranfield University, Silsoe College, 1993.
12. Levine, H. and Slade, L., in *Water Science Reviews*, Vol. 3, ed. F. Franks. Cambridge University Press, Cambridge, 1988, pp. 79–185.
13. Williams, M. L., Landel, R. F. and Ferry, J. D., *Journal of the American Chemical Society*, 1955, **77**, 3701.
14. Sperling, L. H., in *Introduction to Physical Polymer Science*. Wiley-Interscience, New York, 1986.
15. Kalichevsky, M. T., Blanshard, J. M. V. and Marsh, R. D. L., in *The Glassy State in Foods*, ed. J. M. V. Blanshard and P. J. Lillford. Nottingham University Press, Nottingham, 1993, pp. 133–156.
16. Lopes da Silva, J. A., Gonçalves, M. P. and Rao, M. A., *Carbohydrate Polymers*, 1994, **23**, 77.
17. Wagner, K. W., *Elektrotech. Z.*, 1915, **135**, 163.
18. Tobolsky, A. V. and Andrews, R. D., *J. Chem. Phys.*, 1943, **11**, 125.
19. Ferry, J. D., *J. Am. Chem. Soc.*, 1950, **72**, 3746.
20. Beveridge, W. I. B., in *The Art of Scientific Investigation*. Heinemann Educational Books, London, 1968.
21. Nishinari, K., Watase, M., Kohyama, K., Nishinari, N., Oakenfull, D., Koide, S., Ogino, K., Williams, P. A. and Phillips, G. O., *Polymer Journal*, 1992, **24**, 871.
22. Nishinari, K. and Watase, M., *Thermochimica Acta*, 1992, **206**, 149.
23. Papageorgiou, M., Kasapis, S. and Richardson, R. K., *Carbohydrate Polymers*, 1994, **25**, 101.
24. Walkinshaw, M. D. and Arnott, S., *J. Mol. Biol.*, 1981, **153**, 1075.
25. Chronakis, I. S., Kasapis, S. and Abeysekera, R., *Food Hydrocolloids*, 1997, **10**, 000.
26. Haque, A. and Morris, E. R., *Carbohydrate Polymers*, 1993, **22**, 161.
27. Ong, M. H., Whitehouse, A. S., Abeysekera, R., Al-Ruqaie, I. M. and Kasapis, S., *Food Hydrocolloids*, 1997, in press.
28. Eyring, H. and Hirschfelder, J. O., *J. Phys. Chem.*, 1937, **41**, 249.
29. Doolittle, A. K. and Doolittle, D. B., *J. Appl. Phys.*, 1957, **28**, 901.
30. Berry, G. C., Fox, T. G., *Adv. Polym. Sci.*, 1967, **5**, 261.
31. Arridge, R. G. C., in *Mechanics of Polymers*. Oxford University Press, Oxford, 1975, pp. 24–50.
32. Kovacs, A., *Adv. Polym. Sci.*, 1964, **3**, 394.
33. Miller, A. A., *J. Polym. Sci.*, 1964, **A2**, 1095.
34. Vogel, H., *Phys. Z.*, 1921, **22**, 645.
35. Ferry, J. D., in *Viscoelastic Properties of Polymers*. Wiley, New York, 1980, pp. 264–320.
36. Shen, M. C. and Eisenberg, A., *Prog. Solid State Chem.*, 1966, **3**, 407.