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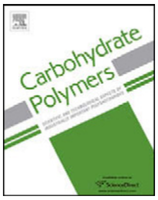

Studies on macromolecular interactions in ternary mixtures of konjac glucomannan, xanthan gum and sodium alginate

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ABSTRACT

Covalent and non-covalent macromolecular interactions were studied in the proprietary commercial product, PGX® (PolyGlycopleX®) which is manufactured from konjac glucomannan, xanthan gum and sodium alginate using a proprietary manufacturing process (EnviroSimplex®). Analytical techniques established the absence of changes in primary covalent structure of any of the component polysaccharides which may have occurred when the product was manufactured. However, non-covalent interactions between the polysaccharides were apparent in aqueous solutions of proprietary ternary mixtures by comparing their flow behaviour with those of unmixed controls. The interactions indicated by the flow behaviour confirmed those observed in the sedimentation behaviour, published earlier (Abdelhameed, A. S., Ang, S., Morris, G. A., Smith, I., Lawson, C., Gahler, R., et al. (2010). *Carbohydrate Polymers*, *81*, 145–148). Non-covalent macromolecular interactions between konjac glucomannan and xanthan gum are already known in binary mixtures but the flow behaviour of solutions of laboratory-prepared ternary powder mixtures with variable sodium alginate content was consistent with a ternary macromolecular interaction involving sodium alginate at below 5% but this was significantly enhanced by heat treating the solutions when the sodium alginate content exceeded 5%. Sedimentation coefficient distribution analysis from analytical ultracentrifugation supports these observations showing a clear shift to higher sedimentation coefficients in the heat-treated solutions of ternary mixtures at sodium alginate concentrations >8%, and over a greater range compared with unheated material. Calcium ion addition failed to precipitate calcium alginate from solutions of PGX® (containing all three polysaccharides) but precipitates were obtained from solutions of binary mixtures prepared with either konjac glucomannan or xanthan gum plus sodium alginate. The results are consistent with an interaction between a konjac glucomannan–xanthan gum complex and sodium alginate to form a new, ternary complex.

1. Introduction

There is considerable interest in the use of mixed food grade polysaccharide systems for use in functional foods and dietary or health products. The unexpectedly high viscosity of one particular proprietary product used for supplementation, PolyGlycopleX®, (α-D-glucurono-α-D-manno-β-D-manno-β-D-gluco), (α-L-gulurono-β-D-mannurono), β-D-gluco-β-D-mannan (PGX®) led us to investigate whether macromolecular interactions were occurring between the three components of this product, *viz.* konjac glucomannan, xanthan gum and sodium alginate, which would account for this unexpected viscosity behaviour. PGX® and PolyGlycopleX® are both trade names belonging to InovoBiolgic Inc., Calgary, Alberta, Canada. PGX® is produced from a mixture of polysaccharide powders composed of proprietary proportions of konjac glucomannan, xanthan gum and sodium alginate that has been subjected to a proprietary process (EnviroSimplex®) including heat input after mixing the solid components.

The hydrodynamic properties of glucomannans (Kök, Abdelhameed, Ang, Morris, & Harding, 2009), xanthan (Berth et al., 1996; Dhami, Harding, Jones, & Hughes, 1995; Mannion et al., 1992; Morris et al., 2001) and alginates (Harding, 1992a; Horton, Harding, & Mitchell, 1991; Horton, Harding, Mitchell, & Morton-Holmes, 1991; Kelly, Gudo, Mitchell, & Harding, 1994) are well understood. It is known from rheological studies that mixtures of polysaccharides in concentrated solution can interact synergistically. Shatwell, Sutherland, Ross-Murphy, and Dea (1991), for example, have shown significant non-covalent interactions between xanthan gum and konjac glucomannan to form a strong thermoreversible gel network. These observations have

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Table 1
Single polysaccharides and proprietary mixtures.

Polysaccharide samples	Lot no.	Moisture content (%, w/w)	Covalent interactions			Non-covalent interactions				
			GCMS	HPAEC-PAD	^{1}H NMR	Flow behaviour		Sedimentation coefficient		Ca^{2+} ion precipitation
			Single & proprietary mixtures	Single & proprietary mixtures	Single & proprietary mixtures	Single & proprietary mixtures	Ternary mixture components	Single & proprietary mixtures	Ternary mixture components	Single & proprietary mixtures
Konjac glucomannan	2538	10.0	√	√		√	√	√	√	√
	2681	10.6				√				
Xanthan gum	2504	10.8	√	√	√	√	√	√	√	√
	2505	11.8				√				
Sodium alginate	2455	16.7	√				√		√	
	2638	11.6		√	√	√				√
	2639	11.9				√		√		
TM1	900285	–	√							
	900416	10.0		√		√		√		√
	1112050809	10.5	√			√				
	Lab mix[a]	–			√					
PGX®	900495	4.9		√		√		√		√
	901008	–			√					
	2029070523	6.2	√√			√				

[a] Mixed in the laboratory using konjac glucomannan (lot no. 2538) supplemented with proprietary quantities of xanthan gum (lot no. 2504) and sodium alginate (lot no. 2455).

been supported by dilute solution interaction studies using sedimentation velocity in the analytical ultracentrifuge on mixtures of the same molecules by Dhami (1996, chap. 8). He observed a very strong interaction in dispersions of xanthan gum and konjac glucomannan with xanthan gum as the dominant component but an interaction that was very sensitive to the ionic strength of the aqueous medium. In a more recent study (Abdelhameed et al., 2010) we observed changes in the sedimentation velocity behaviour of PGX® compared with unmixed controls of each single component polysaccharide in the analytical ultracentrifuge. These interactions were also consistent with the formation of macromolecular complexes which were found to be sensitive to the ionic strength of the aqueous supporting solvent but were clearly significant at low ionic strength.

Analytical studies reported here investigate whether any of the macromolecular interactions between the polysaccharides are covalent arising from possible chemical changes involving new covalent linkages or new sugars within the polysaccharides which may have formed by chemical reactions during the manufacture of PGX®. Rheological and analytical ultracentrifugation measurements were then used to probe the magnitude of non-covalent intermolecular interactions in aqueous solution. Since binary interactions between konjac glucomannan and xanthan gum are already known to exist, results from these techniques and the response of solutions to added Ca^{2+} ions were also used to determine whether the third polysaccharide, sodium alginate, also adds a further contribution to these interactions.

2. Materials and methods

2.1. Single polysaccharides and proprietary mixtures

All the polysaccharides used in the study were supplied by InovoBiolgic Inc., (Calgary, Alberta, Canada). Single polysaccharides were: konjac glucomannan, lot nos. 2538 and 2681; xanthan gum, lot nos. 2504 and 2505; and sodium alginate, lot nos. 2455, 2638 and 2639. Commercial proprietary polysaccharide mixtures were composed of konjac glucomannan, xanthan gum and sodium alginate which had been subjected to the EnviroSimplex® process, including heat input after mixing the solid components (PGX®, lot nos. 900495, 901008 and 2029070523). Mixtures of the same composition were also taken prior to the EnviroSimplex® process (TM1, lot nos. 900285, 900416 and 1112050809 and a further TM1 sample mixed in the laboratory using the proprietary quantities of lot nos. 2538, 2504 and 2455 of konjac glucomannan, xanthan gum and sodium alginate, respectively). The specific sample details of each of the materials used in each analysis, together with their moisture contents determined by Karl Fischer titration are summarised in Table 1.

2.2. Possible covalent interactions in PGX®

Several analytical techniques were used to determine whether any covalent chemical changes had occurred in PGX® during processing. In this study, it was assumed that the mixture of the single polysaccharides prior to processing (TM1) was unchanged at the level of primary structure. TM1 was therefore used as a ‘standard’ for comparative purposes thus avoiding the need for a rigorous structural analysis. GCMS analysis of partially methylated alditol acetates was used to reveal the monosaccharide components of the neutral polysaccharide component in PGX® and their positions of linkage in comparison with those in TM1 (Björndal, Lindberg, & Svensson, 1967). HPAEC-PAD and ^{1}H NMR spectroscopy were then used to compare not only the neutral sugars but also the uronic acids produced after partial hydrolysis of PGX® and TM1.

2.2.1. Sample preparation for GCMS analysis

Weighed amounts of single polysaccharides and proprietary mixtures (approximately 450 μg of each) were taken and a few drops of dimethyl sulphoxide added to each. The samples were permethylated using sodium hydroxide (NaOH)/methyl iodide (MeI) with the samples shaken and then sonicated a total of four times over a period of 2 h. The samples were purified by chloroform extraction, then hydrolysed with 2 M trifluoroacetic acid (TFA) for 2 h at 120 °C and reduced with sodium borodeuteride ($NaBD_4$) in 2 M NH_4OH for 2 h at room temperature. The borate produced on the decomposition of the borodeuteride was removed by 3 additions of a mixture of methanol in glacial acetic acid (90:10) followed

Table 2
Eluent gradient used in HPAEC column.

Eluent
A: water
B: 500 mM sodium acetate in 100 mM NaOH
C: 100 mM NaOH

Gradient

Time (min)	%B	%C
0	0	15.5
20	0	15.5
21	50	0
32	50	0
32.5	0	100
42	0	100
42.5	0	15.5
52	0	15.5

by lyophilisation. The samples were then acetylated using acetic anhydride (1 h at 100 °C) and purified by extraction into chloroform.

2.2.2. *GCMS analyses*

The partially methylated alditol acetates (PMAAs) were separated and identified by GCMS. Authentic standards of the PMAAs of all the expected monosaccharides were also analysed for comparison. GC separation was performed with a DB5 column, on-column injection at 45 °C and a temperature programme of 1 min at 40 °C, then 25 °C min^{-1} to 100 °C, then 8 °C min^{-1} to 290 °C and finally holding 290 °C for 5 min. MS identification was performed with an ionisation voltage of 70 eV in scanning mode over a mass range of 50–620 Da with unit resolution.

2.2.3. *Partial hydrolysis conditions*

Samples of single polysaccharides and proprietary mixtures for HPAEC-PAD analysis were partially hydrolysed with 2 M TFA at 100 °C for 1 h in sealed tubes and the TFA was then removed by lyophilisation. For NMR analysis, samples of single polysaccharides (excluding konjac glucomannan) and proprietary mixtures were hydrolysed with TFA for extended times of 4 h and 24 h.

2.2.4. *Solution preparation for HPAEC-PAD analysis*

Polysaccharide hydrolysates were dissolved in deionised distilled water (30 mg ml^{-1}) and diluted to 0.0225 mg ml^{-1} with deionised distilled water for analysis. Standard solutions of each of the expected hydrolysate components from the three polysaccharides, *viz.* glucose and mannose (from konjac glucomannan and xanthan gum), glucuronic acid (from xanthan gum) and mannuronic and guluronic acids (from sodium alginate), were similarly prepared. However, in the case of guluronic acid, the quantity of material available was very limited and the concentration analysed was <0.0225 mg ml^{-1}.

2.2.5. *HPAEC-PAD analyses*

Aliquots (10 μl) of hydrolysate and standard solutions were injected onto a Dionex CarboPac PA1 (250 mm × 4 mm) column with guard column (50 mm × 4 mm) at 30 °C. The column was eluted with a solvent gradient (Table 2) formed with A: deionised filtered water, B: 50 mM sodium acetate (anhydrous, ≥99.5%) in 100 mM NaOH (HPLC Electrochem grade) and C: 100 mM NaOH at a flow rate of 1 ml min^{-1}. Run times were 52 min.

2.2.6. *Solution preparation for ^{1}H NMR analysis*

Polysaccharide hydrolysates were dissolved in D_2O (30 mg in 1 ml) and lyophilised before redissolving in D_2O and placing in NMR tubes. Standard solutions of the two expected monosaccharides (glucose and mannose) and two of the three expected uronic acids (glucuronic and mannuronic acids) were similarly prepared. In the case of the third uronic acid (guluronic acid), available quantities were very limited and the concentration prepared was <0.5 mg in 1 ml of D_2O.

2.2.7. *^{1}H NMR spectroscopy*

NMR spectra were acquired on hydrolysate and standard solutions at 298.1 K with a Brüker 400 MHz Avance III spectrometer with auto tune broadband multinuclear probe and variable temperature accessory running Brüker Topsin software. Sixteen scans were run on the majority of samples except for guluronic acid which, because of its low concentration, was given 256 scans.

2.3. *Non-covalent macromolecular interactions*

The presence of non-covalent macromolecular interactions between the three polysaccharides in PGX® in solution was investigated by three techniques. Since binary interactions between konjac glucomannan and xanthan gum were expected from previous studies, some experiments were also included to specifically probe any participation of the third polysaccharide, sodium alginate, in possible ternary interactions. Studies began with measurements to reveal synergistic effects in the flow behaviour of aqueous solutions of proprietary mixtures by comparison with single polysaccharide controls. The participation of sodium alginate was then specifically probed using flow behaviour and sedimentation coefficient measurements on ternary mixtures of variable composition and finally by observations of any precipitation of calcium alginate from solutions of PGX®, TM1 and binary blends of sodium alginate with either konjac glucomannan or xanthan gum in response to the addition of Ca^{2+} ions.

2.3.1. *Solution preparation of single polysaccharides and proprietary mixtures for flow behaviour measurements*

Single polysaccharides and proprietary mixtures were studied in solution in deionised distilled water. Accurately weighed samples were dispersed at concentrations of 0.1, 0.2 and 0.5 g in 100 g of water (0.1%, 0.2% and 0.5%, respectively) as received and allowed to hydrate for 2 h with stirring. Solutions were sheared with a high speed mixer for 1 min to fully dissolve all the particulates and finally stirred for a further 1 h.

2.3.2. *Solution preparation of ternary mixtures for flow behaviour and sedimentation coefficient measurements*

Single polysaccharide powders were mixed together in the laboratory in a range of compositions as received varying the sodium alginate content in a range from 0% to 33% whilst maintaining, in the remainder, the same constant high konjac glucomannan to xanthan gum weight ratio as in PGX®. Aqueous solutions of each composition were prepared at a single concentration of 0.5% as received (0.5 g in 100 g of water); one subset of solutions was unheated; a second was given a heat treatment in full, sealed Sterilin containers for 1 h at 90 °C; a third small subset of solutions was given an extended heat treatment (4 h). Unheated solutions were identified as A0 to A33 according to the alginate content and heated solutions as either A0H1 to A33H1 or A0H4 to A33H4 depending on the duration of the heat treatment.

2.3.3. *Flow behaviour measurements*

Solution flow behaviour was measured with a Bohlin Gemini Rheometer using C14 DIN 53019 concentric cylinder measuring system at 25.0 ± 0.1 °C. Steady state shear rates were measured at a series of constant applied shear stresses ascending from 0.1 Pa to 10 Pa. Flow behaviour was characterised initially using flow curves of log viscosity vs log shear rate.

2.3.4. Measurement of the distribution of sedimentation coefficients

The technique of sedimentation velocity in the analytical ultracentrifuge was used as the probe for the interaction studies. This free-solution method has the advantage over other methods as it does not need columns, membrane materials, other separation media or immobilisation (Harding, 1992b, 2005a) which might otherwise disrupt or interfere with interaction phenomena. Sedimentation velocities were measured with a Beckman XL-I ultracentrifuge equipped with Rayleigh interference optics. Data were captured using a CCD camera system. Initial scans were made at a low rotor speed of 3000 rpm to confirm the elimination of very high molecular weight particulates before adjustment to a rotor speed of 45,000 rpm. Sedimentation coefficients were corrected to the standard conditions of the density and viscosity of water at 20.0 °C (Schachman, 1959). Scans were taken at 2 min intervals for a run time of ~12 h. Data were analysed in terms of distributions of sedimentation coefficient $g(s)$ vs s (see e.g. Harding, 2005b) using the "least squares $g(s)$" SEDFIT algorithm (Dam & Schuck, 2003) based on the finite-element analysis method of Claverie, Dreux, and Cohen (1975). Analysis of the change in sedimentation coefficient distributions was used to ascertain the presence of an interaction. A total loading concentration 0.5% (0.5 g in 100 g of water) was used.

2.3.5. Precipitation behaviour with Ca^{2+} ions

Aliquots of solutions of TM1 and PGX® in deionised distilled water at 0.5% (0.5 g in 100 g water) were diluted to 0.1% (w/w), 0.05% (w/w) and 0.01% (w/w). At each concentration, 5 ml of 10% $CaCl_2 \cdot 2H_2O$ solution were added and thoroughly mixed into the solution to achieve a Ca^{2+} ion concentration of 0.5%. The same addition of Ca^{2+} ions was also made to:

1. a parallel series of control solutions of sodium alginate alone containing the same alginate concentrations as those of the TM1 and PGX® solutions;
2. solutions of binary mixtures of either konjac glucomannan or xanthan gum with sodium alginate containing the same sodium alginate concentrations (measured in g in 100 g water) and the same relative proportions of the two polysaccharides as in the 0.5% solutions of PGX® and TM1.

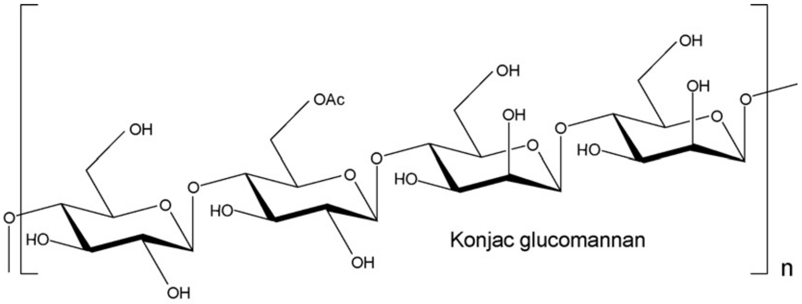
The solutions were allowed to stand for 30 min before visual inspection for the presence/absence of a precipitate.

3. Results and discussion

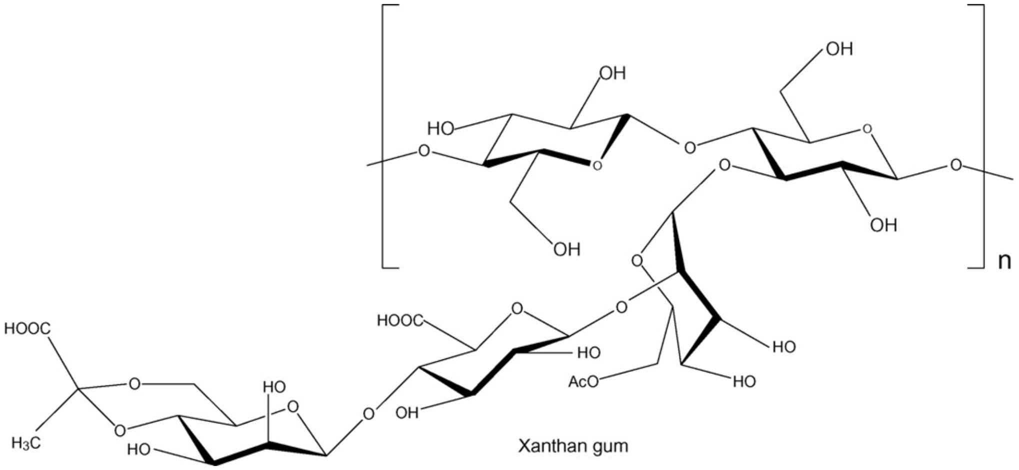
3.1. Possible covalent interactions in PGX®

3.1.1. Primary structures of single polysaccharides

Konjac glucomannan is a partially acetylated (1,4)-β-D-glucomannan obtained from the tubers of *Amorphophallus konjac* or Konnyaku root (Bewley & Reid, 1985; Dea et al., 1977).



Xanthan gum is a microbial polysaccharide produced by *Xanthomonas campestris*. It has unique rheological and gel forming properties and finds many applications particularly in the food and oil industries. The structure of xanthan is based on a cellulosic backbone of β-(1,4)-linked glucose units which have a trisaccharide side chain of mannose–glucuronic acid–mannose linked to every second glucose unit in the main chain. Some terminal mannose units are pyruvylated and some of the inner mannose units are acetylated (Andrew, 1977).

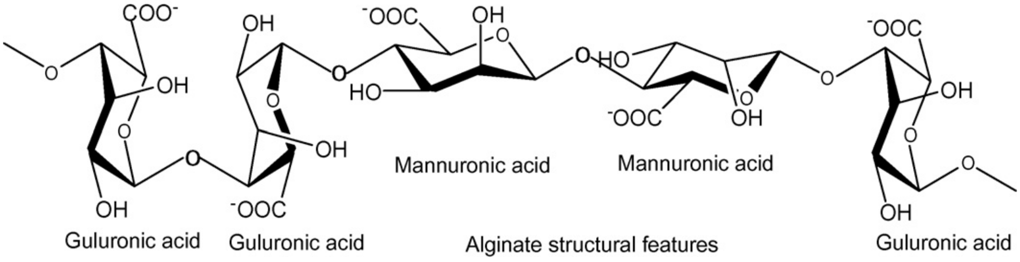


Sodium alginate is a salt of alginic acid, a polysaccharide obtained from the brown seaweeds (e.g. *Laminaria hyperborea*, *Fucus vesiculosus*, *Ascophyllum nodosum*). The chemical structure consists of blocks of (1,4) linked-β-D-polymannuronic acid (poly M), (1,4) linked-α-L-polyguluronic acid (poly G) and alternating

Table 3
Retention times (in min) of PMAAs corresponding to the **s**ugars and linkages in various sample lots identified by GCMS (tr: trace, ND: not detected).

Polysaccharide samples	Lot no.	Terminal mannose or glucose	2-Linked mannose	4-Linked mannose	4-Linked glucose	3,4-Linked glucose
Konjac glucomannan	2538	12.52	ND	13.78	13.86	ND
Xanthan gum	2504	12.54	13.68	ND	13.85	14.65
Sodium alginate	2455	ND	ND	13.78	ND	ND
TM1	900285	ND	13.65 tr	13.77	13.85	14.65
	1112050809	12.47	13.62 tr	13.74	13.83	14.63 tr
PGX®	2029070523	12.51	13.66 tr	13.78	13.86	14.65
	2029070523	12.48	ND	13.74	13.83	14.62 tr

blocks of the two uronic acids (poly MG) (Grasdalen, Larsen, & Smidsrød, 1981). The main use for alginate is in textile printing where it is used as a thickener in the printing of cottons with reactive dyes. It is also used as a thickener in the food industry (Clare, 1993). Alginates form strong gels with divalent metal cations and the 'egg box' model has been used to describe this form of gelation (Grant, Morris, Rees, Smith, & Thom, 1973).



3.1.2. *GCMS analyses*

The GCMS analyses of the PMAAs of konjac glucomannan and xanthan gum demonstrated the presence of the characteristic sugars and linkages expected from their known primary structures. Konjac glucomannan gave GC peaks corresponding to 4-linked glucose, 4-linked mannose and terminal glucose and/or mannose (mainly from side chains). Xanthan gum gave strong peaks corresponding to terminal mannose and/or glucose (from side chains) and 4-linked glucose (in the main chain) plus a peak for 3,4-linked glucose and a weak peak for 2-linked mannose (both from side chains). Virtually all of these peaks were also detected in the GCMS analyses of the PMAAs of TM1 and PGX® (Table 3) showing that they both contain konjac glucomannan and xanthan gum. The trace peak eluting at the position of 2-linked mannose from the xanthan gum component was too weak to assign categorically from the mass spectrum but the signals at retention times of about 12.47 and 14.65 min were consistent with the terminal mannose and the 3,4-linked glucose, respectively, of xanthan gum. Crucially, these analyses did not reveal any additional unexpected sugars or sugar linkages in TM1 or PGX® that might have emanated from other component biopolymers or from any new sugars or sugar linkages that might possibly have formed during processing. As expected, these analyses were unable to identify sodium alginate and xanthan gum uronic acid components.

3.1.3. *HPAEC-PAD analyses*

Table 4 shows the measured retention times of the standards comprising the expected hydrolysate components of TM1 and PGX®. The quantity of guluronic acid available was too low to provide a meaningful chromatogram but its retention time was ascertained from the chromatogram of the two hydrolysate components of sodium alginate having previously determined the retention time of mannuronic acid. Chromatograms of the single polysaccharide hydrolysates each contained only peaks for the expected components. Chromatograms for hydrolysates of TM1 and PGX® (Fig. 1a and b) clearly showed four of the possible hydrolysate components (glucose, mannose, glucuronic acid and mannuronic acid) but, probably due to the relatively low sodium alginate content of the proprietary mixtures, the fifth, guluronic acid, was not detected. No unexpected hydrolysate components were detected. These results supported those from GCMS analyses of PMAAs in identifying chemically unchanged konjac glucomannan and xanthan gum and the presence of mannuronic acid suggested the additional presence of chemically unchanged sodium alginate.

3.1.4. *^{1}H NMR spectroscopy*

The ^{1}H NMR spectra for the monosaccharide and uronic acid standards were well resolved and their characteristic chemical shifts were found in both the hydrolysates of xanthan gum and sodium alginate (Fig. 2a and b) and in the hydrolysates of TM1 and PGX® (Fig. 2c). The chemical shifts observed for glucose and mannose from xanthan gum could be resolved into anomeric resonances (4.6–5.2 ppm) and into sugar ring resonances (3–4 ppm) but those for mannuronic and guluronic acids from sodium alginate were closer together between 3.6 and 5.2 ppm. The uronic acid resonances found in TM1 and PGX® hydrolysates were clearly a combination of those found in hydrolysates of xanthan gum and sodium alginate adding further support to the presence of chemically unchanged sodium alginate in PGX®.

3.2. *Non-covalent macromolecular interactions*

3.2.1. *Flow behaviour of individual polysaccharides and proprietary mixtures*

Solutions of the individual polysaccharides in water show quite distinct differences in their flow behaviour with respect

Table 4
Retention times of standards used in HPAEC.

Standard	Retention time (min)
Glucose	13.98
Mannose	15.22
Guluronic acid[a]	25.13
Glucuronic acid	25.58
Mannuronic acid	25.75

[a] Ascertained from the hydrolysate of sodium alginate assuming that the significant peak occurring before that for mannuronic acid was for guluronic acid.

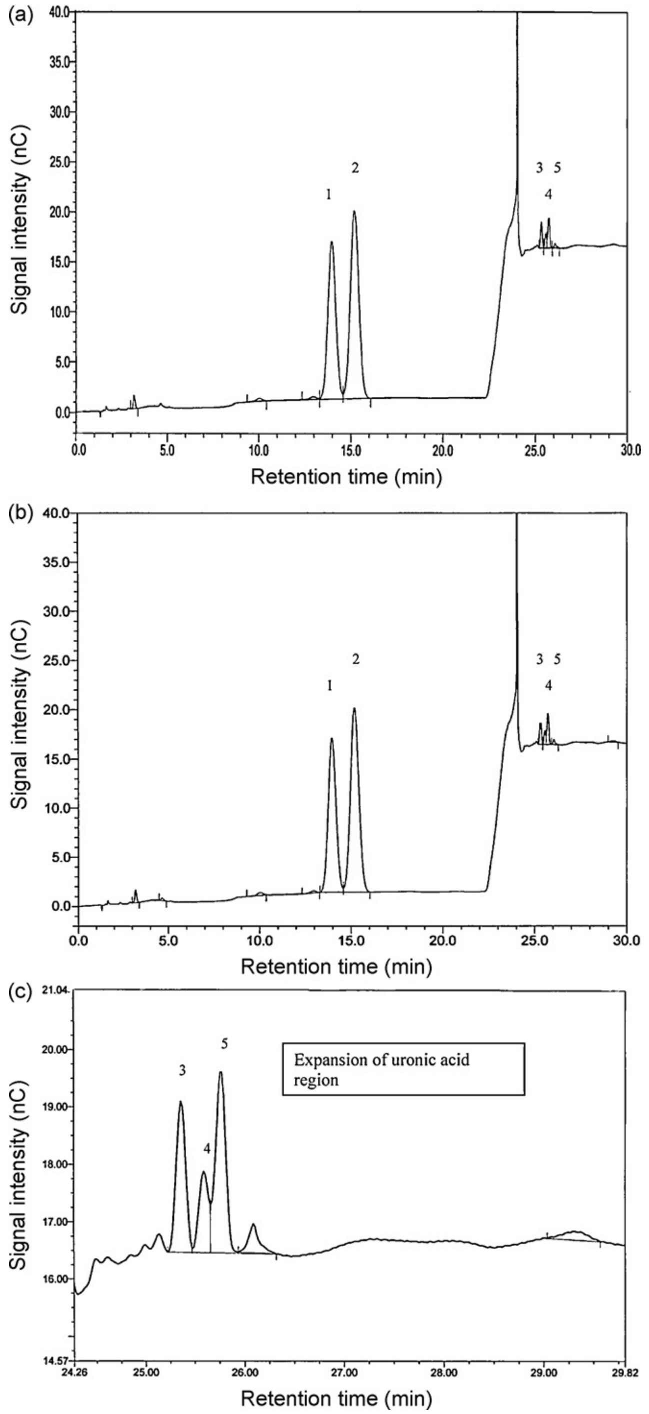


Fig. 1. HPAEC-PAD chromatograms of hydrolysates of (a) TM1 and (b) PGX®: the two profiles are indistinguishable. (c) Expansion of the uronic acid region. Retention time values: glucose (peak 1) = 13.98 min; mannose (peak 2) = 15.20 min; glucuronic acid (peak 4) = 25.68 min: mannuronic acid (peak 5) = 25.75 min. The peak labelled 3 (=25.35 min) is assumed to be guluronic acid.

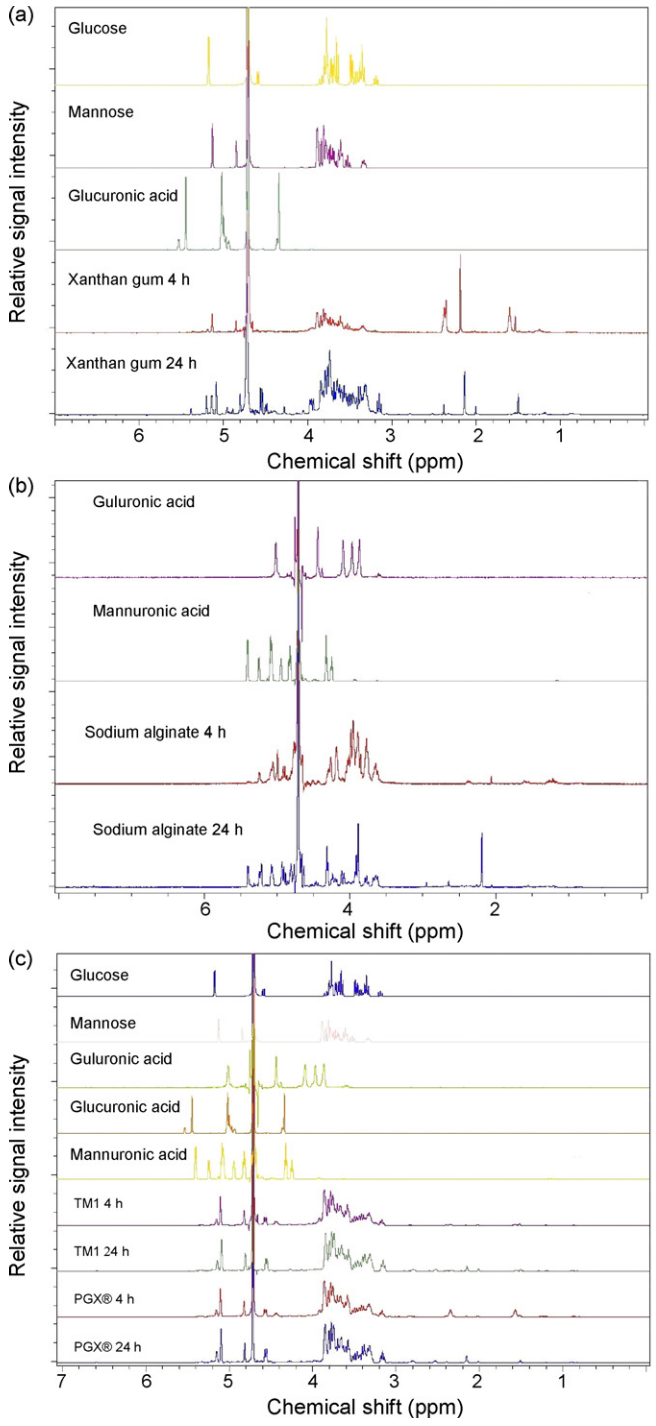


Fig. 2. ^{1}H NMR spectra of hydrolysates of (a) xanthan gum, (b) sodium alginate and (c) TM1 compared with PGX®.

to concentration (Fig. 3a–c). Little difference was seen in flow behaviour between solutions of different lots of the same single polysaccharide. Xanthan gum was clearly the most powerful viscosifying and shear thinning agent, followed by konjac glucomannan and finally sodium alginate. Xanthan gum solutions also had the most extensive shear thinning regions across many decades of shear rate where the logarithmic plots were linear. These were fitted to a power-law model between viscosity η and shear rate D:

$$\eta = KD^{n-1}$$

where K is the consistency index and n is the flow behaviour index derived from the intercept and slope, respectively, of the log–log plot. The K value indicates the overall consistency and n indicates the deviation from Newtonian behaviour ($n = 1$); the smaller the n value the greater the degree of shear thinning.

Solutions of the two lots of TM1 and PGX® in water were all found to be similar in flow behaviour. The flow curves of these proprietary mixtures were closest to those of xanthan gum; they

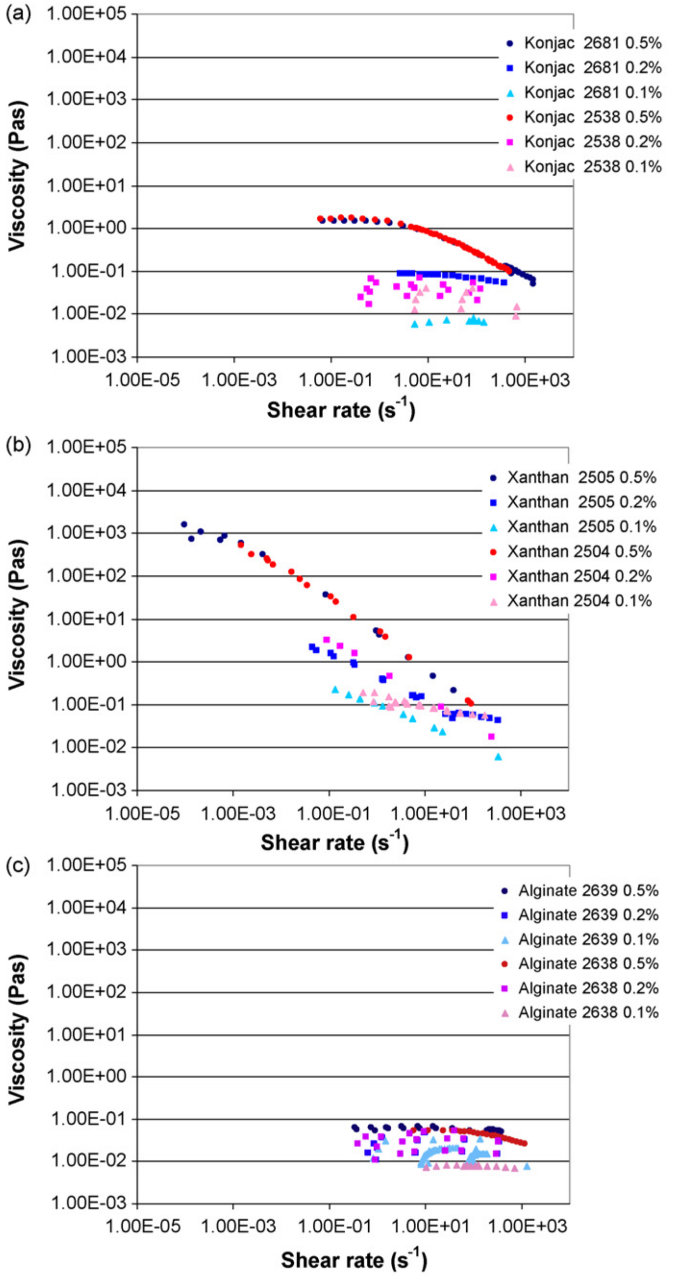


Fig. 3. Flow curves of aqueous solutions of (a) konjac glucomannan (2 lots), (b) xanthan gum (2 lots) and (c) sodium alginate (2 lots) at concentrations of 0.5%, 0.2% and 0.1%.

all conformed to the power law and showed extensive shear thinning behaviour but the magnitude of the viscosities and the degree of shear thinning at each concentration were actually higher than those for xanthan gum alone. These results are clearly shown by the similarity of power law *K* and *n* values for the TM1 and PGX® solutions and the difference in *K* and *n* values between solutions of these proprietary mixtures and those of xanthan gum (Fig. 4a and b); the higher *K* values corresponding to greater viscosities and the lower *n* values corresponding to greater degrees of shear thinning over the concentration range. Given that konjac glucomannan is the predominant polysaccharide in the proprietary ternary mixtures, and xanthan gum and sodium alginate are both minor components, the flow behaviour of TM1 and PGX® solutions provides a clear indication of an interaction between at least two of the polysaccharides in these solutions.

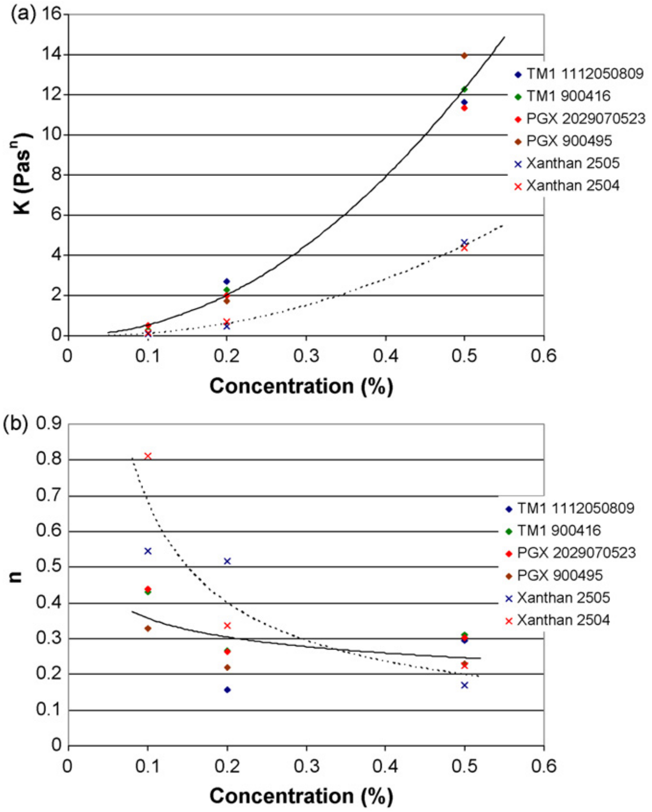


Fig. 4. Relationship between power-law parameters and concentration of aqueous solutions of TM1 (2 lots), PGX® (2 lots) and xanthan gum (2 lots). The curves show (a) consistency index *K* and (b) flow behaviour index *n*.

3.2.2. *Flow behaviour of ternary mixtures*

The flow curves for solutions of the laboratory-prepared ternary mixtures which had not been heat-treated conformed to the power law and principally showed a decrease in viscosity with increase in the content of sodium alginate. However, the power law *K* value showed a small initial increase but this was followed by a major decrease with increase in sodium alginate content (Fig. 5a). There appeared to be a maximum *K* value at about 3–5% sodium alginate. The decline in *K* and increase in *n* values above about 5% sodium alginate shown in Fig. 5a and b would be expected when the two more powerful viscosifiers (konjac glucomannan and xanthan gum) were being replaced by a weaker, less shear thinning viscosifier (sodium alginate). Clearly, there is still an interaction between the konjac glucomannan and the xanthan gum, as demonstrated by the flow behaviour of the binary mixture solution at zero alginate (A0) but any interactive effect there may be involving sodium alginate was only sufficient to overcome the effect of dilution of the other two polysaccharides in the ternary mixture up to a sodium alginate content of about 6%. At higher sodium alginate contents, any interactive effects on *K* value were outweighed by dilution.

Similar data for the solutions that had been heat-treated for 1 h (Fig. 5a) indicated an initial decline in *K* value due to heat treatment at sodium alginate contents below about 5% but, at 11% and above, the decline in *K* value was much less steep than that for unheated solutions (Fig. 5a) and here the rise in *K* value caused by heat treating the solutions became increasingly positive with increase in sodium alginate content. A maximum in *K* value also occurred in heat-treated solutions but at the slightly higher sodium alginate content of 8–11% in the ternary mixture. The maximum *K* value was similar to that found in the unheated solutions. The *n* value of heat-treated solutions remained low and unchanged across

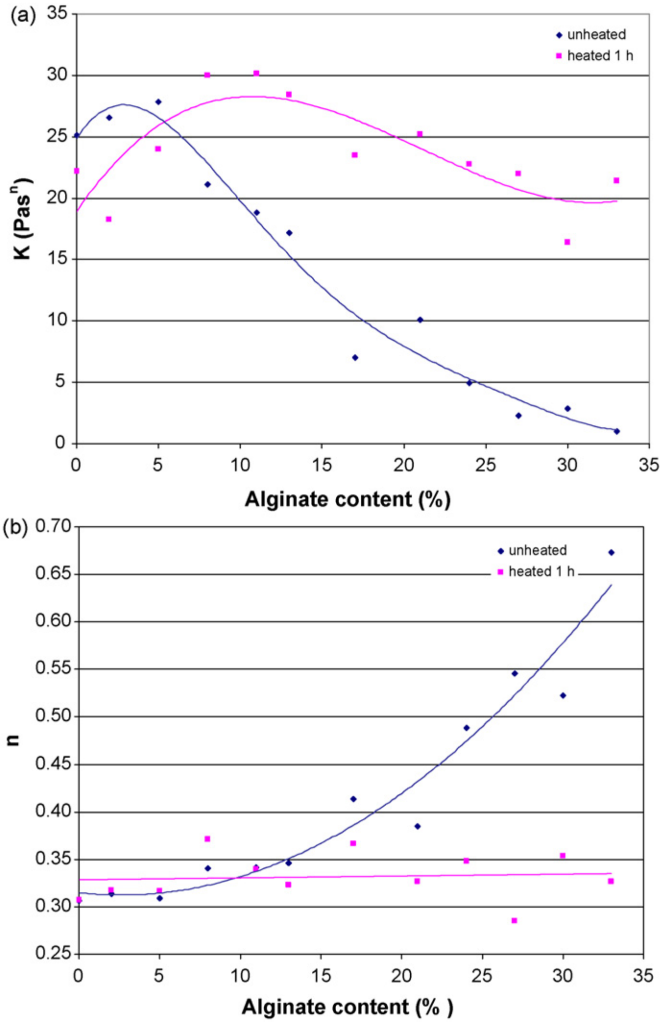


Fig. 5. Relationship between power-law parameters and the sodium alginate content of ternary powder mixtures in aqueous solutions at a concentration of 0.5%. Ternary powder mixtures were composed of konjac glucomannan and xanthan gum mixed in a constant high, proprietary ratio plus a varying amount of sodium alginate. The curves show (a) consistency index *K* and (b) flow behaviour index *n*.

the range of sodium alginate contents (Fig. 5b). For the limited number of solutions heated for 4 h, the *K* and *n* values were similar to those obtained for the same solutions heated for only 1 h.

The heat treatment conditions imposed in the laboratory were designed to be more severe than those involved in the commercial EnviroSimplex® process in order to evaluate if they could change complex formation effects on viscosity. The data indicate that the laboratory heat treatment of the solution of freshly mixed powders did significantly increase the overall level of macromolecular interactions. Since the *K* value for solutions of powder mixtures containing between 0% and about 5% sodium alginate actually declined after heat treatment, this higher level of interactions was unlikely to be due to an enhancement of the interaction between konjac glucomannan and xanthan gum. It would appear that the laboratory heat treatment process had enhanced the interaction of sodium alginate with one or both of the other two polysaccharides.

3.2.3. Distribution of sedimentation coefficients of ternary mixtures

Sedimentation coefficient distributions for solutions of unheated mixtures and heat-treated mixtures are shown in Fig. 6. In the absence of sodium alginate, no significant interaction product was observed in the solution containing the binary mixture of konjac glucomannan and xanthan gum either before (A0) or after heat treatment for 1 h or 4 h (A0H1 and A0H4), showing a sedimentation coefficient distribution essentially that of the konjac glucomannan control (see Abdelhameed et al., 2010). The situation is different in the presence of sodium alginate. The unheated ternary mixtures showed some interaction for a sodium alginate content of 13, 17, 21 and possibly 24% from the appearance of higher sedimentation coefficient material but no significant effects were observed above a sodium alginate concentration of 27%. For the heat-treated samples, complexes were observed above a sodium alginate concentration of ~8%, consistent with the rheological measurements. Some of the higher sodium alginate content samples that had been heat-treated for 1 h A21H1 (21% sodium alginate mixture heated for 1 h), A24H1, A27H1, A30H1 and all 4 h heat-treated samples apart from A0H4 (no alginate) had formed gels after the heat treating process and could not be analysed by the sedimentation velocity method. This implies the presence of interactions in the original solutions of sufficient strength to flip these into the gel state – by contrast the molecular interactions in the unheated samples were insufficient to promote such gelation phenomena.

Our observations based on analytical ultracentrifugation in solution are in broad agreement with the rheological experiments except in terms of one point of detail: unlike with the rheological experiments we failed to see clear evidence for an interaction in solution in the absence of sodium alginate. It is possible that there was a weak interaction which was disrupted by pressure effects at the higher rotor speeds employed (Schachman, 1959), effects which were insufficient to disrupt the interactions in the presence of sodium alginate for the mixtures specified.

3.2.4. Precipitation behaviour of PGX® with Ca^{2+} ions

Solutions of sodium alginate alone are known to react strongly and instantaneously with added Ca^{2+} ions to form either gels or precipitates depending on the mode of calcium addition (Clare, 1993; Haug & Smidsrød, 1965). Slow Ca^{2+} ion release, for example using insoluble calcium phosphate in conjunction with a slow-release acid such as glucono-δ-lactone, tends to form a gel but fast release, for example by calcium chloride addition, causes an instantaneous precipitate of calcium alginate. The polyguluronate segments of the alginate macromolecule are known to bind most strongly with Ca^{2+} ions (Kohn, Furda, Haug, & Smidsrød, 1968) but, if these segments were to become less accessible due to interactions with one or both of the other two polysaccharides, calcium alginate precipitation might be restricted.

Calcium alginate precipitates were not observed when Ca^{2+} ions were added to either TM1 or PGX® solutions ranging from 0.5% to 0.01%. Calcium alginate precipitates were formed in the corresponding sodium alginate solutions except for the most dilute solution which contained insufficient alginate to be precipitated by 0.5% Ca^{2+} ions. This finding implies that the polyguluronate segments of the alginate macromolecule that normally bind strongly with Ca^{2+} ions in solution were less available for such interaction in TM1 or PGX® solutions where sodium alginate was in the presence of konjac glucomannan and xanthan gum. This may be due to these segments of the macromolecule being less accessible to Ca^{2+} ions due to alternative interactions with one or both of the other two polysaccharides. Calcium alginate precipitates were observed when Ca^{2+} ions were added to the binary solutions of either konjac glucomannan or xanthan gum with sodium alginate which suggested that the sodium alginate requires the presence of both the other polysaccharides to interact and may only interact with an existing konjac glucomannan–xanthan gum complex.

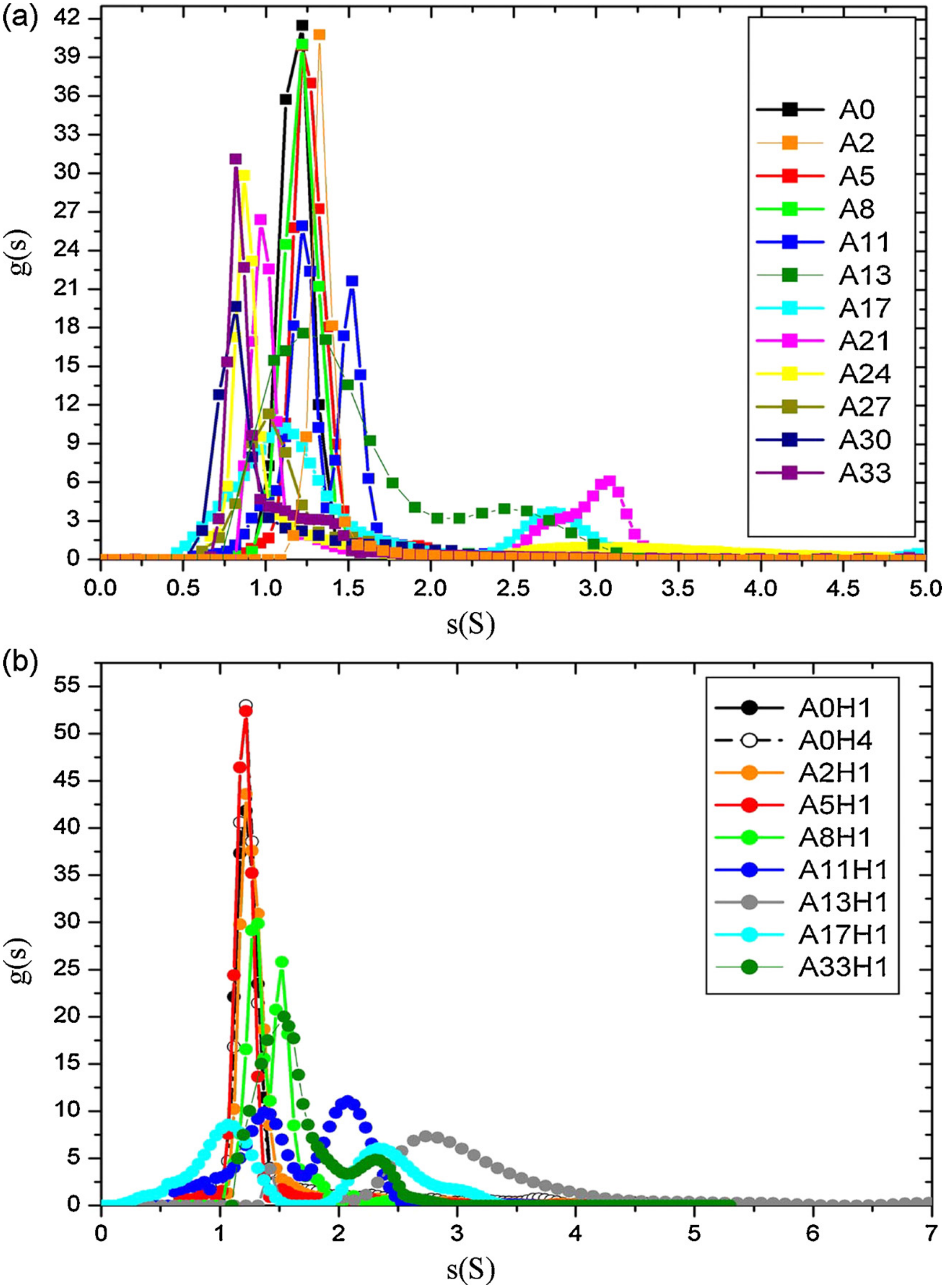


Fig. 6. (a) Sedimentation coefficient distribution profiles *g*(*s*) vs *s* for non-heat-treated mixtures of konjac glucomannan, xanthan gum and sodium alginate with a fixed ratio of konjac glucomannan:xanthan gum but increasing proportions of sodium alginate (0%, 2%, 5%, 8%, 11%, 13%, 17%, 21%, 24%, 27%, 30%, 33%). (b) Corresponding plot for mixtures that had been heat-treated for 1 h or 4 h. In (b) only 9 profiles have been shown because A21H1 (21% sodium alginate mixture heated for 1 h), A24H1, A27H1, A30H1 and all 4 h treated samples apart from A0H4 (no sodium alginate) had gelled after the heat treating process. Loading concentrations 0.5% in deionised distilled water.

4. Conclusions

Analytical studies are consistent with the absence of covalent interactions between polysaccharide macromolecules in a proprietary powder mixture of konjac glucomannan, xanthan gum and sodium alginate (although the methylation method used was not able to reveal any involvement from the uronic acids present) either before (TM1) or after a proprietary manufacturing process involving heat input (PGX®). However, rheological measurements confirmed that non-covalent interactions do occur in aqueous solutions of PGX®. Such synergistic interactions are already known for two of the components, konjac glucomannan and xanthan gum but a ternary interaction involving the third polysaccharide, sodium alginate, was suspected when addition of Ca^{2+} ions failed to precipitate calcium alginate from PGX® solutions. Further studies of the rheological and sedimentation behaviour of solutions of ternary mixtures containing variable alginate content prepared in the laboratory confirmed a ternary interaction and this was enhanced by heat treating the solutions particularly when the sodium alginate content of the mixture was greater than about 5%. Further Ca^{2+} ion addition experiments showed the presence of both the other two polysaccharides was required to prevent calcium alginate precipitation. The overall conclusion is that, in solution, sodium alginate is interacting with a konjac glucomannan–xanthan gum complex.

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