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Dilute Solution Viscometry Studies on a Therapeutic Mixture of Non-digestible Carbohydrates

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Abstract: Recent work has shown the beneficial effects of a proprietary mixture of three non-digestible carbohydrates: konjac glucomannan, xanthan and alginate and these effects have been linked with a synergistic interaction observable with analytical ultracentrifugation, rheological and NMR measurements. These observations have been supported by fundamental dilute solution viscosity studies. Preparations of konjac glucomannan, xanthan and alginate have been checked with regards their molecular integrity (molar mass distribution) using a newly established method based on the analytical ultracentrifuge. The intrinsic viscosity behaviour for each of the individual polysaccharides were estimated at low ionic strength I (10^{-3} M) and found to be (2090 ± 120) ml/g, (4430 ± 340) ml/g and (3460 ± 330) ml/g for konjac glucomannan, xanthan and alginate respectively and at $(10^{-1}$ M) (2350 ± 200) ml/g, (3370 ± 310) ml/g and (1210 ± 50) ml/g respectively. The intrinsic viscosity $[\eta]$ was then determined for a proprietary mixture of the three (known as "PGX[®]") at both ionic strengths and compared with the predicted values for a non-interacting mixture. In $I=10^{-3}$ M solvent a significant difference was observed (3090 ± 250) ml/g compared with the predicted value (2350 ± 300) ml/g, although at higher ionic strength the interaction appears to have gone: $[\eta] = (1990 \pm 250)$ ml/g compared with the predicted value of (2180 ± 300) ml/g. This appears to reinforce the earlier observations that in PGX[®] there is a synergistic interaction which is ionic strength sensitive.

Keywords: Konjac glucomannan, alginate, xanthan, synergistic interaction, intrinsic viscosity, PGX[®].

INTRODUCTION

There is growing interest in the use of combinations of non-digestible carbohydrate or "NDC's" – also referred to as "dietary fibre" – in the development of functional food materials particularly in their use in satiety based products. Obesity is now a major problem in many countries and the need to address this is acute: dietary or satiety products can help. One particular proprietary product used for food product supplementation, namely 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[®]) is one such product. PGX[®] and PolyGlycopleX[®] are both trade names belonging to InovoBiologic Inc., Calgary, Alberta, Canada. PGX[®] is produced from a mixture of proprietary proportions of powders 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 higher than expected absolute

viscosities inspired a recent investigation to explore 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 behaviour and interactions appeared to be observed based on sedimentation velocity in the analytical ultracentrifuge [1,2]. We now seek to explore if precision dilute solution intrinsic viscosity measurements on these solutions reinforce the earlier observations.

The hydrodynamic properties of glucomannans [3], xanthan [4-7] and alginates [8-11] are now well understood. It has also been inferred from rheological studies that mixtures of polysaccharides in concentrated or gel like systems can interact synergistically. Shatwell *et al.* (1991), for example, have shown significant non-covalent interactions between xanthan gum and konjac glucomannan to form a strong thermoreversible gel network [12]. These observations have been supported by dilute solution interaction studies using sedimentation velocity in the analytical ultracentrifuge by Dhama on mixtures of the same molecules [13]. He observed a strong interaction in dispersions of xanthan gum and konjac

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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 [1], we observed changes in the sedimentation velocity behaviour of PGX[®] compared with unmixed controls of each single component polysaccharide in the analytical ultracentrifuge. Combination with nuclear magnetic resonance (NMR) and rheological measurements [2] showed that the interactions which give a ternary complex were clearly non-covalent and were found to be sensitive to the ionic strength of the aqueous supporting solvent and were clearly significant at low ionic strength.

We explore these observations further by examining fundamental dilute solution viscosity characteristics of the PGX[®] in comparison with the individual polysaccharides. The intrinsic viscosity is a sensitive function of conformation, volume (including any swelling or expansion due to interaction with surrounding solvent) and for non-spheroidal particles, to molar mass [14]. After checking the molecular integrity (molar mass distribution) of individual preparations of konjac glucomannan, xanthan and alginate using a newly established method based on the analytical ultracentrifuge [1], the intrinsic viscosity behaviour for each of the individual polysaccharides were measured at low ionic strength I (10^{-3} M) and higher I (10^{-1} M). The intrinsic viscosity $[\eta]$ was then determined for PGX[®] at both ionic strengths and compared with the predicted values for a non-interacting mixture.

MATERIAL AND METHODS

Preparation of Buffer Solutions

Phosphate-chloride buffer solutions (PBS) were prepared by dissolving 9.19g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 3.122g KH_2PO_4 and 5.846g NaCl in 2 litres of deionised distilled water at pH=7.0 and ionic strength 0.1M according to Green [16], with appropriate dilution for 10^{-3} M.

Polysaccharides

All the polysaccharides used in the study were supplied by InovoBiologic Inc, (Calgary, Alberta, Canada) and were as previously described in [2]. The konjac glucomannan was lot No. 2538; xanthan gum, lot No. 2504 and sodium alginate, lot No. 2455/2639. The PolyGlycopleX[®] (PGX[®]) mixtures of polysaccharides contained konjac glucomannan,

xanthan gum and sodium alginate in a proprietary ratio, heat treated and granulated. The samples were dissolved in deionised distilled water then dialysed into buffer solution at pH=7.0 and ionic strength I (10^{-1} M) or I (10^{-3} M). Concentrations were measured (after dialysis) using an Atago (Fairfax, Canada) DD-5 refractometer calibrated with glucose standards.

Sedimentation Velocity in the Analytical Ultracentrifuge

The molecular integrity and polydispersity of the polysaccharide solutions were probed by using sedimentation velocity in the analytical ultracentrifuge [17-18] using a Beckman instruments (Palo Alto, California, U.S.A.) Optima XL-I ultracentrifuge. Polysaccharide samples (~400 μ l) at 0.2 mg/ml concentration and phosphate buffer dialysate (400 μ l) at pH 7.0 at either $I = 10^{-1}$ M or $I = 10^{-3}$ M were injected into the sample and reference channels respectively of double-sector 12 mm optical path length cells. The Rayleigh interference optical system was used for recording concentration profiles and the movement of the sedimentation boundary in the analytical ultracentrifuge cell [19]. An initial low rotor speed of 3000 rpm was used to monitor for the sedimentation of any supramolecular materials and then adjusted to a rotor speed of 45000 rpm. Scans were taken at 2 min intervals for a run time of ~ 24 hours. The standard conditions of density and viscosity of water at 20.0^o C were used for normalization of the sedimentation coefficients s [20]. The data was analysed using the "least squares $g(s)$ model" SEDFIT algorithm in terms of distributions of sedimentation coefficient distribution $g(s)$ vs s [19-21] to provide an assessment of sample polydispersity. Analysis of the change in sedimentation coefficient distributions was used to ascertain the presence of an interaction. Apparent molecular weight distributions (at $c=0.2$ mg/ml) were evaluated from the $g(s)$ vs s distributions using the Extended Fujita approach [15].

Capillary Viscometry

The relative viscosities η_r of a series of polysaccharide solutions ranging in concentration (c), from 0.2-1.0 mg/ml were measured from the ratio of flow times of solution to solvent using a 2ml Ostwald viscometer. The U-tube viscometer was suspended in an accurate temperature regulated water bath. The temperature was kept constant at $(20.0 \pm 0.01)^\circ\text{C}$ throughout by using a coolant system. Because of the low concentrations no correction for solution density

was necessary [14]. The reduced viscosity η_{red} ($=\eta_r-1)/c$ and inherent ($\ln(\eta_r)/c$) viscosities were then extrapolated to zero concentration using the relations of Huggins (Eq. 1) and Kraemer (Eq. 2), respectively [22-23].

$$\eta_{sp}/c = [\eta] (1 + K_H [\eta] c) \quad (1)$$

$$\ln(\eta_{rel})/c = [\eta] (1 - K_K [\eta] c) \quad (2)$$

where the intrinsic viscosity $[\eta]$ is taken as the mean of intercepts from Eqs. (1) and (2) and K_H and K_K are the Huggins and Kraemer constants, respectively. To avoid possible ambiguities through transition from the dilute to the semi-dilute region $[\eta]$ was also estimated from the Solomon-Ciuta (1961) relation [24]:

$$[\eta] \approx (1/c) (2\eta_{sp} - 2\ln(\eta_{rel}))^{0.5} \quad (3)$$

at a concentration $c=0.2\text{mg/ml}$

RESULTS AND DISCUSSION

To assess the homogeneity of the preparations apparent sedimentation coefficients ($s_{20,w}$) and

sedimentation coefficient distributions were obtained for all samples at one concentration (0.2 mg/ml) using the least squares $g^*(s)$ distribution method.

Figure 1 shows the apparent sedimentation coefficient distribution or $g^*(s)$ vs s profiles for the three polysaccharides samples at different ionic strengths $I=10^{-3}\text{M}$ and $I=10^{-1}\text{M}$. Note that as the ionic strength is increased non-ideality effects are suppressed through charge shielding (with the exception of konjac glucomannan which is uncharged). The corresponding weight average $s_{20,w}$ values are shown in Table 1 (for $I=10^{-1}\text{M}$). If the conformation type of the molecule is approximately known then it is possible to obtain an estimate for the apparent molecular weight distribution and an apparent weight average molecular weight [15], based on the power law relation:

$$s = \kappa_b M^b \quad (4)$$

Figure 2 shows the corresponding apparent molecular weight distributions. The apparent weight average molecular weights, $M_{w,app}$ and polydispersity ratios ($M_{z,app}/M_{w,app}$) corresponding to these

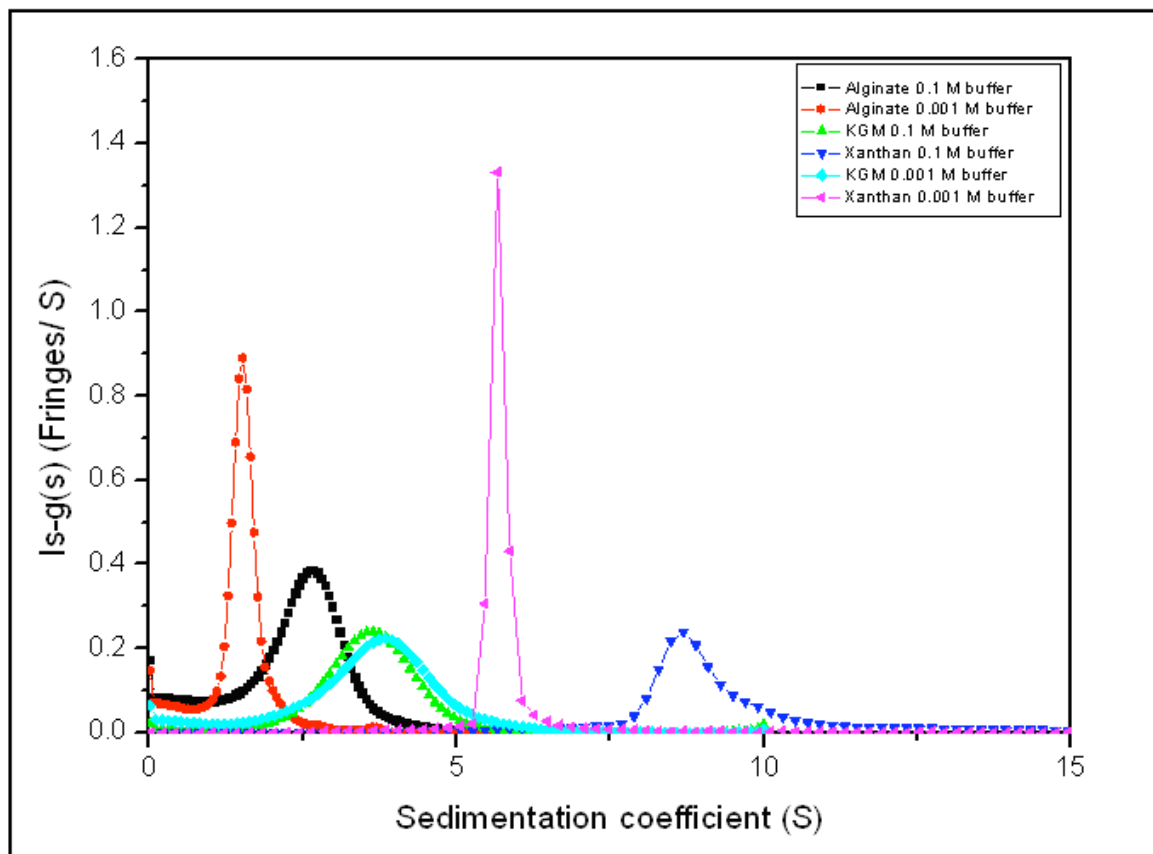


Figure 1: Apparent sedimentation coefficient distribution $g(s)$ vs s profiles (at $c=0.2\text{mg/ml}$) for konjac glucomannan, xanthan and alginate at $I=10^{-3}\text{M}$ and $I=10^{-1}\text{M}$. Note that as the ionic strength is increased non-ideality effects are suppressed through charge shielding (with the exception of konjac glucomannan which is uncharged).

Table 1: Hydrodynamic Properties of Konjac Glucomannan, Xanthan and Alginate. Phosphate chloride Buffer I=10⁻¹M, pH =7.0.

Sample	^a $s_{20,w}$ (S)	^b $M_{w,app}$ (g/mol)	^c Polydispersity
konjac glucomannan	3.52±0.08	840,000± 70,000	1.5±0.2
xanthan	9.26±0.02	2,300,000± 200,000	2.3±0.5
alginate	2.56±0.01	140,000± 10,000	1.6±0.2

a: apparent sedimentation coefficient at 0.2mg/ml; b: apparent weight average molecular weight at 0.2mg/ml; c: $M_{z,app}/M_{w,app}$.

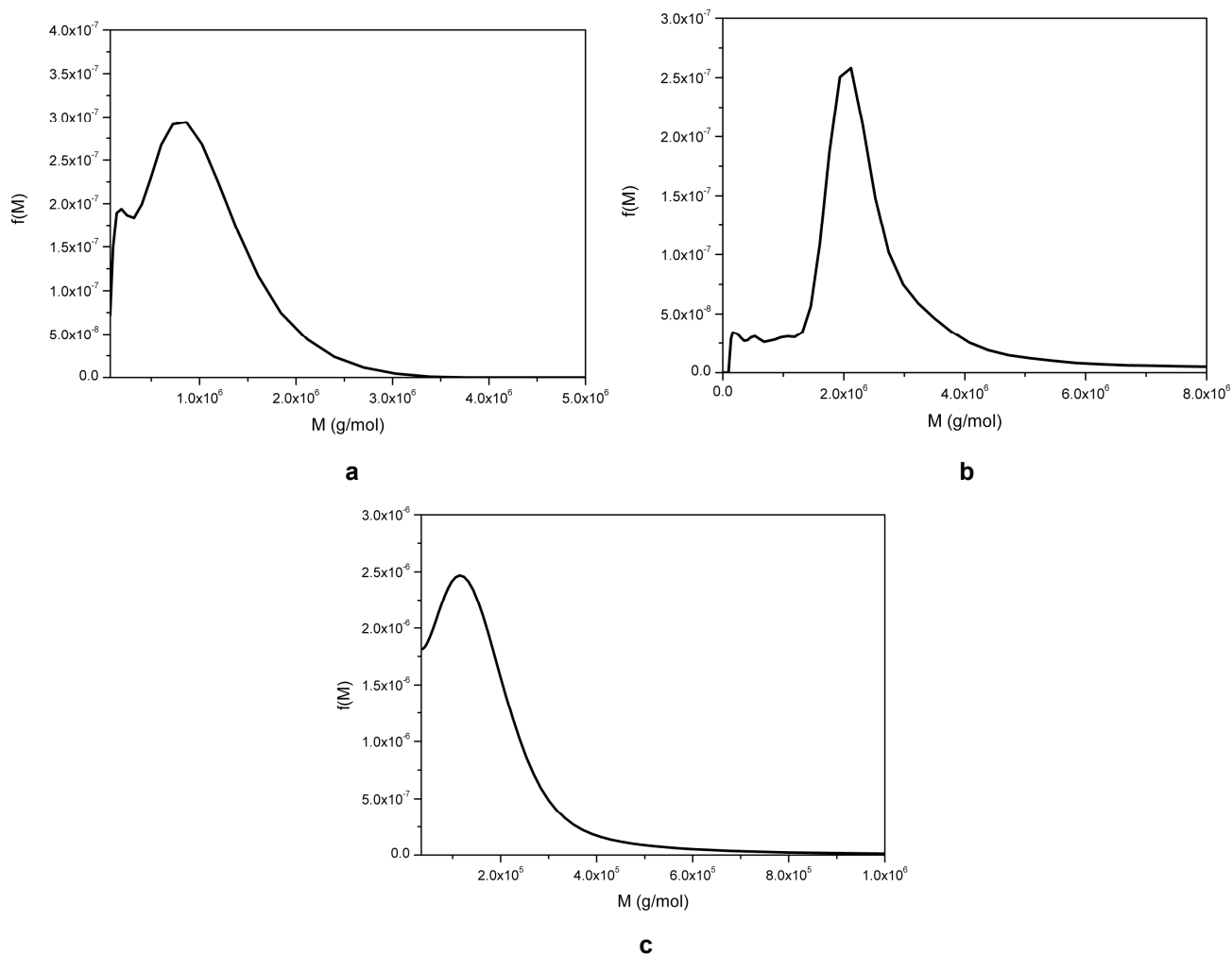
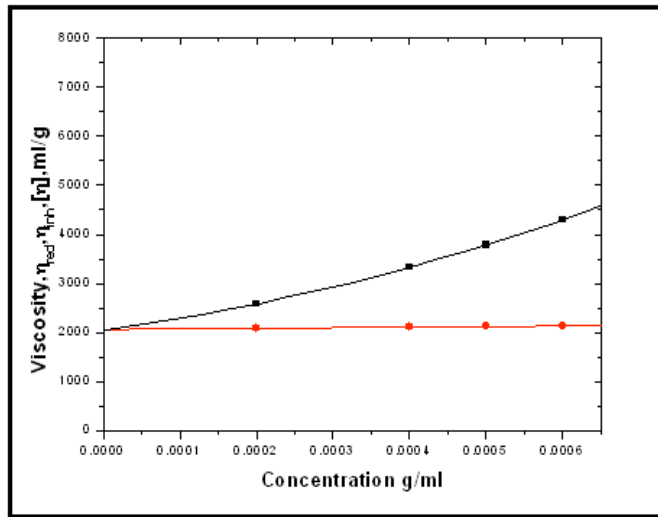


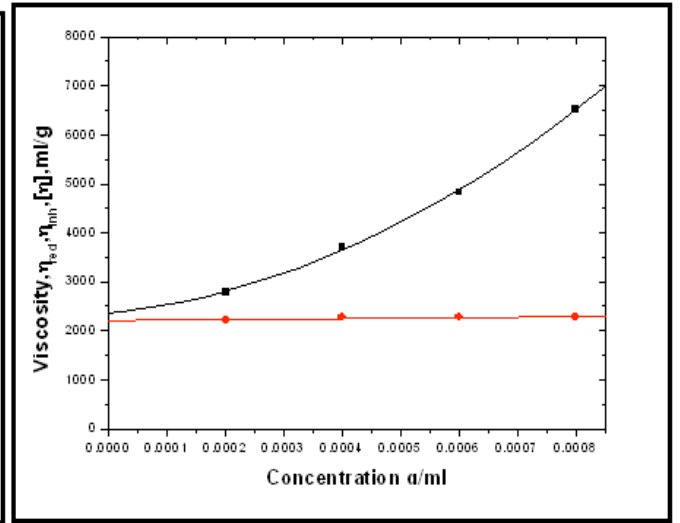
Figure 2: Apparent molecular weight (molar mass) distributions (at $c=0.2\text{mg/ml}$) for (a) konjac glucomannan (b) xanthan and (c) alginate using the *Extended Fujita* method [15]. The following values for the power law conversion parameters were used. For (a): $\kappa_s = 0.044$, $b = 0.32$; (b): $\kappa_s = 0.197$, $b = 0.26$; (c): $\kappa_s = 0.052$, $b = 0.33$. Values for b and κ_s were obtained from ref [15].

distributions are provided in Table 1. Even at such a low concentration (0.2mg/ml) the effects of non-ideality may still be significant so these values and also those of $s_{20,w}$ given in Table 1 are apparent ones. The $s_{20,w}$ and $M_{w,app}$ values for xanthan approximately correspond with those values that would be expected at this concentration from the study on keltrol xanthan by Dhama *et al.* [5].

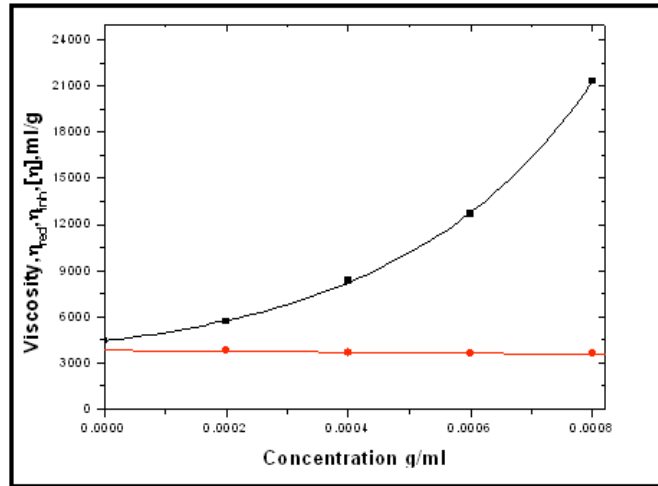
Intrinsic viscosity values resulting from the Huggins and Kraemer extrapolation methods for each of the samples (at both 10⁻³M and 10⁻¹M) investigated (Figures 3a-f) are reported in Table 2. Within the error estimates there is a good agreement between the intrinsic viscosity results obtained from Huggins extrapolation compared with Kraemer extrapolation (for both ionic strengths) using capillary viscometry. Clear



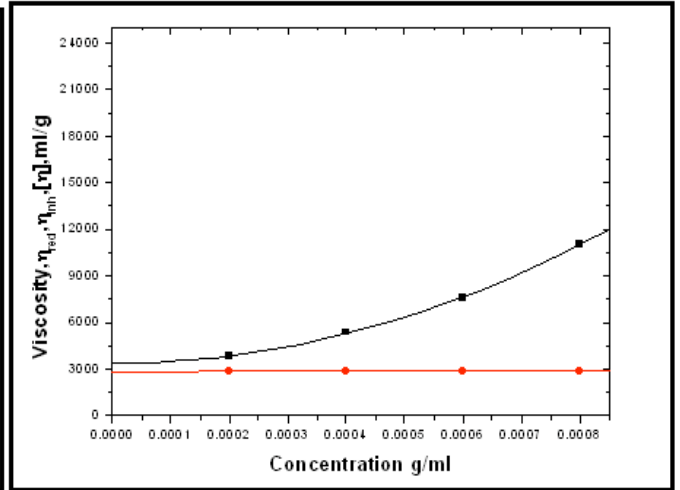
a



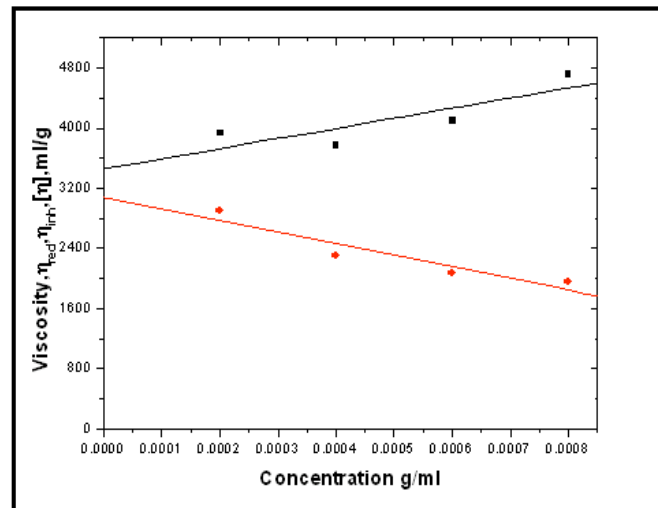
b



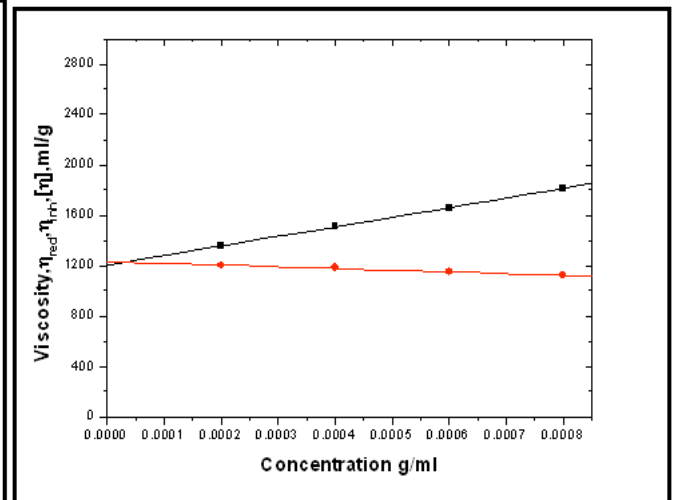
c



d



e



f

Figure 3. continued.....

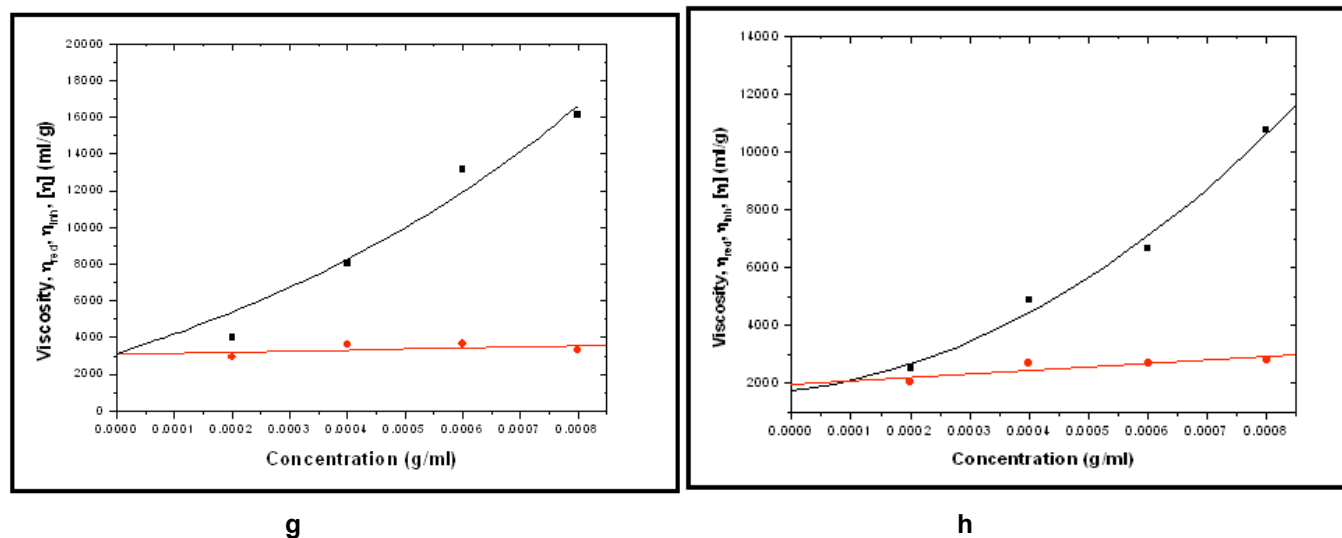


Figure 3: Intrinsic viscosity evaluations for (a) konjac glucomannan, $I=10^{-3}M$, (b) konjac glucomannan, $I=10^{-1}M$; (c) xanthan, $I=10^{-3}M$, (d) xanthan, $I=10^{-1}M$; (e) alginate, $I=10^{-3}M$, (f) alginate, $I=10^{-1}M$; (g) PGX, $I=10^{-3}M$, (h) PGX, $I=10^{-1}M$.

differences between the results for the two ionic strengths were seen for xanthan and alginate, resulting from the lack of suppression of the primary charge effect at the lower ionic strength. By contrast good agreement was observed for the glucomannan – which is an uncharged polysaccharide. For the glucomannan and xanthan the Huggins plots were non-linear – this may indicate a transition from dilute to semi-dilute behaviour [14] – with some interchain coil overlap at the higher concentrations. For all cases in addition to the Huggins and Kraemer extrapolations the intrinsic viscosity was also estimated by the method of Solomon-Ciuta [24] – eqn. 3 – at the lowest concentration used (0.2mg/ml) – the results (also presented in Table 2) were close to the extrapolated values, reinforcing that data.

Intrinsic viscosity values for PGX[®] are also reported in Table 2 for the two ionic strengths (Figure 3g,h). Because of the significant non-linearity of the Huggins plots, the values given are the extrapolated values from

the Kraemer plot and the estimates from Solomon-Ciuta. Also included in Table 2 are the values expected based on a non-interacting mixture of konjac glucomannan, xanthan and alginate. This data shows that there is clearly an interaction at $I=10^{-3}M$ which disappears at $I=10^{-1}M$ showing that the interaction is electrostatic in nature. In $I=10^{-3}M$ solvent a significant difference was observed (3090±250) ml/g compared with (2350±50) ml/g, although at higher ionic strength the interaction appears to have gone: $[\eta] = (1990±250)$ ml/g compared with the predicted value of (2180±20) ml/g. Although some caution needs to be expressed as at $10^{-3}M$ there is not complete suppression of charge effects, by appropriate comparison of the controls done at the same ionic strengths the measured intrinsic viscosity for the complex does appear considerably larger than predicted based on the behaviour of the individual polysaccharides under otherwise identical solvent conditions. This appears to reinforce the earlier observations that in PGX[®] there is a synergistic interaction which is ionic strength sensitive.

Table 2: Intrinsic Viscosities of Konjac Glucomannan, Xanthan and Alginate and PGX

Sample	$[\eta]$ ($I=10^{-3}M$) (ml/g)	^a $[\eta]$ ($I=10^{-3}M$) (ml/g)	$[\eta]$ ($I=10^{-1}M$) (ml/g)	^a $[\eta]$ ($I=10^{-3}M$) (ml/g)
konjac glucomannan	2090±120	2230	2350±200	2390
xanthan	4430±340	4350	3370±310	3130
alginate	3460±330	3210	1210±50	1250
PGX	3090±250	3250	1990±250	2200
PGX (predicted if no interaction)	2350±300	2540	2180±300	2350

a: from Solomon-Ciuta estimation of $[\eta]$ at $c=0.2mg/ml$.

CONCLUDING REMARKS

The intrinsic viscosity studies reported here seem to reinforce the earlier studies based on analytical ultracentrifugation, nuclear magnetic resonance and rheological measurements [1-2]. There is clearly a non-covalent interaction which is sensitive to the ionic strength of the supporting solvent. We can make an approximate estimate of the increase in hydrodynamic volume caused by the complexation process, assuming there is no alteration in conformation.

$$[\eta] = \nu v_s \quad (5)$$

where v_s is the (swollen) specific volume (ml/g) and ν is the Einstein-Simha shape factor.

If we make the approximation there is no alteration in overall shape then $[\eta]$ varies approximately with v_s , which means on mixing at low ionic strength v_s seems to be ~30% larger than expected if there had been no interaction. Although this approximation is quite crude the difference in intrinsic viscosity does indicate there is a significant increase in macromolecular volume through solvent interaction – which is fully reversible if the ionic strength is increased.

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