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Vitrification of κ-carrageenan in the presence of high levels of glucose syrup

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We present evidence that co-solute (glucose syrup) can cause massive changes in the time/temperature dependence of structures formed by κ-carrageenan. Modest concentrations of glucose syrup ($< 40\%$) promote conventional disorder-to-order transitions of its hydrogels and reinforce the final network strength, as seen by small deformation dynamic oscillation. In contrast, at higher concentrations of co-solute, there is a leap in thermal stability which is associated with a drop in network strength. Differential scanning calorimetry demonstrates that the rise in network strength is accompanied by more pronounced enthalpic events, but the change in enthalpy declines in accordance with the drop in storage modulus at higher levels of co-solute. Cooling of the samples triggers a transformation from low modulus solid-like to high viscosity liquid-like behaviour and at 85% co-solute, shear moduli increase by about five decades. Eventually the storage modulus overtakes the loss modulus completing the progression from the rubbery plateau and glass transition region to the glassy state, in the way reported for amorphous synthetic polymers. Vitrification was equivalently followed by changing the temperature or frequency of measurement thus implementing the time–temperature superposition principle. The Williams–Landel–Ferry/free volume theory was operative in the glass transition zone, thus allowing calculation of useful parameters, e.g. the thermal expansion coefficient, but it gave way to an Arrhenius relationship for the relaxation processes in the glassy state. It is proposed that the addition of co-solute induces a transformation from an enthalpic aggregated structure to a partially cross-linked structure of more flexible chains where the entropic contribution to elasticity becomes dominant, thus allowing vitrification of the polysaccharide network. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: κ-carrageenan; glucose syrup; dynamic oscillation; glass transition; WLF/free volume theory)

INTRODUCTION

It is almost 30 years since the pioneering work by Rees and his co-workers shed light on the conformation and structural properties of κ-carrageenan in solution and in the gel state[1]. Sigmoidal changes in optical rotation verify the disorder-to-order transformation of κ-carrageenan chains during cooling. X-ray diffraction studies and model building, taking into account the angles of glycosidic bonds, the closeness of adjacent atoms and the space available to the partner chain, argue for the formation of a double helical structure with three disaccharides per turn of helix[2]. This is further stabilised with hydrogen bonding, perpendicular to the helix axis, between the OH groups at positions 6 and 2 of the galactose rings of the two strands[3]. Helices come together to form large aggregates which constitute the structural unit of the κ-carrageenan network. The occasional presence of 6-sulphate and/or 2,6-disulphate residues in the chain introduces a kink in the molecular arrangement which is not compatible with double helix formation and serves as a 'solubilizing point' in the network[4]. K^+ are the main stabilising counterions of the aggregates and according to the domain model they form an array between adjacent helices[5]. It is further postulated that in the case of a potassium κ-carrageenan gel the double helices do not survive as single entities but further stabilization is possible upon association, thus effectively leading to a coil → aggregate transition[6].

Compared with the above, the area of high-solid carrageenan systems remains relatively unexplored with work so far being carried out by Nishinari and his group[7]. Mechanical measurements recorded the longitudinal vibrations of cylindrical discs of κ-carrageenan during heating runs. High concentrations of the polysaccharide were used (2–6%) with the co-solute content (sucrose or glucose) ranging from 0 to 45%[8]. It was found that the values of Young's modulus increased with co-solute concentration, which was attributed to the formation of additional and more thermally stable polymer–polymer interactions. Studies were extended to several sugars and polyols using differential scanning calorimetry and focusing on trends in the setting and melting temperatures of gels[9]. The range of co-solute was rather limited (up to 2 M for monosaccharides and 0.5 M for di/trisaccharides) but sufficient to show a positive relationship between the thermal stability and the average count of equatorial hydroxyl groups (e-OH) of the possible conformations which sugars adopt in solution. Taking into account that the distance between two adjacent e-OH fits well the space of water molecules in an ice lattice (4.9 Å)[10], it was proposed that the increasing number of equatorial hydroxyl groups serves as a more efficient anchorage of water molecules to the co-solute, thus shifting the balance from polymer–water to polymer–polymer interactions with accentuated thermal and mechanical stability.

Recently, work on high-solid systems included a wide range of sugar concentrations (0–85%) and polysaccharide at levels of industrial use (0.1–1%). Thus, deacylated gellan

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and high methoxy pectin samples were prepared at high temperatures and changes in viscoelasticity were monitored during cooling and subsequent heating[11]. Experiments showed that over the upper range of co-solute, mechanical properties break away from the conventional ion-dependent sugar-stabilized sol → gel transition[12,13], and instead a rubbery structure is formed at high temperatures which, upon cooling, transforms into a glassy consistency. Fixed temperature mechanical spectra taken at intervals over the available range reproduced the sample vitrification according to the time–temperature superposition principle[14]. This allowed fitting of shift factors over a wide temperature or frequency range using the Williams–Landel–Ferry equation (WLF), and eventually viscoelastic functions were rationalized with the free volume theory in the way that has been done so successfully for synthetic polymers[15,16]. The present study looks at the structural properties of κ-carrageenan networks as modified with increasing concentrations of glucose syrup. We feel that successful treatment of the κ-carrageenan results (in addition to those of gellan and pectin) with the combined WLF/free volume scheme will go a long way towards establishing a unifying theory for the glass transitions of polysaccharide/sugar systems.

EXPERIMENTAL

Materials. The sample of κ-carrageenan was a gift from Hercules (batch X6960). ^{1}H NMR analysis was performed according to the technique of Welti[17] and showed that ι-carrageenan-like segments, i.e. with a sulphate group at position 2 of the 3,6-anhydride residue, constitute about 8% of the polymer. An Amberlite IR-120 exchanging resin from BDH was used to prepare the polysaccharide in the potassium form. The resin was first eluted with HCl to convert it to the H^+ form and then with a solution of KCl (2 M) to bring it to the K^+ form. Excess salt was removed by elution with water until the washings no longer gave a precipitate with silver nitrate, i.e. chloride free. Ion exchanging was carried out at high temperature (>85°C) to ensure that the polysaccharide remains in the disordered form. That was achieved by pre-heating the resin with hot (95°C) distilled water and keeping it as a short column in a separating funnel surrounded by steam. Hot κ-carrageenan solutions were made at a concentration of 3%, introduced into the column and kept there for 30 min. Finally, solutions were dialysed against distilled water and freeze-dried to produce the material of this investigation.

κ-carrageenan in the potassium form was characterized with intrinsic viscosity measurements $[\eta]$ on the basis of the Huggins, Kraemer and single-point derivations. We used the Contraves Low Shear 30 viscometer with a cup-and-bob geometry of inner and outer radii of 5.5 mm and 6.0 mm, respectively. Readings were taken at a constant ionic strength (0.01 M KCl) and at 40°C, to ensure that the polymer exists in the disordered coil conformation, yielding a $[\eta]$ value of 10.5 ± 0.2 dl g^{-1}.

The glucose syrup used was a Cerestar product (Batch NX8472). 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. GPC analysis provides the following relationship between degree of polymerization and surface area (%) of the glucose syrup spectrum:

DP1	17.54
DP2	12.99
DP3	10.55
DP4	8.79
DP5	7.29
DP6	5.28
DP7	4.78
DP8	4.21
DP9	3.19
DP10	1.96
> DP10	23.40

Methods. The freeze-dried material of κ-carrageenan was dissolved in distilled water at 90°C using gentle stirring for 10 min. Following solvation, the temperature was lowered to about 80°C and appropriate amounts of glucose syrup were added taking into account the concentration of solids in the stock. Finally a KCl solution (2 M) was used to bring the counterions to a constant level of 0.01 M. The polysaccharide concentration was also kept constant at 0.5%, whereas that of glucose syrup varied from 0 to 85%. Samples were loaded on the pre-heated platen of a commercial controlled stress Carri-Med CSL500 rheometer or a prototype rheometer developed in this laboratory[18]. The measuring geometries were cone-and-plate (cone angle 0.05 rad; 50 mm diameter) and parallel plate. Using a range of plate sizes with diameter from 4 to 0.8 cm and measuring gaps of 1–2 mm, the compliance of the machine was minimised and accurate measurements were taken within the range of 10^0 to approx. 10^9 Pa. Samples were cooled from 80°C to 5°C (gels at lower levels of co-solute) or to −20°C (vitrified systems at the upper range of co-solute) at a scan rate of 1°C min^{-1}, frequency of 1.6 Hz (equivalent to the experimentally convenient angular frequency of 10 rad s^{-1}) and strain of 0.1%. Mechanical spectra were taken from 0.01 to 16 Hz at the end of the cooling runs followed by controlled heating (1°C min^{-1}) to 80°C. A silicone fluid (Dow Corning 510/50 cs) was used to cover the exposed edges of our samples thus preventing loss of moisture. For the implementation of the time–temperature superposition, new samples were made, scanned down at 1°C min^{-1}, and frequency sweeps were recorded at temperature intervals of 10°C.

Differential scanning calorimetry (DSC) measurements were taken on a Setaram "batch & flow" calorimeter. Approximately 0.8 g of the κ-carrageenan/glucose syrup mixture was sealed in the sample pan and the appropriate amount of co-solute was used as thermal reference, the sample and reference pans being balanced to within 0.1 mg. Samples were scanned at 0.1°C min^{-1} from 95 to 5°C. Values of ΔH were determined by numerical integration of peak area, using baselines interpolated from higher and lower temperatures by a polynomial curve-fitting procedure. The transition midpoint temperature (T_m) was taken as the temperature at which half the total heat change had occurred.

RESULTS AND DISCUSSION

Rheological evidence for the vitrification of κ-carrageenan networks at high levels of glucose syrup

This work started with the verification of results taken by Nishinari et al. who monitored the longitudinal modulus of disk gels of κ-carrageenan during heating from 15 to 80°C.

These showed stronger and more thermally stable gels as a result of increasing the co-solute content, e.g. values of Young's modulus for a 3% κ-carrageenan gel varied from $\approx 3.8\times10^5$ to 5.4×10^5 Pa at 15°C with raising the glucose content from 15 to 45%[7,8]. Cohesive gels for compression testing required Young's modulus values well in excess of 10^4 Pa and the above κ-carrageenan preparation with 15% glucose exhibited firmness of 7×10^4 Pa at 56°C, whereas networks with 45% co-solute weakened gradually to this level at 80°C. However, use of the polysaccharide at levels of commercial interest (<1%) generates solutions at high temperatures whose transformation into relatively weak gels during cooling can be followed by the technique of dynamic oscillation[18].

Figure 1 shows changes in the storage modulus (G') for 0.5% κ-carrageenan systems on cooling, as monitored by low-amplitude oscillatory measurements (1.6 Hz; 0.1% strain). At 0% co-solute, a sharp sol–gel transition commences at ≈28°C (scan rate of 1°C min^{-1}) which levels off towards the end of the cooling run. In the presence of 20% glucose syrup, the temperature at the onset of the gelation process shifts to 42°C and this is accompanied by higher G' values which eventually approach equilibrium, as in the aqueous carrageenan network. This, of course, is in agreement with the observations of the preceding paragraph and it appears that the presence of co-solute enhances the macromolecular association in the system. A similar trend in the mechanical and thermal stability of concentrated agarose gels (2–12%) was also observed as a function of increasing the sugar content from 15 to 30%[19].

Increasing amounts of co-solute in *Figure 1* develop further the network strength and in the case of 60% glucose syrup systems form much earlier and become firmer compared with the aqueous formulation. In both 40% and 60% samples, however, the temperature dependence of the transition becomes more gradual and networks still consolidate themselves at the bottom end of the temperature range. At the top level of co-solute in particular, it appears that a second process is coming in at temperatures below 20°C. Next we focused on this extra feature of viscoelastic behaviour in an effort to identify its origin.

Figure 2 illustrates the mechanical spectra of storage modulus monitored for 0.5% κ-carrageenan gels on completion of the cooling runs (5°C) described in *Figure 1*. Addition of glucose syrup up to 60% results in a steady increase in the network firmness. A flat frequency dependence of G' is observed over the accessible frequency range (from 0.01 to 16 Hz) for the 20 and 40% samples, which is typical of networks of hydrogels[20]. Similarly, the G' trace at 0% co-solute remains independent of frequency (ω) at about 50 Pa ($\log G'=1.7$) but this has not been plotted here so that we can zoom in on the events around 60% co-solute. At this level there is the first evidence of some frequency-dependence of modulus. Strikingly, the solid-like response collapses from ≈0.8 kPa at 60% glucose syrup to ≈0.28 kPa on addition of an extra 5% co-solute to the system. Furthermore, the development of a strong dependence of modulus on frequencies higher than 0.5 Hz distorts the gel-like profile seen at lower levels of co-solute. The values of storage modulus recover partially at the 70% formulation and a power law dependence on log ω is shown which at 75% glucose syrup results in substantial acceleration of the mechanical response within the experimental frequency window.

A similar trend in modulus variation with co-solute content was recorded for the gellan polysaccharide[21]. In that series of experiments, the maximum in the flat frequency-dependence of G' was seen at 30% sucrose followed by a drop in network rigidity with its lowest point occurring at 60% sucrose. Meanwhile the frequency dependence of modulus becomes apparent and the recovery ensues in gellan samples with 60 to 85% co-solute, along the lines described for *Figure 2*. As mentioned in the introduction, this characteristic frequency dependence was associated with the vitrification of high-solid gellan structures and treated with the WLF/free volume theory engineered to rationalize the rubber-to-glass transition of amorphous synthetic polymers[11,15]. It appears, therefore, that there is

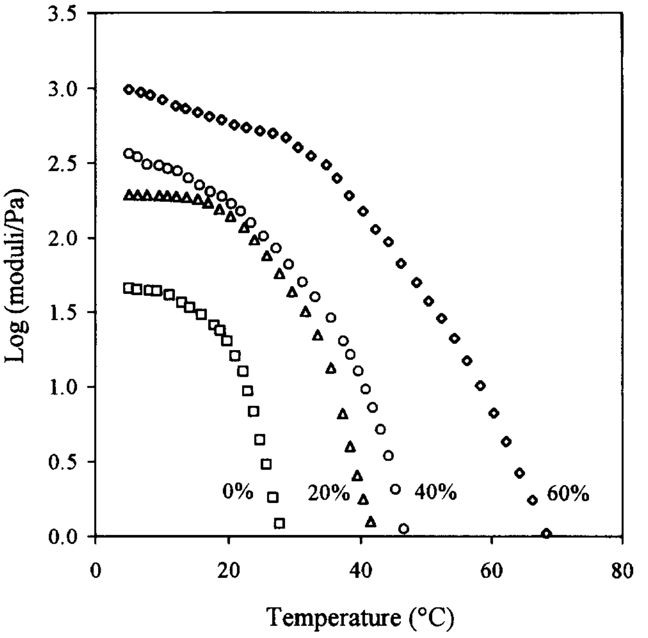

Figure 1 Cooling profiles of G' for 0.5% κ-carrageenan at 0.01 M KCl with the levels of glucose syrup shown by the individual traces (scan rate, 1°C min^{-1}; frequency, 1.6 Hz; 0.1% strain)

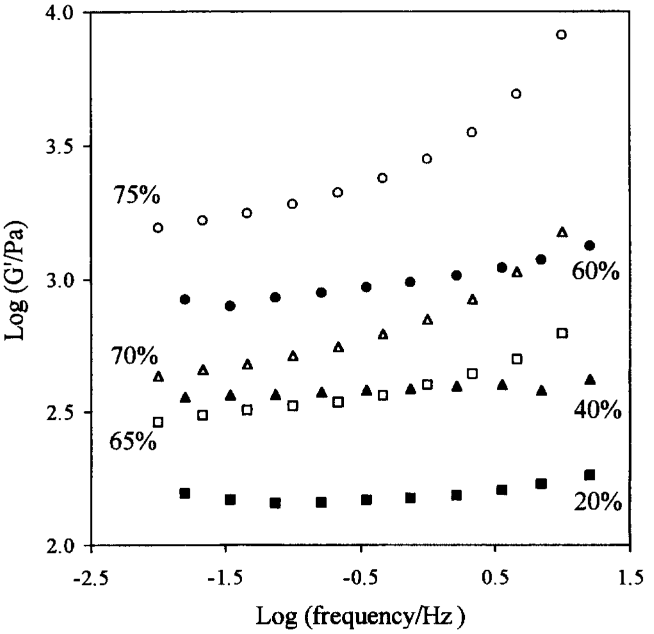

Figure 2 Frequency sweeps of G' for 0.5% κ-carrageenan on completion of the cooling runs at 5°C with the levels of glucose syrup shown by the individual traces (0.01 M KCl; 0.1% strain)

a unifying theme behind the addition of sugar to a polysaccharide network where the building up of polymeric associations and thus the network strength is eventually halted and subsequently reversed. Molecules of water are believed to stabilize associations between double helices of gellan[22] and κ-carrageenan aggregates[23,24], the structural knots of their networks, and its shortage with increasing levels of co-solute offers an obvious explanation of the phenomenon in *Figure 2*. In addition, the space between two adjacent e-OH of a sugar molecule matches the span of water molecules in the regular ice lattice (4.9 Å), an arrangement that enhances the stability of hydrogen bonding between sugar and water[10] and further deprives the tertiary polysaccharide associations of their hydration layer. Diminishing enthalpic interactions might create more flexible polysaccharide chains with a significant entropic contribution to the viscoelasticity, and this might lead to a glass-related consistency upon cooling of the high-sugar system. This hypothesis was examined by extending measurements of mechanical properties to sub-zero temperatures and by recording changes in the enthalpic content of κ-carrageenan networks as a function of co-solute content.

Figure 3 illustrates the temperature course of G' and G'' (loss modulus) for our polysaccharide at the upper range of glucose syrup (65–80%). Conformational ordering and the onset of network formation is well on its way at around 42°C for the 65% sample, with both moduli developing continuously and creating a structure of a high $\tan \delta$ value (G''/G'). For example, the viscoelastic ratio at 5°C is equal to 0.46 (experimental frequency of 1.6 Hz) which is substantially higher than the values expected for aqueous κ-carrageenan gels, i.e. $\tan \delta < 0.1$[25] and from the unplotted G'' trace of *Figure 1*. Gratifyingly, this transforms into a second transition, this time from solid- to liquid-like character, at temperatures below −10°C. Subsequent heating traces back the part of the cooling curve around the second transition but departs from it at higher temperatures and creates thermal hysteresis; hence, the network does not melt below 60°C.

The following elements also characterise the second process: Increasing the amount of glucose syrup from 65% to 80% shifts the point where the two moduli cross over from about −10°C to almost 30°C. The viscous component becomes increasingly dominant and, within the co-solute range of *Figure 3*, covers three orders of magnitude at −20°C. Replacement of glucose syrup, for example at 70%, with a mixture of 50% sucrose and 20% glucose syrup drives back the onset of the second transition from −3°C (*Figure 3b*) to −17°C (unplotted results). Finally, the thermal hysteresis between temperatures at the onset of network formation and melting closes from a gap of 17 to

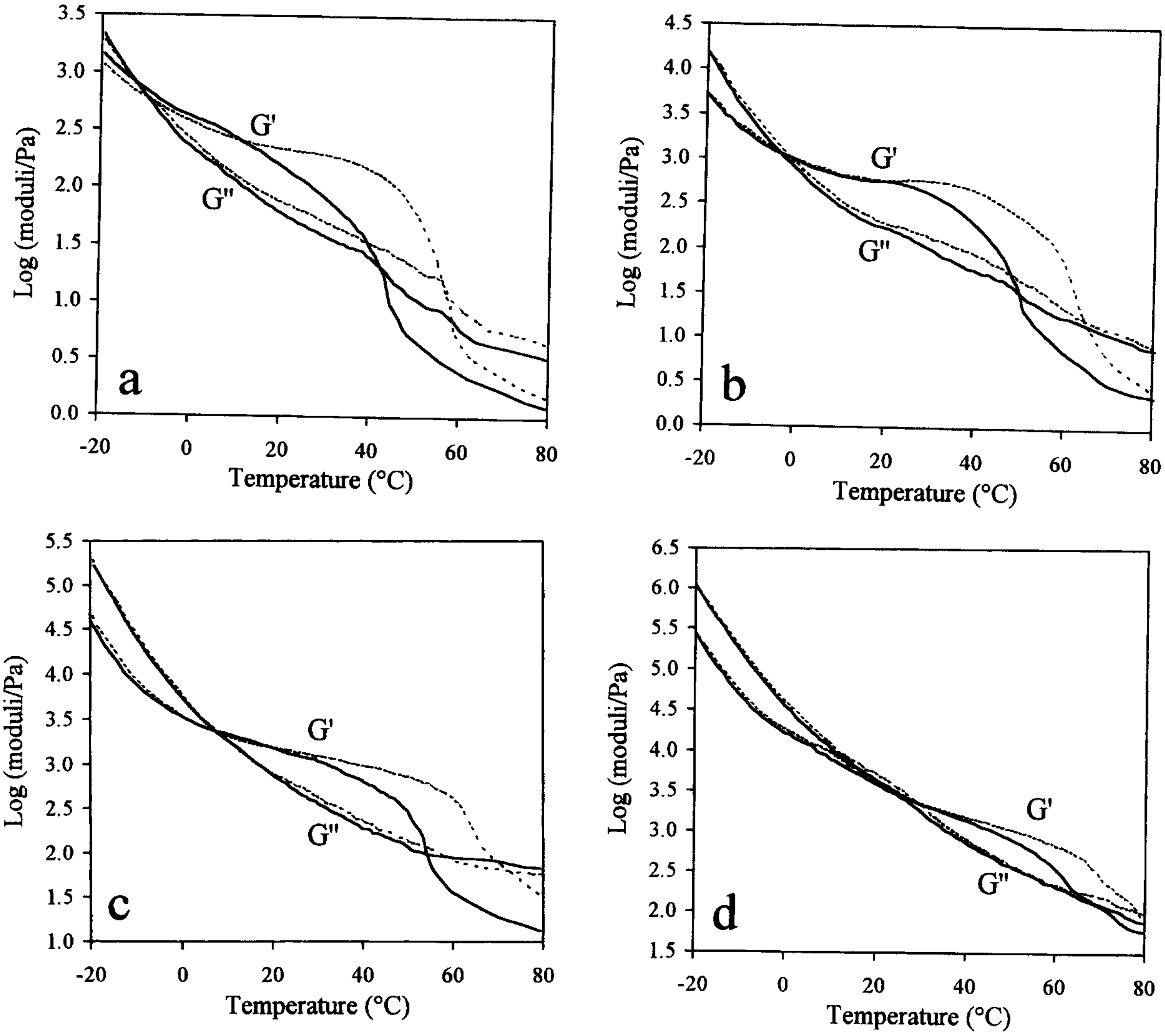

Figure 3 Cooling (————) and heating (– – – –) profiles of G' and G'' for 0.5% κ-carrageenan with: a) 65%, b) 70%, c) 75% and d) 80% glucose syrup, plus 0.01 M KCl (scan rate, 1°C min^{-1}; frequency, 1.6 Hz; 0.1% strain)

10°C at 65% and 80% glucose syrup, respectively. Collectively, these changes in rheology reflect the gradual development of a glass transition region where increasing concentrations of co-solute (and molecular weight as seen by the replacement of sucrose with glucose syrup) effectively antiplasticize the κ-carrageenan network. This retains a degree of aggregation judged by the occurrence of thermal hysteresis between cooling and heating runs at the upper temperature end, as opposed to perfectly reversible spectra of the glass transition region.

The glass transition was captured in its entirety when κ-carrageenan was mixed with 85% glucose syrup. As shown in *Figure 4*, solid-like samples with a rubbery texture are obtained at 80°C and upon cooling give rise to an early onset of vitrification, i.e. at about 60°C which is 30 degrees higher than the corresponding point at 80% co-solute (*Figure 3d*). The values of modulus rise steeply, thus covering four orders of magnitude as the temperature drops from 60 to 0°C. Qualitatively, the glass transition is the regime where the long-range movements of the backbone (rotational, thread-like) are limited, whereas thermal motions of shorter segments, e.g. pendant groups, survive and dissipate energy, thus contributing mainly to the viscous component[26], as seen in *Figure 4*. At sub-zero temperatures, however, the two moduli cross over once more and the elastic component again becomes dominant ($G' = 10^{8.3}$ Pa at 1.6 Hz). This transformation corresponds to the onset of the glassy state where only stretching and bending of chemical bonds is allowed and the resistance to motion develops a solid-like rather than a liquid-like nature[27]. Values of G' around $10^{8.5}$ Pa have been reported for the glassy state of poly(*n*-octyl methacrylate) at −14.3°C[28].

Figure 4 also depicts the baseline behaviour of the co-solute without the polymeric component. Similarly, the thermal behaviour of the co-solute was compared with that of the polymer/co-solute mixture in the vitrification studies of the gellan polysaccharide[11]. There the upper level of co-solute was a solution of 50% sucrose with 35% glucose syrup which produced a Newtonian viscosity of 0.6 Pa s at 90°C. At the other end of the cooling routine the sample developed a predominantly solid-like profile which was attributed to the partial crystallization of sucrose in the system. A comparable result was obtained for frozen ice cream due to crystallization of lactose, with the dominant storage modulus varying between 10^6 and 10^7 Pa as a function of increasing frequency of oscillation[29]. Single preparations of glucose syrup at 70% reproduced the transformation from liquid- to solid-like behaviour with cooling, and DSC heating scans showed an endothermic transition at approximately −9°C[30]. This temperature is congruent with the depression in the freezing point of water expected at the above level of co-solute. Thus, addition of 0.5% κ-carrageenan in *Figure 3b* prevents the water molecules separating out in the form of ice crystals and the system as a whole enters the glass transition region at sub-zero temperatures. Use of glucose syrup at the highest concentration (85% in *Figure 4*) sees another development. This time cooling gradually transforms a pourable solution into a system with extremely high viscosity where the viscous component remains dominant at −20°C. It appears, therefore, that the polydispersity of glucose syrup (see *Materials*) and, at this level, its strong interaction with water molecules inhibit crystallization and encourage vitrification in the system. On the other hand, the presence of κ-carrageenan results in a rubbery structure which undergoes rapid vitrification and the onset of the glassy state is now captured at approximately −10°C.

Calorimetric evidence for the reduction in the enthalpy of κ-carrageenan associations in a high solids environment

In studies of the gellan polysaccharide with increasing concentrations of co-solute, we were unable to carry out DSC experiments since the compartment of the calorimeter can only be loaded at ambient temperature and networks, in the presence of calcium ions with normally twice the stoichiometric equivalent of the polymer, remain thermally irreversible at temperatures below 98°C. However, the current formulations of κ-carrageenan/glucose syrup with 0.01 M KCl allow formation and melting of structures within the freezing and melting points of water.

Figure 5a illustrates typical cooling scans for κ-carrageenan as a function of increasing levels of co-solute with the calculated baselines subtracted from the experimental traces. Drawing a baseline allows accurate estimation of the enthalpy (ΔH) and the midpoint temperature (T_m) of the gelation process which are reproduced in *Figure 5b*. The aqueous polysaccharide spectrum has not been plotted because the transition is still happening at 5°C and this prevents the reliable estimation of the above parameters. Work on the same material by Goycoolea et al.[31] has shown that high levels of potassium chloride (0.1 M) offset the exotherm to a relatively high temperature ($T_m = 49.7$°C) with the ΔH being 29 J g^{-1} which, similar to the trend in T_m values, should be well above the enthalpic content of the transition at 0.01 M KCl.

The positive development in the temperature/frequency dependence of G' with co-solute (0% to 60%) in *Figures 1 and 2* is matched by a rise in enthalpy from ≈ 26 to 34 J g^{-1} at 10% and 50% glucose syrup, respectively. The beginning

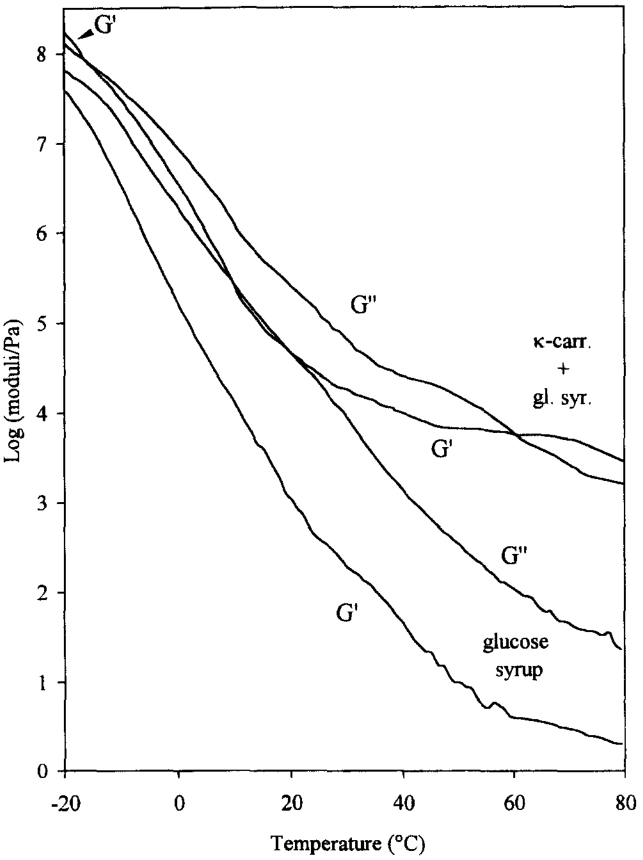

Figure 4 Cooling profiles of storage and loss moduli for 0.5% κ-carrageenan (0.01 M KCl) plus 85% glucose syrup, and a single glucose syrup preparation of 85% (conditions as in *Figure 3*)

of the transition is sharp (especially at 10% and 30% co-solute in *Figure 5a*), thus suggesting a co-operative process of coil-to-aggregate formation. In accordance with optical rotation profiles, the end of the transition is quite broad and forms a tail which is attributed to polydispersity and the formation of a 'heterogeneous' network encapsulating a range of temperature-induced relaxation processes[32]. The break point in the rheology of *Figure 2* is accompanied by a drop in the enthalpy of the thermal transition which is reduced to 13.8 J g^{-1} at 85% glucose syrup. Furthermore, a gradual ordering process develops on the high-temperature side of the peak which argues for reduction in co-operativity, the exotherms now being perfectly symmetrical (80% and 85% co-solute). In spite of the diminishing order at the upper range of glucose syrup there is no abatement in the shift of exotherms to higher temperatures, as shown in *Figure 5a* and defined in terms of the T_m in *Figure 5b*; values follow a linear dependence from ≈ 21.3 to 66.3°C at 10% and 85% co-solute respectively. Thermal transitions leading to the establishment of potassium-induced κ-carrageenan networks involve, according to the domain model[6], a direct coil → helix → aggregate reaction where the existence of helices is prevented unless they are further stabilized by lateral associations. This requires the presence of a hydration layer around the aggregates[23,24]. Therefore, the tendency to form progressively longer helices at high levels of co-solute might be increasingly counterbalanced by the inability to survive as stable entities (aggregates) in the scarcity of water molecules. In accordance with this suggestion, temperatures at the onset of network formation in *Figure 3* are shifted from 42 to 69°C, whereas the thermal hysteresis between cooling and heating profiles at the same range of co-solute (65 to 80%) diminishes 6 degrees (approx. 38%).

The application of the time–temperature superposition principle to the vitrification of high-glucose-syrup κ-carrageenan systems

The discussion so far has dealt with the effect of temperature on the structural properties of κ-carrageenan and demonstrated that the network's diminishing order at high levels of glucose syrup is associated with the development of vitrification phenomena in the mixture. Cooling of low acyl gellan or high methoxy pectin in the presence of co-solute also generated a glass transition which was related to a time/frequency effect ($t = 1/\omega$) by means of the time-temperature superposition principle (TTS). The same approach was employed in the present investigation.

Figure 6a and *b* illustrates the frequency dependence of G' and G'' for preparations of 0.5% κ-carrageenan with 85% glucose syrup (0.01 M KCl). Samples were cooled at 1°C min^{-1} and mechanical spectra were obtained from 60

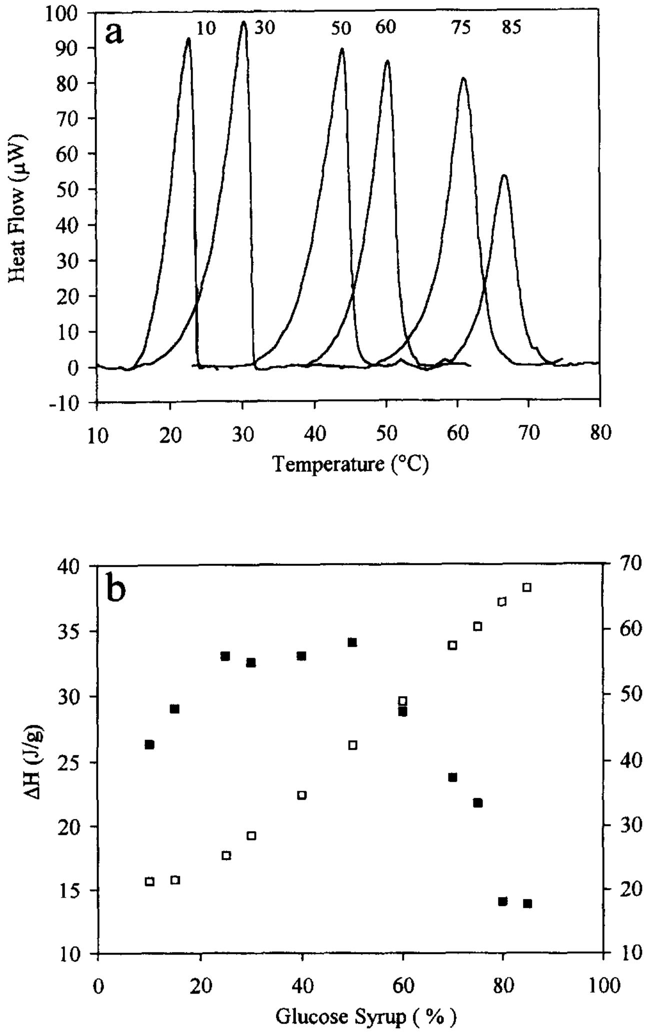

Figure 5 a) Cooling exotherms of 0.5% κ-carrageenan samples (0.01 M KCl) at levels of glucose syrup shown by the individual traces, and b) enthalpy changes (■) and midpoint transition temperatures (□) as functions of co-solute concentration (scan rate, 0.1°C min^{-1})

to −20°C at temperature intervals of ten degrees. The transition zone from rubber- to glass-like consistency is shown clearly and in the case of storage modulus it entails a relatively flat frequency response at 60°C, rising solid-like character in the second part of the frequency window at 40 and 30°C, and a slowing in the build-up of rigidity as the glassy state is reached at sub-zero temperatures. To derive a single composite curve, data were normalized at 60°C (reference temperature) using the TTS. The method assumes that all relaxation times of processes in a system have the same temperature dependence and involves a horizontal shift of the curves along the log frequency axis (for the theoretical deduction of the TTS see the original paper by Tobolsky[14]). He also implemented a minor vertical superposition by multiplying data with the factor T_o/T that takes into account the rubber-like nature of elasticity in this part of the composite curve (T_o is the reference temperature in degrees Kelvin).

The same horizontal shift was used for both viscoelastic parameters of successive frequency sweeps which put together the composite (or master) curve of *Figure 6c*. Data include the vertical adjustment although they can be transposed to the same effect without it. Fitting of the experimental points yields *Figure 6d* where we also plot the damping factor ($\tan \delta = G''/G'$). Clearly, the matching of adjacent curves produces a spectacular development in viscoelasticity up to $10^{8.75}$ Pa, with the frequency window stretching almost ten orders of magnitude from 10^{-2} to 10^{8} Hz. The rubbery region gives way to a glass transition at frequencies higher than 1 Hz and the values of tan δ become greater than 1. They reach a maximum and start descending to a plateau value around 1 as the moduli traces cross over for a second time. Thus the storage modulus dominates once more and enters the glassy state, with the crossing point defining the glass transition frequency (Ω_g).

The empirical approach used to follow the vitrification of amorphous synthetic polymers was pioneered by Williams, Landel and Ferry and can be summarised in the following equation[33]

$$\log a_T = C_1^0(T - T_0)/(T_\infty - T) \tag{1}$$

where a_T is the shift factor emanating from the transposition of two adjacent mechanical spectra, T_∞ is the Vogel temperature[34] which is equal to $T_o - C_2^0$, with C_1^0 and C_2^0 being constants. Then the WLF equation acquired physical significance by relating it to the theory of free volume[15]. Briefly, this deals with the temperature dependence of total specific volume (u) of a macromolecule and postulates that

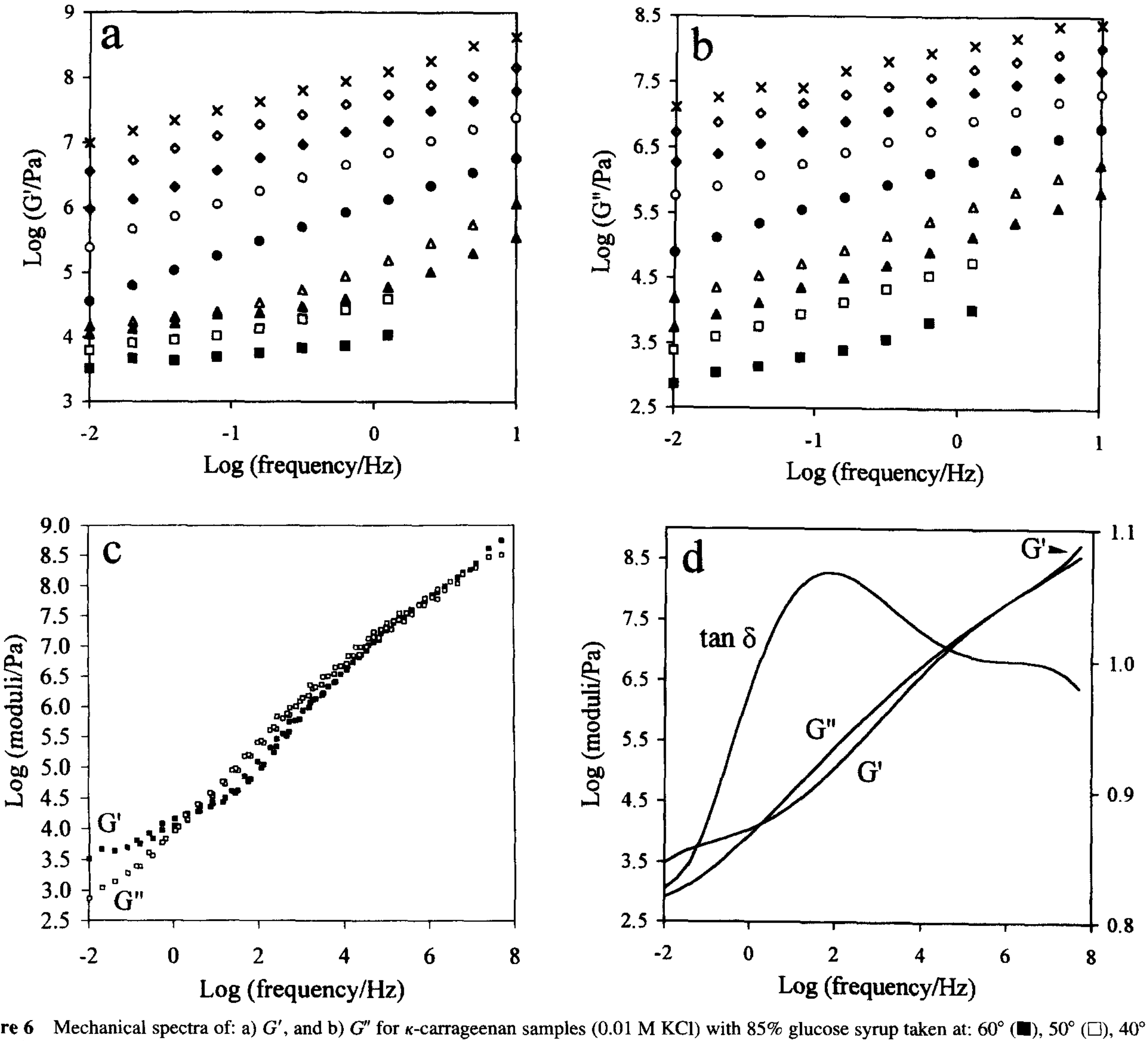

Figure 6 Mechanical spectra of: a) G', and b) G'' for κ-carrageenan samples (0.01 M KCl) with 85% glucose syrup taken at: 60° (■), 50° (□), 40° (▲), 30° (△), 20° (•), 10° (○), 0° (♦), − 10° (◇) and − 20°C (X). The time–temperature superposition of both moduli at the reference temperature of 60°C is given in c), and d) shows the composite curve with the tan δ

at a narrow region around the glass transition temperature (T_g), the thermal expansion coefficient (α_f) undergoes a discontinuity leading to a collapse of the free volume (u_f) in relation to the occupied volume. One can now rework the WLF equation thus finding that

$$C_1^0 = B/2.303f_0 \text{ and } C_2^0 = f_0/\alpha_f \qquad (2)$$

For simplicity the value of B is taken equal to one, and f (or f_0 at the reference temperature) is the fractional increase in free volume given by the dimensionless ratio u_f/u.

Equation (1) was logged in a minimization routine which fitted the experimental temperatures against the estimated shift factors by varying the T_∞ until the best straight line was obtained ($r^2 = 0.988$). As shown in *Figure 7a*, however, the WLF approach is not applicable at sub-zero temperatures where a systematic deviation from the linear relationship is observed. Data are also compared with the corresponding fits of low acyl gellan and high methoxy pectin at the same level of co-solute (85%). Within the experimentally accessible temperature range of our rheological setting (90 to −20°C), master curves for gellan and pectin covered the rubbery plateau and most of the glass transition zone, but the glassy state was not reached. The glass transition temperatures were predicted by the linear fits of *Figure 7a* and were −53 and −26°C for pectin and gellan, respectively[11]. Since the onset of the glassy state was captured in the master curve of κ-carrageenan/glucose syrup mixtures (second cross-point of moduli in *Figure 6d*), the T_g should lie within the experimental temperature range. The linear part of the temperature dependence in *Figure 7a* yields a T_∞ value of 216 K, and based on the observation that the Vogel temperature always appears to be fifty degrees below T_g[15], that was estimated to be −7°C. This temperature is very close to the onset of the glassy state recorded during continuous cooling at 1°C min^{-1} of the κ-carrageenan plus glucose syrup sample in *Figure 4* (G' becomes greater than G'' at approx. −10°C).* Therefore, the initially complicated dependence of mechanical properties on both frequency and temperature was resolved into two separate functions, one of frequency alone and another of temperature alone (*Figures 6d* and *7a*, respectively).

Intertwining the WLF approach with the theoretical framework of free volume allows calculation of the fractional free volume at the glass transition temperature (f_g). Equation (1) is valid within the temperature range T_g to T_g + 100°C, thus generating the following relationship for two reference temperatures

$$C_1^0 = C_1^g C_2^g/(C_2^g + T_0 - T_g) \qquad (3)$$

$$C_2^0 = C_2^g + T_0 - T_g$$

At the reference temperature of 60°C, a straightforward calculation gives $C_1^0 = 5.75$ and $C_2^0 = 117$ deg. Substitution in equation (3) produces the corresponding parameters for the glass transition temperature with values in accordance with experience[11,15], i.e. $C_1^g = 13.5$ and $C_2^g = 50$ deg. Similarly, use of the last two parameters in equation (2) makes the thermal expansion coefficient, α_f, equal to 6.5×10^{-4} per degree centigrade. Further, f_g is constant at 0.032 and agrees well with estimates for glasses of synthetic and biological origin, i.e. the free volume is expected to be about 3% of the total volume at the glass transition temperature.

Going back to the κ-carrageenan data of *Figure 7a*, the development of a curvature at sub-zero temperatures demarcates the applicability of the WLF treatment. As recorded in *Figure 4* and predicted from the free volume approach, however, this temperature range is associated with the advent of the glassy state where it is theorized that the WLF kinetics become inoperative[16]. The WLF exercise, of course, is one of the quantitative frameworks used to follow the shifting of curves into a composite arrangement. Dynamic or transient mechanical measurements below the glass transition region (terminal to rubbery zone in the master curve) are rationalized traditionally with the Arrhenius relation which describes the dependence of relaxation processes on temperature. Thus for two experimental temperatures the ratio a_T of any relaxation times should comply with the Arrhenius equation as follows[35]

$$\log a_T = \frac{E_a}{2.303R}\left(\frac{1}{T} - \frac{1}{T_0}\right) \qquad (4)$$

where E_a refers to a constant activation energy. Gratifyingly, recasting the κ-carrageenan/glucose syrup data of

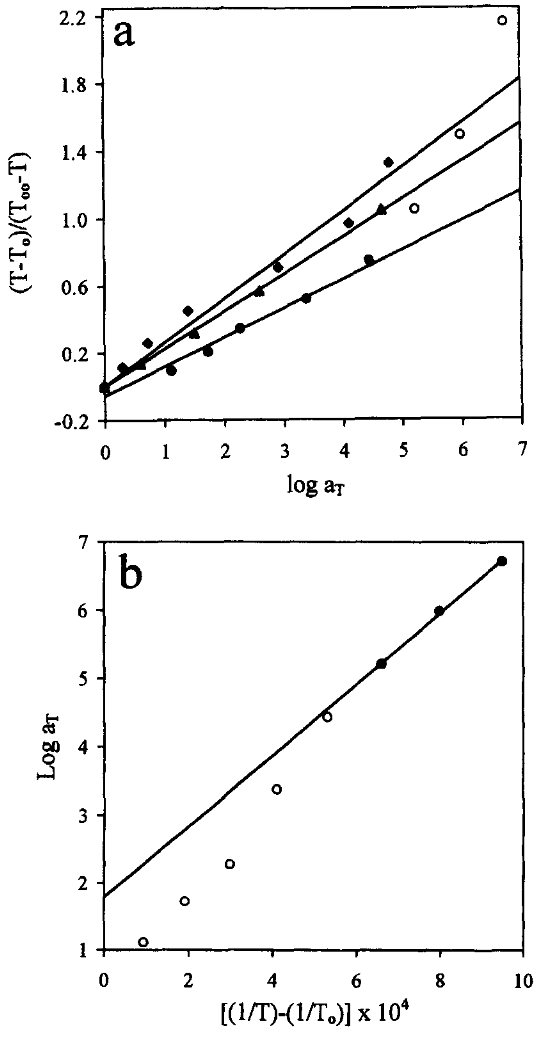

Figure 7 a) Shift factors of storage and loss modulus traces plotted in relation to T_∞ for κ-carrageenan (•,○), low acyl gellan (▲) and high methoxy pectin (◆) at 85% co-solute with the straight lines reflecting the WLF fit, and b) the shift factors of κ-carrageenan plotted against $1/T$ with the straight line reflecting the Arrhenius fit

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* According to Ferry, the glass transition temperature, T_g, is the third cross-point of G' and G'' traces as they progress from the relaxation to the glassy state (master curve), where the solid-like response again becomes dominant.

Figure 7a transforms the curvature into a linear relationship of log a_T vs. $1/T$ in *Figure 7b*. Furthermore, the WLF linearity of the glass transition regime in *Figure 7a* departs increasingly from the Arrhenius straight line relationship of *Figure 7b*. Therefore, the large configurational adjustments that contribute to changes in free volume give way at subzero temperatures to a constant activation energy of processes associated with a barrier to rotation in the solid-like environment of the glassy state.

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