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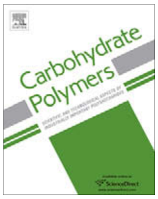

Short communication

An analytical ultracentrifuge study on ternary mixtures of konjac glucomannan supplemented with sodium alginate and xanthan gum

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ABSTRACT

Mixed solutions of konjac glucomannan supplemented with xanthan and alginate were studied using the technique of sedimentation velocity in the analytical ultracentrifuge. Interactions – as manifested by changes in the sedimentation coefficient distributions compared to unmixed controls – were observed for ternary mixtures in the presence of alginate. The complexes observed also appeared sensitive to the presence of the ionic concentration of the supporting solvent, but were clearly significant at low ionic strength.

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 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 interactions were occurring between the three components to account for this unexpected viscosity behaviour.

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, 1991a; Horton, Harding, Mitchell, & Morton-Holmes, 1991b; 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 interactions between xanthan and konjac glucomannan to form a strong thermoreversible gel network. These observations have been supported by dilute solution interaction studies using sedimentation velocity in the analytical ultracentrifuge on mixtures of the same molecules by Dhami (1996). He observed a very strong interaction in dispersions of xanthan and konjac glucomannan with xanthan as the dominant component but an interaction that was very sensitive to the ionic strength of the aqueous medium. In the present study, we investigate the properties of mixtures in which glucomannan is the dominant component, supplemented by xanthan and alginate.

2. Materials and methods

2.1. Polysaccharides

All the polysaccharides used in the study were supplied by InovoBiolgic Inc., (Calgary, Alberta, Canada) namely: konjac glucomannan, lot No. 2538; xanthan gum, lot No. 2504; and sodium alginate, lot No. 2455/2639. The polysaccharides were studied individually and as ternary mixtures comprising PGX®, a proprietary processed mixture of the three polysaccharides which had been heat treated (and granulated) and the same proprietary mixture of the same polysaccharides as for PGX® which remained untreated (TM1). PGX® and PolyGlycopleX® are both trade names belonging to InovoBiolgic Inc., Calgary, Alberta, Canada. Samples were dissolved in deionised distilled water and then dialysed into solutions of ionic strength 0.0001 M, 0.001 M, 0.01 M, 0.1 M and 0.2 M in phosphate–chloride buffer at pH ∼ 6.8 and made up according to Green (1933). Ionic strengths >0.05 M were supplemented by the addition of NaCl.

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2.2. Analytical ultracentrifugation

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; Harding, 2005a) which might otherwise disrupt or interfere with interaction phenomena. A Beckman XL-I ultracentrifuge was used 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 monitor for the presence of very high molecular weight particulates – this was not the case – before adjustment to a rotor speed of 45,000 rpm. Sedimentation coefficients *s* were corrected to standard conditions of the density and viscosity of water at 20.0 °C to yield $s_{20,w}$. 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 of 2.0 mg/ml was employed for the controls and mixtures.

3. Results and discussion

3.1. Integrity of the reactants

Konjac glucomannan, xanthan and alginate were first of all characterised separately by the analytical ultracentrifuge to establish their molecular integrity. Unimodal plots were seen in all cases for the apparent sedimentation coefficient distributions (Fig 1a–c). Under these conditions konjac glucomannan has an apparent weight average sedimentation coefficient $s_{20,w}$ of ~1.6S, alginate ~1.3S and xanthan ~3.5S, where $1S = 10^{-13}$ s.

3.2. Complex formation and the effect of added electrolyte

Sedimentation coefficient distribution plots were then generated for the ternary mixtures TM1 (unheated mixtures, Fig 2a and b) and PGX® (heated and granulated, Fig 2c and d) at the same total loading concentration used in the controls (2 mg/ml), up to a maximum of 10S. As our criterion for interaction, we estimate the amount of material with apparent sedimentation coefficients greater than that of the highest sedimenting species in the controls – xanthan: material sedimenting at >3.5S is regarded as an interaction product. Table 1 shows the clear increase for both the TM1 and PGX® mixtures, although there is still a considerable

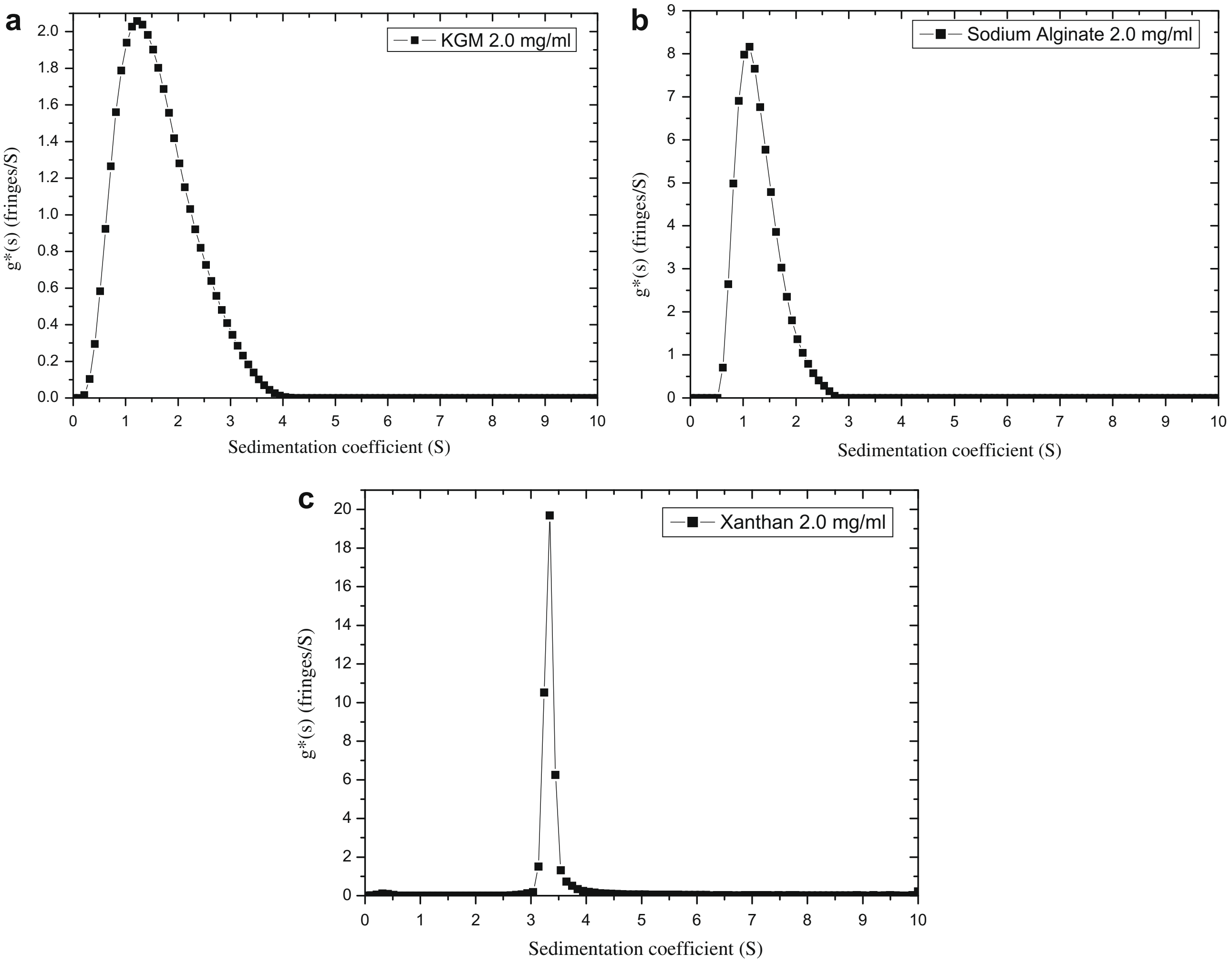

Fig. 1. Apparent sedimentation concentration distributions g*(s) vs *s* for (a) glucomannnan (b) sodium alginate and (c) xanthan at a loading concentration of 2 mg/ml and at *I* = 0.0. Rotor speed 45,000 rpm, temperature = 20.0 °C. The ordinate is expressed in fringe units per Svedberg (S) and the abscissa is in Svedberg units.

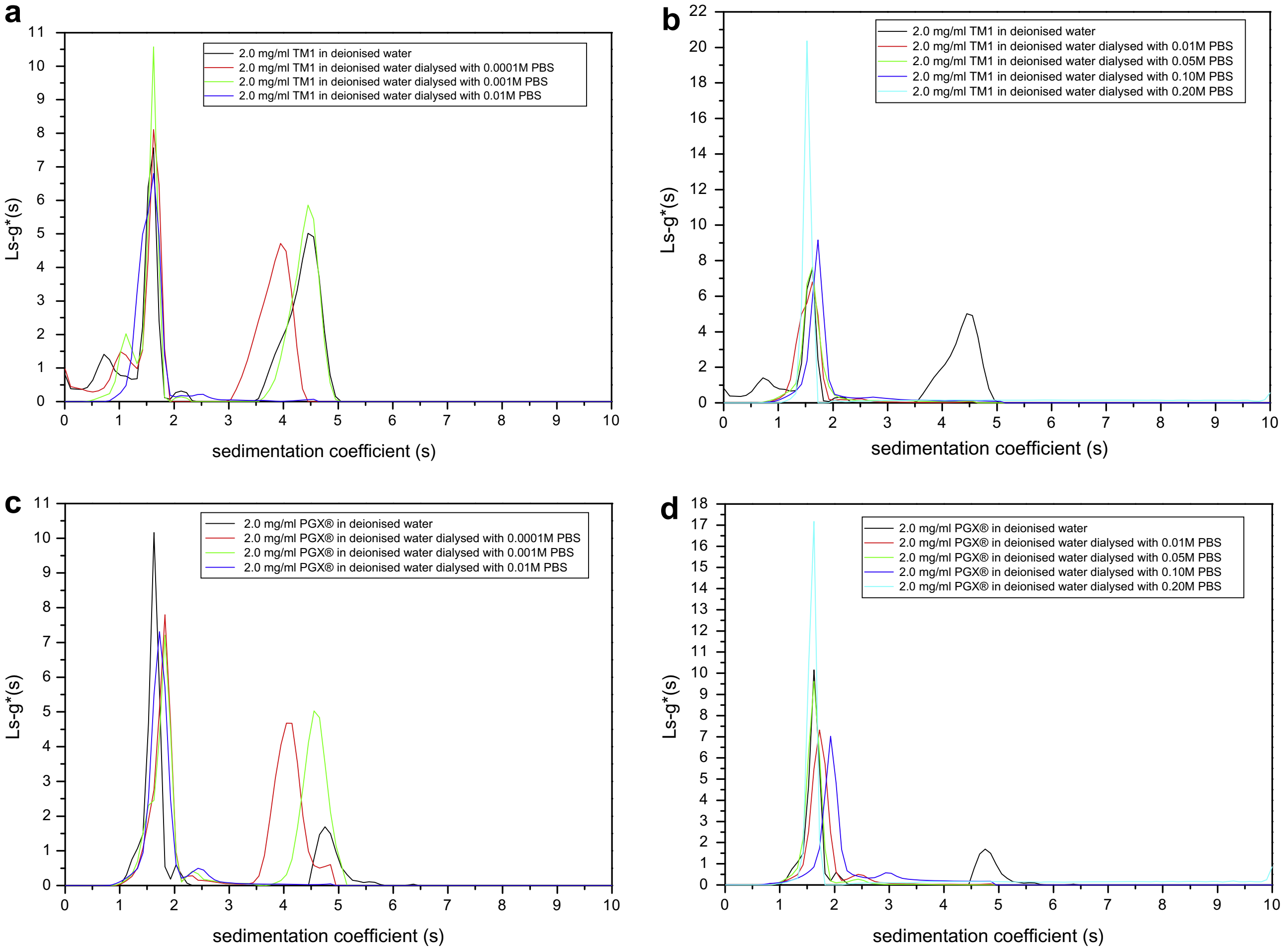

Fig. 2. Apparent sedimentation concentration distributions for (a) TM1 at ionic strengths 0–0.01 M (b) TM1 at ionic strengths 0–0.2 M (c) PGX® at ionic strengths 0–0.01 M (d) PGX® at ionic strengths 0–0.2 M. Rotor speed 45,000 rpm, temperature = 20.0 °C.

Table 1
Concentration of sedimenting material >3.5S. Ultracentrifuge cell loading concentration in each case was 2.0 mg/ml.

Sample	$c_{>3.5S}$ (fringe units)
Glucomannan	0
Alginate	0
Xanthan	0.1 ± 0.1
TM1	3.4 ± 0.1
PGX®	0.8 ± 0.1

Table 2
Effect of ionic strength on TM1. Ultracentrifuge cell loading concentration in each case was 2.0 mg/ml.

Ionic strength (M)	$c_{>3.5S}$ (fringe units)
0.0	3.4 ± 0.1
0.0001	3.2 ± 0.1
0.001	3.4 ± 0.1
0.01	0
0.05	0
0.1	0
0.2	0

proportion of unreacted material particularly at low sedimentation coefficients (~2S). Fig. 2 and Table 2 also show the effect of an increase in ionic strength on the appearance of the higher sedimenting material.

It can be seen that, for both mixtures, significant amounts of higher sedimenting material were observed up to an ionic strength of 0.01 M above which the appearance of such material was suppressed (Fig 3a and b) (Table 3).

4. Conclusions

Mixtures of glucomannan supplemented by xanthan and alginate show the presence of interaction products which are removed on the addition of moderate amounts of electrolyte. These observations are consistent with an interaction within the ternary mixtures which can be suppressed by inclusion of a supporting electrolyte beyond an ionic strength of 0.01 M. The interaction is not stoichiometric as there is a considerable proportion of material sedimenting at lower sedimentation coefficients (<3.5S under the conditions we have studied). Further study may reveal the specific nature of the interaction between the components, and in particular the role of alginate in these mixtures, and its relation to rheological conditions.

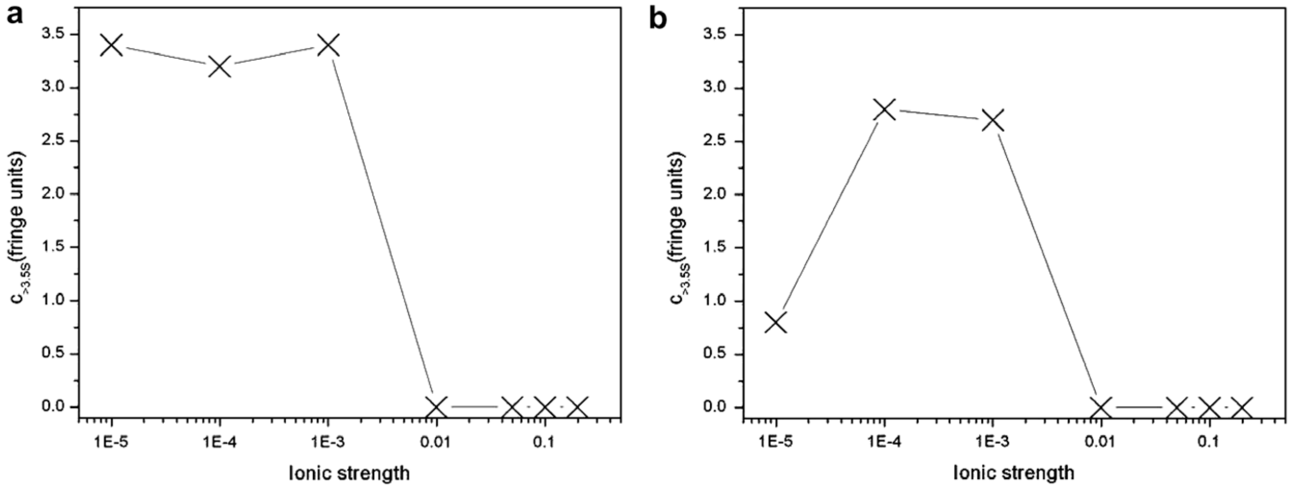

Fig. 3. Effect of ionic strength (expressed in molar concentration units M) on the amount of material with a sedimentation coefficient >3.5S for (a) TM1 (b) PGX®. To facilitate the logarithmic scale the $I = 0.00$ value is represented at $I = 0.00001$ M.

Table 3
Effect of ionic strength on PGX®. Ultracentrifuge cell loading concentration in each case was 2.0 mg/ml.

Ionic strength (M)	$c_{>3.5S}$ (fringe units)
0.0	0.8 ± 0.1
0.0001	2.8 ± 0.1
0.001	2.7 ± 0.1
0.01	0
0.05	0
0.1	0
0.2	0

References

Berth, G., Dautzenberg, H., Christensen, B. E., Harding, S. E., Rother, G., & Smidsrød, O. (1996). Static light scattering studies on xanthan in aqueous solutions. *Macromolecules, 29*, 3491–3498.

Claverie, J. M., Dreux, H., & Cohen, R. (1975). Sedimentation of generalized systems of interacting particles. I. Solution of systems of complete Lamm equations. *Biopolymers, 14*, 1685–1700.

Dam, J., & Schuck, P. (2003). Determination of sedimentation coefficient distributions by direct modeling of the sedimentation boundary with Lamm equation solutions. *Methods in Enzymology, 384*, 185–221.

Dhami, R. (1996). Interactions of xanthan with locust bean gum, konjac mannan and guar gum. In Dhami R. (Ed.). *Hydrodynamic studies on xanthan and xylan systems*. Ph.D. Thesis: University of Nottingham; UK [Chapter 8].

Dhami, R., Harding, S. E., Jones, T., & Hughes, T. (1995). Physico-chemical studies on a commercial food-grade xanthan – I. Characterisation by sedimentation velocity, sedimentation equilibrium and viscometry. *Carbohydrate Polymers, 27*, 93–99.

Green, A. A. (1933). The preparation of acetate and phosphate buffer solutions of known pH and ionic strength. *Journal of the American Chemical Society, 55*, 2331–2336.

Harding, S. E. (1992a). News and views: First advanced course on alginates and their applications. *Carbohydrate Polymers, 17*, 155.

Harding, S. E. (1992b). Sedimentation analysis of polysaccharides. In S. E. Harding, A. J. Rowe, & J. C. Horton (Eds.), *Analytical ultracentrifugation in biochemistry & polymer science* (pp. 495–516). Cambridge: Royal Society of Chemistry.

Harding, S. E. (2005a). Analysis of polysaccharides size, shape and interactions. In D. J. Scott, S. E. Harding, & A. J. Rowe (Eds.), *Analytical ultracentrifugation techniques and methods* (pp. 231–252). Cambridge: Royal Society of Chemistry.

Harding, S. E. (2005b). Challenges for the modern analytical ultracentrifuge analysis of polysaccharides. *Carbohydrate Research, 34*, 811–826.

Horton, J. C., Harding, S. E., & Mitchell, J. R. (1991a). Gel permeation chromatography – multi angle laser light scattering characterization of the molecular mass distribution of "Pronova" sodium alginate. *Biochemical Society Transactions, 19*, 510–511.

Horton, J. C., Harding, S. E., Mitchell, J. R., & Morton-Holmes, D. F. (1991b). Thermodynamic non-ideality of dilute solutions of sodium alginate studied by sedimentation equilibrium ultracentrifugation. *Food Hydrocolloids, 5*, 125–127.

Kelly, R., Gudo, E. S., Mitchell, J. R., & Harding, S. E. (1994). Some observations on the nature of heated mixtures of bovine serum albumin with an alginate and a pectin. *Carbohydrate Polymers, 23*, 115–120.

Kök, M. S., Abdelhameed, A. S., Ang, S., Morris, G. A., & Harding, S. E. (2009). A novel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan. *Food Hydrocolloids, 23*, 1910–1917.

Mannion, R. O., Melia, C. D., Launay, B., Cuvelier, G., Hill, S. E., Harding, S. E., et al. (1992). Xanthan/locust bean gum interactions at room temperature. *Carbohydrate Polymers, 19*, 91–97.

Morris, G. A., Li, P., Puaud, M., Liu, Z., Mitchell, J. R., & Harding, S. E. (2001). Hydrodynamic characterisation of the exopolysaccharide from the halophilic cyanobacterium *Aphanothece halophytica* GR02: A comparison with xanthan. *Carbohydrate Polymers, 44*, 261–268.

Shatwell, K. P., Sutherland, I. W., Ross-Murphy, S. B., & Dea, I. C. M. (1991). Influence of the acetyl substituent on the interaction of xanthan with plant polysaccharides-III. Xanthan–konjac mannan systems. *Carbohydrate Polymers, 14*, 131–147.