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Structural properties of pectin–gelatin gels. Part II: effect of sucrose/glucose syrup

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Small deformation dynamic oscillation and bright field microscopy were used to examine the structural properties of single and mixed high methoxy pectin and gelatin systems in the presence of sucrose/glucose syrup blends. Co-solute concentrated (≥78%) systems of the polysaccharide form rubbery structures which are readily transformed into glassy consistencies according to the time–temperature superposition principle. Increasing amounts of co-solute in the gelatin samples induce changes in viscoelasticity from that of conventional hydrogels to mechanical traces that cover much of the plateau region and the beginning of the glass transition area. Furthermore, manipulation of the protein/sugar ratio can result in strong crystalline matrices, or viscoelastic solutions where the co-solute forms the continuous phase and the gelatin inclusions can undertake a conformational transition. The properties of the single components were used to rationalise the phase behaviour of their mixtures. Upon triggering the gelation of pectin, mixtures can be made where either gelatin or both components form a continuous phase. Results are discussed in the light of evidence obtained from the ethylene glycol work in Part I. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The aim of our work on gelatin/high methoxy pectin/co-solute samples is to develop model systems which are close to real foods, with the polymeric component being studied in the presence of co-solutes, and not merely of biopolymers themselves. Upcoming uses for mixed preparations of gelatin and pectin in the presence of sucrose/glucose syrup include wine gums and fruit pastilles. To develop a general understanding and to contrast the behaviour of different co-solutes, Part I of the series deals with the effect of ethylene glycol (EG) on the structure and mechanical properties of these biopolymers (Chronakis *et al.*, 1997).

Preparation of gelatin samples with 0–70% EG results in an immediate rise in gel strength followed by a subsequent network weakening with a maximum point at approx. 30% co-solute. The increase in network strength might be rationalised on the basis of unfavourable interactions between EG and protein segments which can be minimised by enhancing the gelatin self-associations (Gekko and Timasheff, 1981). However, a thermodynamically stable gelatin helix requires a surrounding layer of hydration whose diminishing presence leads to the ultimate drop in rigidity at the top range of ethylene glycol concentrations (Privalov and Tiktopulo, 1970).

Compared with gelatin, high methoxy pectins require a subtle balance of hydrophilic and hydrophobic interactions to sustain a stable gel structure. These involve formation of aggregated helices supported by hydrogen bonds and grouping of methyl ester groups within a cage of water molecules (Walkinshaw and Arnott, 1981; Rolin, 1993). At 30% EG in the system, pectin is capable of forming a soft network which is further reinforced with addition of co-solute up to 60%, presumably due to increasing hydrogen bonding between polymeric segments. Higher levels of ethylene glycol, however, cause a reduction in gel strength since the co-solute can disrupt the water calyx and solvate the methyl clusterings.

Mixtures of gelatin and high methoxy pectin with an EG content of less than 30% form protein continuous gels, as judged from their gelling and melting profiles, which are congruent with the corresponding traces of

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single gelatin networks (Chronakis *et al.*, 1997). Nevertheless, ordered segments of pectin exclude the gelatin chains from their domain, thus inducing a phase separated arrangement of increased mechanical strength. At higher concentrations of EG, the ordered assemblies of pectin are capable of forming a network at higher temperatures than gelatin, with the cooling profiles now showing a bimodal building of structure. Microscopy evidence argues that gelatin manages to create a continuous network alongside the pectin matrix, the mixture thus being a phase separated bicontinuous system.

Regardless of the variation in rigidity of gelatin and high methoxy pectin structures as a function of ethylene glycol composition, the viscoelastic ratio of loss modulus (G'') to storage modulus (G'), tan δ, is only slightly affected. For example, the tan δ values of gelatin and pectin gels containing 70% EG are respectively 0.040 and 0.074 (5°C), and fall well within the range expected (tan $\delta \leq 0.1$) for aqueous biopolymer networks (Almdal *et al.*, 1993). This result might be due to the non-specific thermodynamically unfavourable interaction between EG and these biopolymers that reduces the area of solvent–gelatin/pectin contact, and depending on EG concentration, it allows an extensive or limited formation of relatively similar intermolecular associations.

By contrast, addition of sugars to the gellan polysaccharide generates a maximum in the gel modulus vs co-solute graph and, in addition, transforms the viscoelastic ratio (Papageorgiou *et al.*, 1994). Thus, gellan samples in the presence of 50% sucrose plus 20% glucose syrup not only form weaker networks than those at peak strength (30% sucrose), but also exhibit substantial frequency dependence of shear moduli and a tan δ value of 0.906 at 5°C (marker frequency of 10 rad/s). In contrast to the sigmoidal solution ($G'' > G'$) $\Rightarrow$ gel ($G' > G''$) transition of gelatin/pectin–EG samples, the gellan–sugar system exhibits rubbery viscoelasticity with $G' > G''$ at the highest experimentally accessible temperature of 90°C which develops gradually to an extremely viscous solution (glass) during cooling to subzero temperatures ($G'' > G'$). It was argued that sugars and gellan chains interact closely to reduce crystallinity/aggregation, thus transforming the network to an assembly of flexible polysaccharide segments where the entropic contribution to elasticity is dominant (Whittaker *et al.*, 1997). In the present work we observe a metamorphosis in the behaviour of single gelatin and high methoxy pectin systems by using sucrose/glucose syrup as the co-solute, and attempt to rationalise its effect on both single and binary mixtures.

MATERIALS AND METHODS

The polymeric ingredients have been described in some detail in Part I of the series. Briefly, gelatin was a product of Systems Bio-industries, an acid pigskin extract with an isoelectric point of pH≈8. The citrus peel pectin sample is a high methoxy variety (70% degree of esterification), and came from Hercules (GENU B). It is 'standardised' to specific gel properties by blending with sucrose. The pectin content was found to be approx. 68.1%, and an allowance was made for the polymer and co-solute concentration in the final preparations. Sucrose was of food grade. Cerestar provided glucose syrup with a dextrose equivalent of 42 and a water content of 19%.

Gelatin samples were prepared by soaking the granules in distilled water overnight and then heating to 60°C. Pectin samples were dissolved at 90°C with gentle agitation for 15 min. Then sucrose or sucrose plus corn syrup was added and the pH was adjusted to 3 with 2 M HCl. Binary systems were prepared by mixing appropriate amounts of stock solutions at 75°C. Dynamic oscillation measurements were made on a parallel plate geometry (40 mm diameter; 1 mm gap) of a commercial high-torque Carri-Med CSL 500 rheometer or a cone and plate arrangement (0.02 rad; 50 mm diameter) of an in-house sensitive prototype (Richardson, 1991). Samples were loaded onto the preheated platen of the rheometer and the edges were covered with silicone fluid to prevent evaporation. Cooling and heating runs to 5°C and back were carried out at a scan rate of 1°C/min, a frequency of 1.6 Hz (corresponding to an angular frequency of ≈10 rad/s), and 1% strain (well within the linear viscoelastic region). Mechanical spectra over three or four decades of frequency (in the range from 0.001 to 10 Hz) were also recorded at selected temperatures to extend the window of observation of viscoelastic parameters.

RESULTS AND DISCUSSION

Formation of gels and the transition from rubber- to glass-like consistency in high methoxy pectin induced by increasing amounts of sucrose/glucose syrup blends

As outlined in the Introduction to this work, gel formation in pectin/ethylene glycol systems is seen at co-solute concentrations as low as 30%. However, replacement of ethylene glycol with blends of sucrose/glucose syrup raises the co-solute requirement for structure development. Table 1 shows the variation in viscoelastic parameters as a function of sugar content induced during cooling from 90 to 5°C at 1°C/min, holding there for 100 min, and heating to 95°C at the same scan rate. At 58% co-solute, the onset of gel formation is seen as a steep rise in storage modulus and occurs at 34°C. This gel is thermoreversible and melts at approx. 86°C. Addition of co-solute up to 74% induces an earlier network formation (≈72°C) and reinforces the elastic properties of the systems

Table 1. Small deformation viscoelastic parameters for 1% high methoxy pectin preparations made with increasing levels of co-solute

Sucrose	Glucose syrup	Total solute	Pseudoequilibrium modulus (G′; Pa)		Tan δ at 1 Hz			t_{form} (°C)	t_{melt} (°C)	Onset of glass transition (°C)
(%)	(%)	(%)	90°C	5°C	90°C	5°C	95°C	1°C/min	1°C/min	
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	(83 560)	0.168	1.648	0.201	> 95	> 95	15
50	36	86	12 820	(131 800)	0.514	1.941	0.416	> 95	> 95	24

The concentration of glucose syrup refers to dry solids.

which now remain thermally irreversible at the highest accessible temperature (tan δ values at 95°C are less than 0.5). The transformation from a liquid-like (tan $\delta > 1$ at 95°C) to a solid-like (tan $\delta < 1$ at 5°C) behaviour, reported so far, has also been observed during cooling of the water/EG mixtures of gelatin and pectin, and is the standard process occurring in aqueous polysaccharide preparations, e.g. of agar (Selby and Whistler, 1993) and κ-carrageenan (Therkelsen, 1993). In contrast to the pectin–water/EG mixtures where the values of G′ peak at 60% ethylene glycol and decline continuously thereafter, the upward trend in network strength is not affected at 66 and 74% sucrose/glucose syrup blends. In Part I of the series, we argued that the drop in G′ at this range of co-solute is due to disruption of the hydrogen bond cages around the methyl groups which are now dissolved in ethylene glycol. This statement is endorsed by the current results, where the polyhydric compounds should preserve the hydrogen bond calyx–methyl clustering arrangement, thus reinforcing the network's rigidity.

The general form of modulus development is entirely different when the top two concentrations of co-solute in Table 1 are employed. During sample preparation, it appeared that coherent structures can be formed at any temperature below the boiling point, an observation which is verified by a solid-like response on the rheometer at 90°C (e.g., the tan δ of 1% pectin plus 78% co-solute is equal to 0.168). The antithesis with the results in the preceding paragraph persists at the other end of the cooling run (5°C) where a predominantly liquid-like response is now obtained; e.g. the tan δ at 86% co-solute amounts to 1.941. This unusual behaviour is illustrated in Fig. 1, where the experimental constraints (scan rate of 1°C/min; frequency of 1.6 Hz) reveal a gradual transformation in viscoelasticity, the viscous component eventually

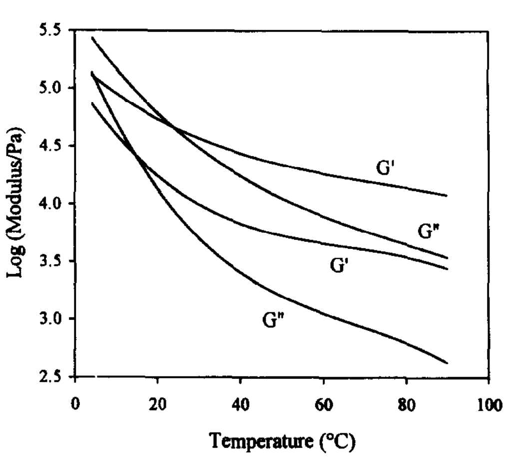

Fig. 1. Development of storage and loss moduli during cooling of 1% pectin sample with 78 and 86% co-solute (bottom and top spectra, respectively). The scan rate is 1°C/min and the frequency is 1.6 Hz.

becoming predominant. Upon heating, both moduli trace back their cooling spectra with no signs of thermal hysteresis, which is evident from the differences in the t_{form} and t_{melt} temperatures at levels of co-solute up to 74% (Table 1).

As mentioned in the Introduction, high sugar gellan preparations show with cooling a similar transformation from solid- to liquid-like behaviour. This, of course, has been reported before for the transition from rubber- to glass-like consistency in amorphous synthetic polymers, and rationalised with the combined WLF/free volume theoretical framework (Williams *et al.*, 1955). The essence of this approach is a change in state, but not in phase of the material, which is manifest in the glass transition profile. The development in viscoelasticity can be used to infer the changing free volume which collapses to approx. 3% of the total volume at the glass transition temperature (Ferry, 1980). It follows that in the absence of a disorder-to-order transition, the vitrification process at a low temperature can be reproduced at a higher temperature, as long as measurements can be carried out at extremely short timescales (usually in the order of tens of kHz). Since conventional rheological measurements cannot be performed at such high frequencies, viscoelastic parameters obtained at regular intervals during a cooling run are shifted along a logarithmic frequency axis, thus depicting a composite curve over a wide frequency window (at least 6–7 decades) for an arbitrary chosen reference temperature (time–temperature superposition; TTS). Application of the TTS principle to aqueous preparations of biopolymers fails because changes in free volume are usually swamped by other temperature-dependent effects, such as enthalpic interactions between the chains (Lopes da Silva *et al.*, 1994).

Following the synthetic polymer approach, therefore, frequency sweeps were recorded at 90, 70, 50, 30, and 10°C for the 1% pectin sample in the presence of 86% co-solute. Figure 2(a, b) illustrates the variation in storage and loss moduli with decreasing temperature. At the top temperature range (90–50°C) and frequency of oscillation, up to 0.1 Hz the spectra of G' remain relatively flat. Beyond this range, however, there is a clear build up of structure culminating at the sharp frequency dependence of G' at 10°C. The G'' values also rise steeply covering three orders of magnitude and eventually overtake those of G' at 10°C. The outcome of superimposing our data at 90°C (reference temperature) is shown in Fig. 2(c). These were fitted in the form of a composite curve and its viscoelastic ratio, $\tan \delta = G''/G'$, covering a frequency range from 10^{-3} to 10^{4} Hz (Fig. 2d). There is a clear progression from a 'rubbery plateau' to a glass transition region with increasing frequency in the way induced by cooling in Fig. 1. As a result, the ratio of loss to storage modulus crosses the threshold from solid- to liquid-like response ($\tan \delta = 1$) and finally levels off at the upper end of the frequency range, indicating that the superposition extends well into the glass transition area (Ferry, 1980). This rather spectacular alteration in the viscoelasticity of high sugar pectin samples will be contrasted with the mechanical profiles of high sugar gelatin samples, and together used as a baseline of behaviour in the discussion of binary mixtures.

Small deformation properties of solutions, gels, and crystalline systems comprising gelatin and sucrose/glucose syrup blends

Gelatin samples were prepared at concentrations of 1.5, 3, and 5% with a view to covering a range of product applications from soft table jellies to firm confectioneries. For each gelatin series, samples were cooled from 70 to 5°C at 1°C/min, left there to equilibrate for 100 min, monitored under increasing angular frequency, and finally heated back. The usual frequency-dependent markers of network formation (t_{form}; sharp rise in the G' trace) and collapse (t_{melt}; G' falling below G'' during heating) were used. Let us reiterate here that our values of t_{form} should not acquire the sense of the gelling point according to the Te Nijenhuis–Winter criterion (i.e., identification of the gelling point with the frequency-independent loss tangent; Te Nijenhuis and Winter, 1989), since, in combination with the t_{melt} values, their use is that of an operational convenience for rationalisation of the phase continuity in biopolymer mixtures.

Table 2 reproduces the changes in viscoelastic functions with increasing levels of co-solute for the 5% gelatin series. The expected stabilisation of a gelatin network is noted as rising storage modulus with increasing co-solute concentrations up to 58%, and it has been associated with the generation of extra collagen-like triple helix structures (Oakenfull and Scott, 1986). There is, however, a significant drop in the rigidity of the gelatin network in the presence of 66% co-solute, which should be attributable to the destabilisation of the triple helix conformation at this level of water shortage (Privalov and Tiktopulo, 1970). Fig. 3(a) shows the asymmetric cooling and heating traces (see also t_{form} and t_{melt} values in Table 2) separated by the isothermal run, at the highest, 66%, sucrose/glucose syrup content. Both the graph and the tabulated values indicate that the $\tan \delta$ at 5°C (0.156) is unusually high for an aqueous gelatin gel (0.011 at 0% sucrose). The high 'sol-fraction' is due to the large quantity of co-solute and its slowing-down effect on the helix fraction of gelatin chains. As a result, a rubbery spectrum of gelatin at 66% co-solute is obtained with the G'' trace showing a pronounced frequency dependence and the G' values start taking off at frequencies higher than 1 Hz (Fig. 3b).

The changes in mechanical characteristics observed

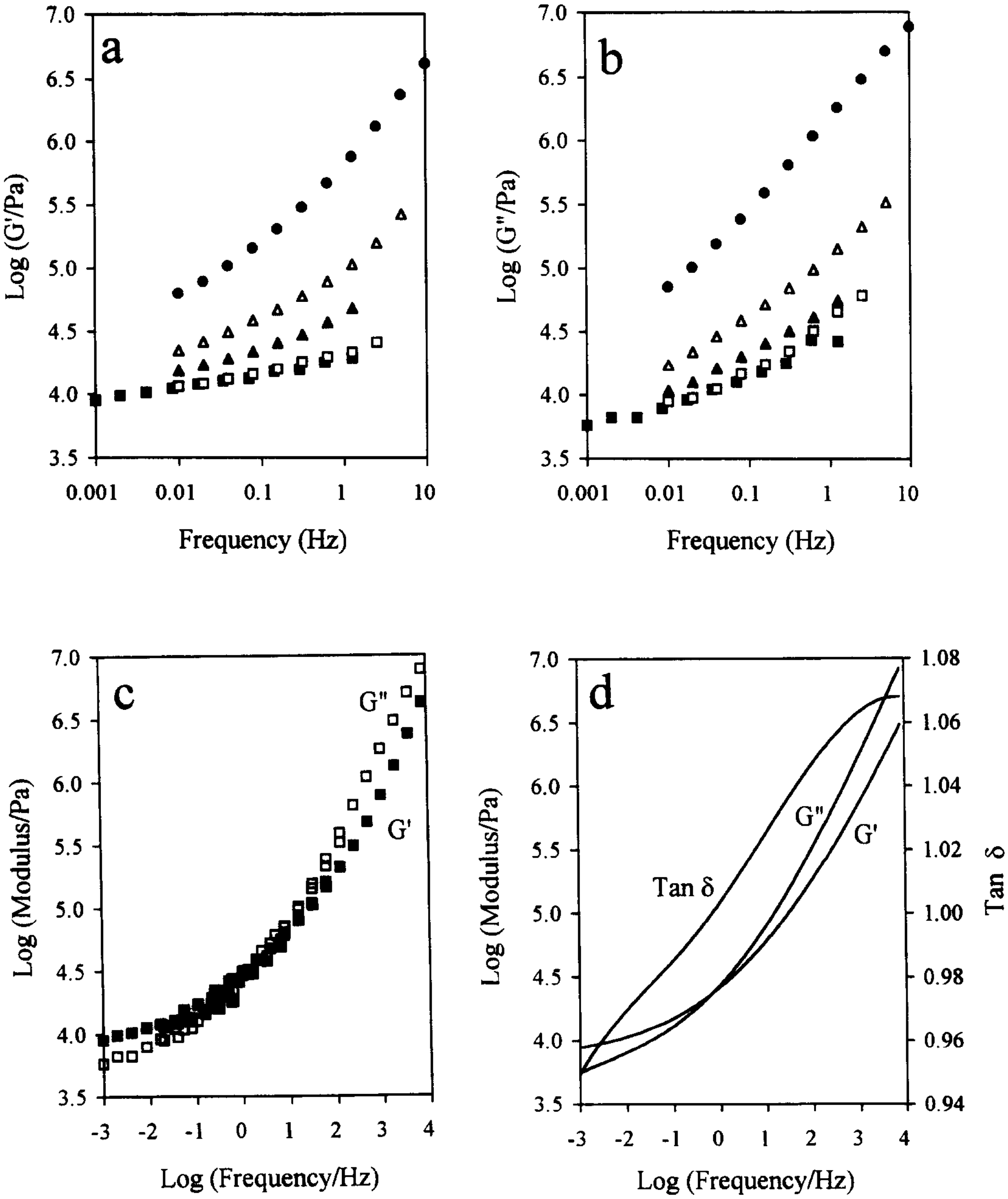

Fig. 2. Frequency sweeps of 1% pectin plus 86% co-solute at 90 (■), 70 (□), 50 (▲), 30 (△), and 10°C (●) for the storage (a) and loss (b) moduli. The time–temperature superposition for the experimental points and a fit with the tan δ trace are shown in (c) and (d), respectively.

Table 2. Small deformation viscoelastic parameters for 5% gelatin preparations made with increasing levels of co-solute

Sucrose (%)	Glucose syrup (%)	Total solute (%)	Pseudo-equilibrium modulus (G'; Pa) 5°C	Tan δ at1 Hz (5°C)	t_{form} (°C) 1°C/min	t_{melt} (°C) 1°C/min
0	—	—	6713	0.011	17	27
20	—	20	7352	0.012	20	30
30	—	30	8637	0.014	22	31
40	—	40	9084	0.016	24	33
50	—	50	9239	0.017	25	34
50	8	58	9553	0.058	28	38
50	16	66	6909	0.156	37	41

The concentration of glucose syrup refers to dry solids.

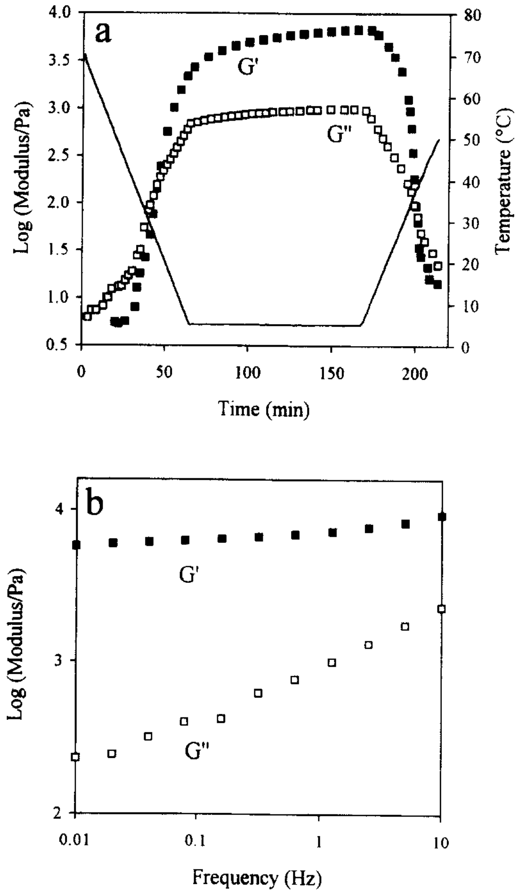

Fig. 3. Variation in storage and loss moduli for 5% gelatin in the presence of 66% co-solute during a cooling–isothermal–heating run (a) and a frequency sweep (b). The scan rate is 1°C/min, the isothermal run lasted 100 min, and the frequency of (a) is 1.6 Hz.

for the 3% gelatin series during the cooling–isothermal–heating routine are given in Table 3. The use of lower levels of protein allowed formulation of systems with up to 78% co-solute. Compared with the inert ethylene glycol molecules which did not alter the setting (t_{form} ≈ 14°C) and melting (t_{melt} = 28°C) characteristics of a 3% gelatin network (Part I), the present values see a rise in t_{melt} of more than ten degrees centigrade with increasing sucrose/glucose syrup solids from 0% to 62%. Similarly, the interactive nature of gelatin–sucrose/glucose syrup molecules raises steadily the 'sol-fraction' of these gels, whereas in the presence of, for example, 70% ethylene glycol, a rather conventional tan δ value of 0.040 is recorded. The high tan δ value (0.643) and the rapidly drooping G′ value (1869 Pa) at 70% sucrose/glucose syrup emphasises the destabilisation of gelatin's native structure at low levels of water. Figure 4(a) illustrates the asymmetric development and demise of viscoelasticity with the G′ trace struggling and eventually overtaking that of G″ only after 35 min within the isothermal run (5°C). Nevertheless, a 'true' structure has taken shape at the end of the isothermal run (100 min; frequency of 1.6 Hz) which produces a characteristic shoulder during subsequent heating, and clearly demonstrates the development of thermal hysteresis between the belated network formation and melting (t_{melt} = 34°C) of this preparation. The outcome of dropping the polymer to co-solute ratio from approx. 0.08 (Fig. 3b) to 0.04 in the sample of 3% gelatin with 70% sugar is shown in Fig. 4(b). This time the values of storage modulus depart from the plateau of the rubbery zone at much lower frequencies (in the order of 0.01 Hz) and converge with those of loss modulus. Eventually, the G″ overtakes the G′ at the high frequency end, an indication of the advent of the glass transition region.

The general form of modulus development during our experimental routine changes dramatically when samples of 3% gelatin are made with 50% sucrose and 28% glucose syrup. Initially, a viscous solution response is obtained during a cooling run and frequency sweeps at elevated temperatures, e.g. at 70°C (not shown here). As depicted in Fig. 5(a), however, at about 10°C (scan rate of 1°C/min) the

Table 3. Small deformation viscoelastic parameters for 3% gelatin preparations made with increasing levels of co-solute

Sucrose (%)	Glucose syrup (%)	Total solute (%)	Pseudo-equilibrium modulus (G′; Pa) 5°C	Tan δ at1 Hz (5°C)	t_{form} (°C) 1°C/min	t_{melt} (°C) 1°C/min
0	—	—	1628	0.034	12	25
20	—	20	2177	0.046	14	28
30	—	30	2776	0.042	20	31
40	—	40	2966	0.048	21	32
50	—	50	2868	0.054	22	33
50	8	58	2904	0.084	24	35
50	12	62	2467	0.117	26	36
50	20	70	1869	0.643	35 min at 5°C	34
50	28	78	1 159 000	0.334	10	10

The concentration of glucose syrup refers to dry solids.

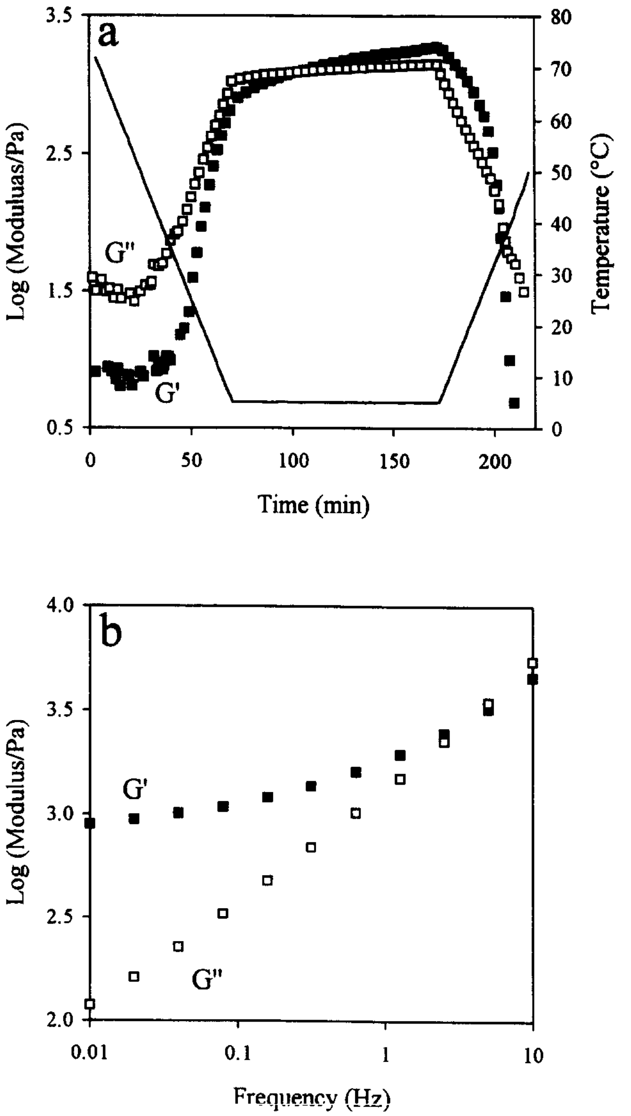

Fig. 4. Variation in storage and loss moduli for 3% gelatin in the presence of 70% co-solute during a cooling–isothermal–heating run (a) and a frequency sweep (b). Conditions as in Fig. 3.

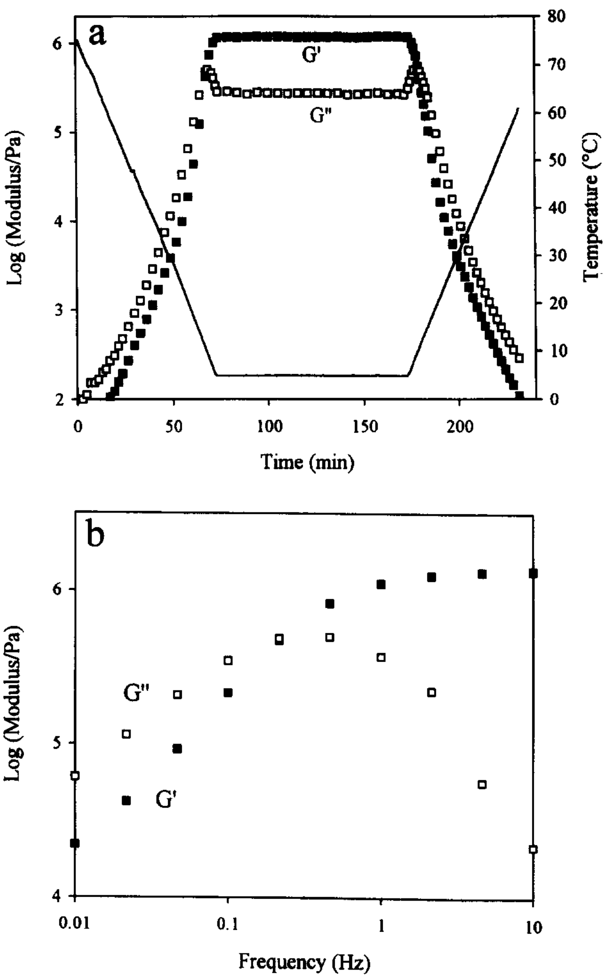

Fig. 5. Variation in storage and loss moduli for 3% gelatin in the presence of 78% co-solute during a cooling–isothermal–heating run (a) and a frequency sweep (b). Conditions as in Fig. 3.

modulus traces cross-over and the solid-like response achieves values in excess of a million Pascals whereas the liquid-like response takes a sudden dive. The picture remains unchanged during the isothermal run and reverses itself on subsequent heating. As a result a perfectly symmetrical time–temperature profile is obtained with no signs of thermal hysteresis pertaining to formation and melting of polymeric networks. This, and the formation of a rigid structure at 5°C, contrasts strongly with the asymmetric profile of the weak and rubbery gelatin gel in Fig. 4; compare also the viscoelastic parameters for the top two co-solute samples in Table 3. Furthermore, the frequency dependence of G' and G'' at 5°C (Fig. 5b) reproduces the metamorphosis from a viscous solution to a rigid solid seen at the final stage of the cooling run. Very similar mechanical spectra have been recorded for concentrated sucrose/glucose syrup blends which were accompanied by endothermic peaks during heating of the samples in a calorimeter (Al-Ruqaie *et al.*, 1997; Ong *et al.*, in press). Work by Dea *et al.* (1984) has also reported this pattern of behaviour for ice cream at subzero temperatures, where it is expected that lactose crystallisation has occurred. Therefore, 'phase inversion' has occurred from a rubbery gelatin consistency at 70% co-solute to a continuous, 78%, co-solute phase whose saturation point is reached during cooling, thus resulting in partial crystallisation and structural reinforcement of the system.

In the last gelatin series we reduced further the protein to co-solute ratio and looked for additional patterns of viscoelastic behaviour. Table 4 summarises the effect of sucrose/glucose syrup blends on the properties of 1.5% gelatin samples. Increasing the sugar content from 0 to 66% results in rubbery gelatin gels with a swollen 'sol-fraction', as seen for the 3% gelatin series (Fig. 4b) before the occurrence

Table 4. Small deformation viscoelastic parameters for 1.5% gelatin preparations made with increasing levels of co-solute

Sucrose (%)	Glucose syrup (%)	Total solute (%)	Pseudo-equilibrium modulus (G′; Pa) 5°C	Tan δ at1 Hz (5°C)	t_{form} (°C) 1°C/min	t_{melt} (°C) 1°C/min
0	—	—	102	0.036	5	27
20	—	20	85	0.090	7	29
30	—	30	139	0.083	12	30
40	—	40	181	0.098	15	33
50	—	50	130	0.190	22	33
50	8	58	139	0.282	28	34
50	16	66	87	0.803	80 min at 5°C	30
50	24	74		1.336	—	—
50	28	78	—	2.352	—	—

The concentration of glucose syrup refers to dry solids.

of crystallisation. There, crystallinity was evidenced by a decrease in the tan δ and a mounting of the G′ values. This time, topping up the amount of co-solute from 66 to 74 and 78% maintained the upward trend in tan δ, and values now are higher than 1. In addition, systems remain viscoelastic liquids throughout the experimental routine, as illustrated for the 1.5% gelatin plus 78% co-solute in Fig. 6(a). Over the allocated time at 5°C there is a slow but steady growth in G′ which in combination with the relatively flat G″ creates a skewed profile. Mechanical spectra at the end of the isothermal run (Fig. 6b) show a rather unconventional frequency response with a shear thinning dynamic viscosity, although the moduli traces are well separated from each other. Subsequent heating of our system creates a sigmoidal trace, especially from the side of G′ whose values collapse well below those of G″.

Combined evidence from Fig. 6(a, b) argues that in the 1.5% gelatin series there is a transformation from a rubbery structure to a quite viscous solution. Clearly, the low levels of gelatin keep the sugars in solution which now form the continuous phase. It seems, though, that the gelatin inclusions undergo conformational ordering and gradual ageing/strengthening during the isothermal run at 5°C. Since blends of sucrose and glucose syrup remain Newtonian during a frequency ramp (Papageorgiou *et al.*, 1994), disruption of the pattern of flow (rubbing surfaces, overlapping fields of countercurrents) between adjacent gelatin particles with increasing frequency might account for the shear thinning behaviour of these composites. Deconvolution of the gelatin helices during subsequent heating of the sample will eliminate the solid-like component along the sigmoidal path monitored in Fig. 6(a). As in the case of a continuous gelatin network (e.g. Table 2), the high melting temperature of gelatin inclusions (mid-transition temperature is between 40 and 45°C) argues that the continuous co-solute phase also imbeds in and cross-contaminates heavily the suspended gelatin particles. By way of concluding this section, we hope that the multitude of viscoelastic profiles exposed by the reduction in gelatin to co-solute ratio and its proposed rationalisation will facilitate a stimulating debate in the area of high solids gelatin systems.

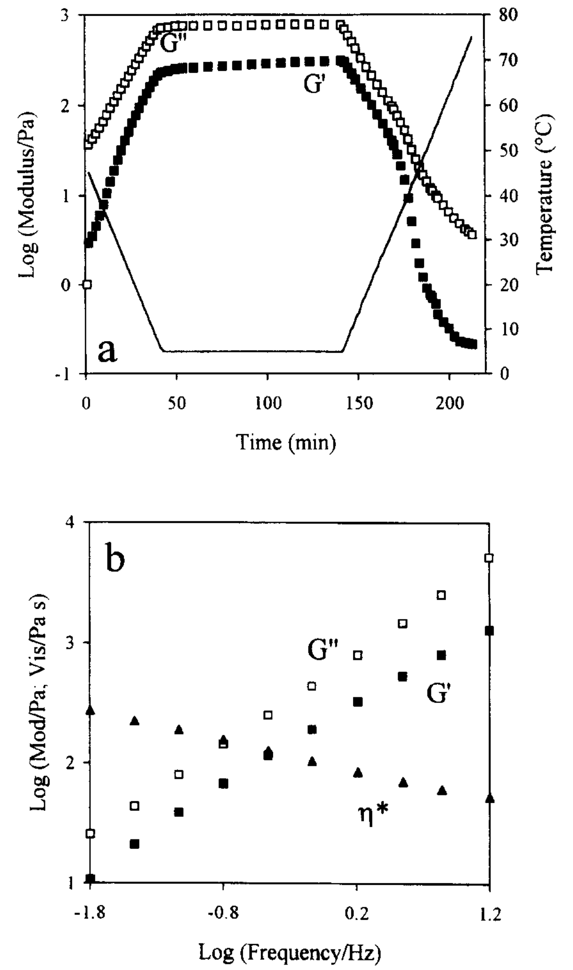

Fig. 6. Variation in G′, G″ and η^* for 1.5% gelatin in the presence of 78% co-solute during a cooling–isothermal–heating run (a) and a frequency sweep (b). Conditions as in Fig. 3.

Table 5. **Small deformation viscoelastic parameters for mixtures of 1% high methoxy pectin and 3% gelatin preparations made with increasing levels of co-solute**

Sucrose	Glucose syrup	Total solute	Pseudo-equilibrium modulus (G′; Pa)		Tan δ at 1 Hz			t_{form} (°C)	t_{melt} (°C)	Onset of glass transition (°C)
(%)	(%)	(%)	85°C	5°C	85°C	5°C	90°C	1°C/min	1°C/min	
0	—	—	—	2079	>1	0.094	>1	13	24	—
20	—	20	—	2132	>1	0.097	>1	16	26	—
30	—	30	—	3081	>1	0.088	>1	24	30	—
40	—	40	—	3966	>1	0.109	>1	26	32	—
50	—	50	—	4387	>1	0.108	>1	28	34	—
50	8	58	—	7076	>1	0.160	>1	35	40	—
50	16	66	—	6939	>1	0.373	0.296	55	>95	—
50	24	74	—	9714	>1	0.817	0.557	65	>95	—
50	28	78	229	(158 600)	0.851	1.831	0.813	>95	>95	52

The concentration of glucose syrup refers to dry solids.

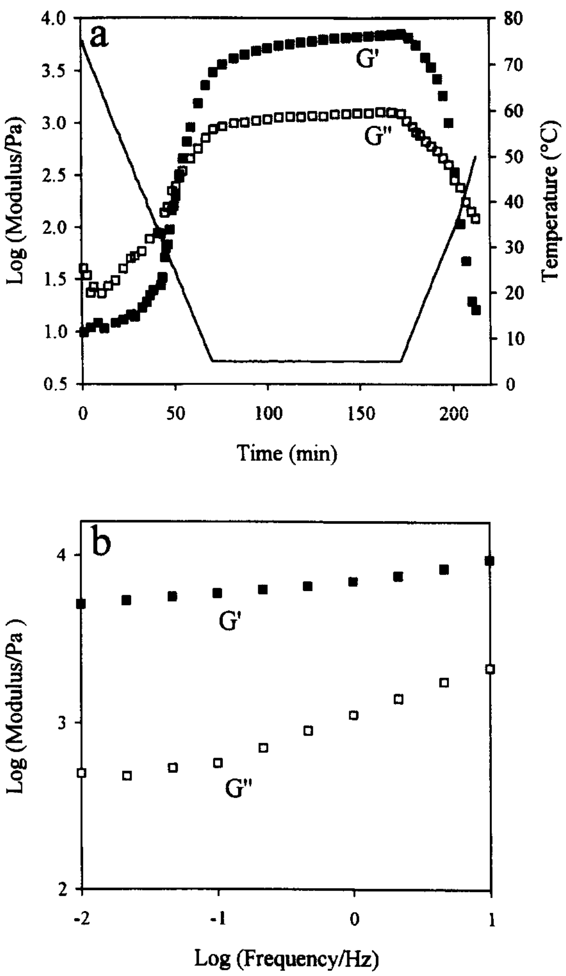

Fig. 7. Variation in storage and loss moduli for the mixture of 1% pectin and 3% gelatin in the presence of 58% co-solute during a cooling–isothermal–heating run (a) and a frequency sweep (b). Conditions as in Fig. 3.

The effect of sucrose/glucose syrup on the phase behaviour of high methoxy pectin/gelatin mixtures

The understanding achieved at the above work of single biopolymers was put to practice in mixtures containing 1% pectin and 3% gelatin. As shown in Table 5, the first point at issue is the significant change in the values of t_{form} and t_{melt} with increasing co-solute content. Thus, addition of sugar up to 58% generates a range of structure formation and melting indices which is covered by the temperature behaviour of gelatin gels. Under our experimental settings, the small-deformation results at 58% co-solute are illustrated in Fig. 7. Gratifyingly, the onset of structuring and the completion of melting, the network reinforcement during the isothermal run, the viscoelastic ratio at the end of the curing process at 5°C, and the ensuing frequency dependence at this temperature are congruent with the properties of a single, 5%, gelatin sample with 66% co-solute (Fig. 3 and Table 2). On the other hand, pectin structures melt at elevated temperatures (≥86°C in Table 1). Furthermore, the

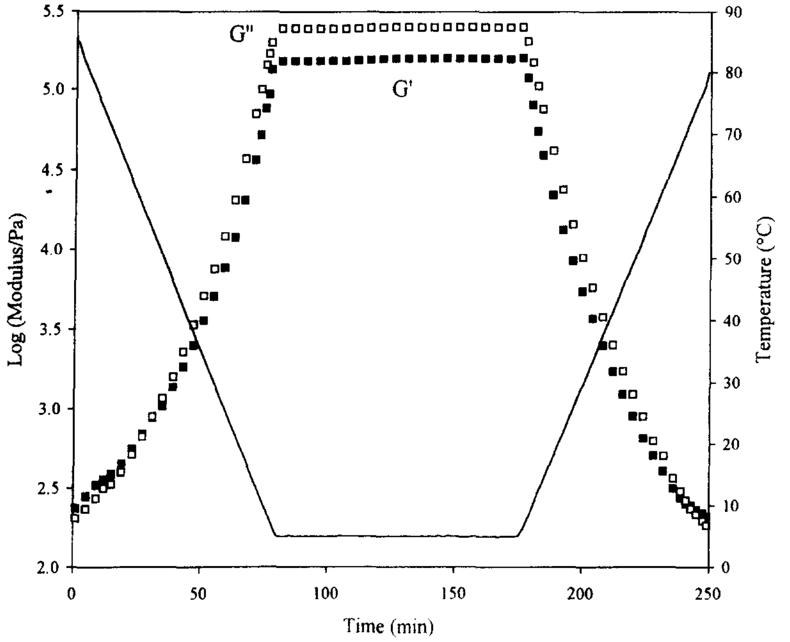

Fig. 8. The development and demise of G′ and G″ for the mixture of 1% pectin and 3% gelatin with 78% co-solute during a cooling–isothermal–heating run. Conditions as in Fig. 3.

mixed gel network is reinforced (≈7 kPa) beyond the level expected from the corresponding sum of rigidities of single pectin and gelatin gels (≈0.5 and 2.9 kPa, respectively). It appears, therefore, that mixtures have phase separated into a continuous gelatin phase suspending the pectin inclusions. At a sucrose/glucose syrup concentration of 58% the pectin domains are capable of gelation, thus excluding efficiently and concentrating up the gelatin matrix, with the system achieving the enhanced elasticity of a single 5% gelatin gel at 66% co-solute.

The step from the gelatin-related t_{form} and t_{melt} values to those in the range of a pectin gel is achieved when the co-solute content is increased to 66 and 74% (Table 5). In the blend with 74% sugar, for example, the onset of gel formation is recorded at 65°C and its structure remains thermally stable at the end of a heating run; tan δ at 90°C is 0.557. Therefore, despite the early melting profile of gelatin gels, mixtures retain overall cohesion at elevated temperatures, a result which argues that the polysaccharide has now formed a continuous network.

The mixture with the highest possible amount of sucrose/glucose syrup (78%) forms, of course, a pectin continuous phase but its behaviour is distinct from that in the preceding paragraph and akin to the transformation observed for single pectin samples at 78 and 86% co-solute (Table 1). Its overall behaviour in our experimental routine is reproduced in Fig. 8. This time a dominant elastic response is recorded at the start of the cooling run (tan δ at 85°C is 0.851) but, as in Fig. 1, the reduction in temperature triggers a metamorphosis in viscoelasticity with the tan δ value at 5°C being equal to 1.831. In the absence of further development during the isothermal run, the gradual reversal to dominant G′ values on subsequent heating concludes a symmetric time–temperature profile. Mirror image temperature runs (i.e., no thermal hysteresis) were also reported for the 3% gelatin plus 78% co-solute in Fig. 5(a). There, however, we observed a transformation from liquid- to solid-like behaviour, owing to co-solute crystallisation, whereas in Fig. 8 the opposite occurs.

Following the methodology of Fig. 2, mechanical spectra of the most concentrated mixture were taken at 70, 50, 30, and 5°C. As in the case of single pectin preparations with ≥78% sugar, the patterns of G′ and G″ advance with decreasing temperature/increasing frequency from relatively flat to quite sharply sloping spectra (Fig. 9a and b). The time–temperature superposition principle was implemented in Fig. 9(c), starting from the reference temperature of 70°C. Clearly, the mixture exhibits a mechanical transition from rubbery to glass-like consistency, as was noted for the high solids pectin systems in Fig. 2(c).[1] Indeed, it is quite remarkable to see that whereas sugar in the presence of gelatin comes out of solution (crystallises) and gradually imparts solid-like properties to the sample of Fig. 5, pectin uses the co-solute as an innate part of its network, thus preventing it from separating out in the form of crystals and allowing the rubber to

[1] In Table 1 and Table 5, the values of pseudoequilibrium modulus in parentheses indicate the dominance of the viscous component at 5°C and the strong frequency dependence of the solid component, as opposed to the relatively flat response of G′ in the plateau region discussed at other places in the paper.

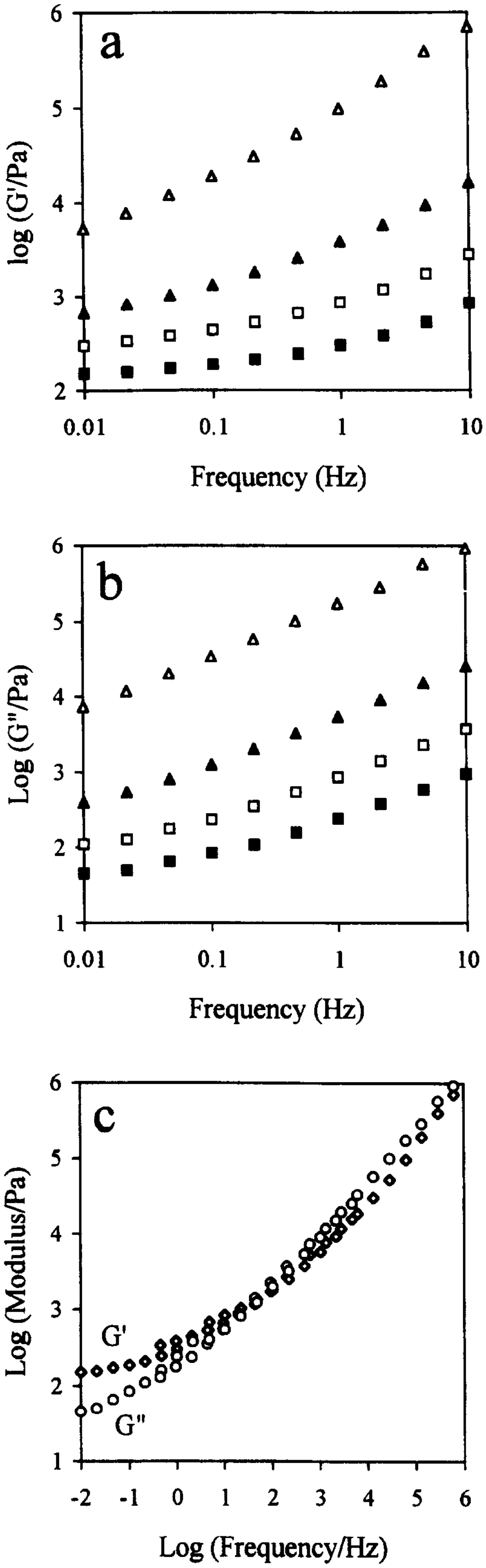

Fig. 9. Mechanical spectra of 1% pectin plus 3% gelatin with 78% co-solute taken at 70 (■), 50 (□), 30 (▲), and 5°C (△) for the storage (a) and loss (b) moduli (the sample was scanned down between frequency sweeps at 1°C/min). The time–temperature superposition of both moduli at the reference temperature of 70°C is shown in (c).

glass transformation to occur in the mixture of Fig. 9.

Although the rheological work has shown comprehensively the formation of a continuous pectin phase at the top range of co-solute ($\geq 66\%$), it is difficult to say if this is a true phase inversion point, i.e., a transformation from a gelatin to a pectin supporting matrix. To determine the topology of the gelatin phase we used bright field microscopy and histochemical dyes to provide contrast to the specific chemical groups of the two polymeric components. These were: Sirius red, which contains sulphonic acid groups and reacts with the basic groups of collagen and Toluidine blue, a metachromatic dye that stains pectin reddish pink (Abeysekera and Robards, 1995). Samples were cooled to 5°C and matured there for 2 h. The dyes reached their target by diffusion for 10 min and gel pieces of 10×10×0.5 mm were taken for examination. Figure 10 reproduces the images of single and mixed gelatin/pectin systems in the presence of co-solute. Single polymer preparations appear as a featureless background, which undoubtedly constitutes an isotropic phase (top and middle micrographs in Fig. 10). Although pectin does not gel at concentrations of co-solute below 58% (Table 1), the reinforced rigidity of the supporting gelatin matrix in mixtures with sugar levels up to 50% (compare values in Table 3 and Table 5) argues that the ordered polysaccharide sequences effectively exclude the protein from their domain. That must correspond to a scale of phase separation below the resolution of our light microscope (maximum magnification of 400×), since mixtures with a low co-solute concentration also appeared as a single entity. Pectin gelation at high levels of sucrose/glucose syrup should further enhance steric exclusion phenomena and more clearly differentiate one phase from another. Thus, in the bottom micrograph of Fig. 10, a phase separated system is obtained with discontinuous inclusions in the range of 1–2 μm. Staining of the samples strongly suggests that gelatin forms a continuous featureless background, with the darker pectin spots being the excluded phase. Therefore, rheology and microscopy have emphasised the phase continuity of the polysaccharide or the protein component at the top range of co-solute ($\geq 66\%$) and together build up a case for a phase separated bicontinuous structure.

CONCLUSIONS

Addition of co-solute, especially in the high solids regime (60–85%), to single and mixed systems of high methoxy pectin and gelatin results in dramatic changes of viscoelasticity. It is encouraging that the methodology of dynamic oscillation and microscopy employed extensively in the description of phase behaviour of

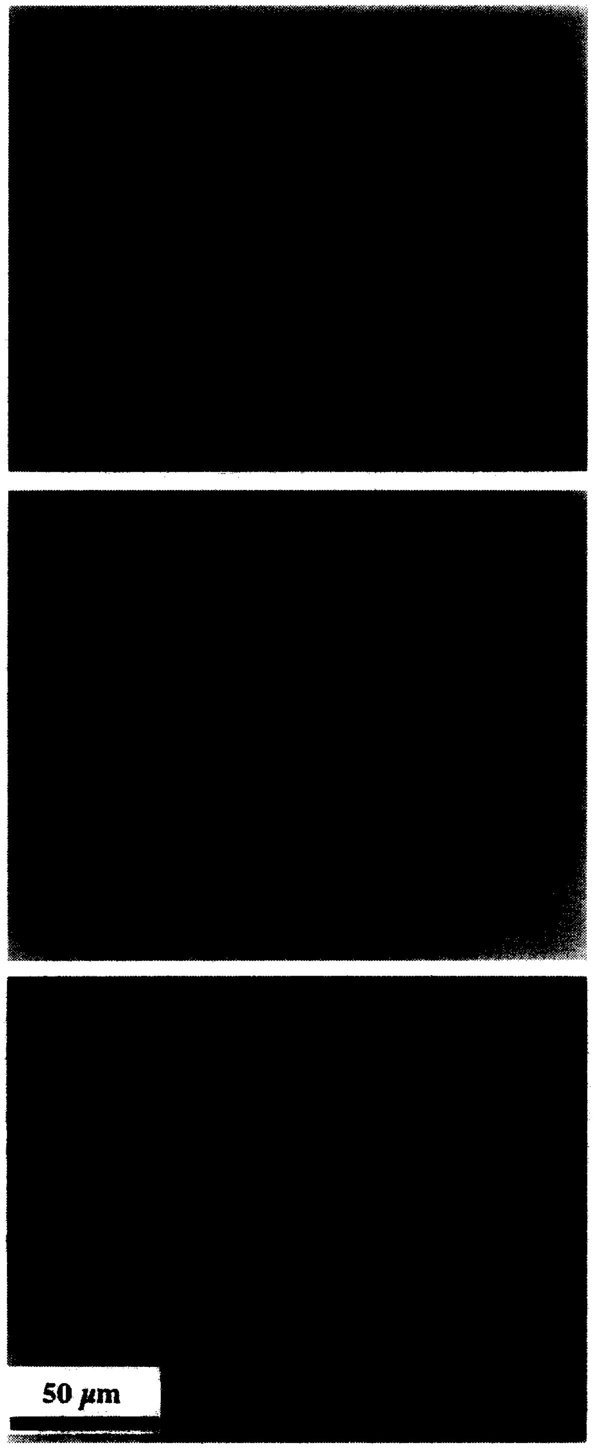

Fig. 10. Bright field micrographs for 1% pectin plus 70% co-solute (top), 3% gelatin plus 70% co-solute (middle), and 1% pectin–3% gelatin plus 70% co-solute (bottom). Images of 400× magnification were acquired with a Nikon inverted microscope Diaphot-TMD.

aqueous systems can be utilised in the area of high solids, where there is renewed scientific interest. In addition, the synthetic polymer approach adapted for the particular needs of biopolymers can guide us in the time/temperature-induced transformation of samples from rubbery to glassy consistencies. Sugar constitutes an integral part of a pectin structure and at highly concentrated levels of co-solute, a rubbery polymer–sugar–water entity is stabilised which can undergo a glass transition. Within our experimental settings, rubbery gelatin–sucrose–glucose syrup systems show signs of vitrification. It appears, however, that the protein–sugar interaction is less stable than with pectin and upon increasing the sugar-to-protein ratio, co-solute precipitates out and creates strong crystalline structures. At conditions below the saturation point, blends of sucrose/glucose syrup can establish a continuous liquid phase with the gelatin inclusions being able to undergo a conformational transition. By comparison, increasing concentrations of ethylene glycol hardly change the viscoelastic ratio (tan δ) of aqueous pectin and gelatin gels, hence suggesting a non-conformative type of hydroxyl groups (mainly *cis* gauch rotamers about the C–C bond with internal hydrogen bonds) for the development of consequential interactions with the two biopolymers.

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