

# Physico-chemical studies on a commercial food-grade xanthan — I. Characterisation by sedimentation velocity, sedimentation equilibrium and viscometry

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A popular commercially available xanthan polysaccharide 'Keltrol RD' has been characterised by sedimentation velocity (Schlieren optics), sedimentation equilibrium (Rayleigh interference optics), and viscometry with regard to: (i) homogeneity; (ii) molecular weight; (iii) gross conformation; and (iv) critical overlap concentration,  $c^*$ . Studies were conducted in phosphate chloride buffer (pH = 6.5,  $I = 0.30$ ). Preparations were shown to be homogeneous with a characteristic hypersharp Schlieren peak from sedimentation velocity. Despite the low  $c^*$  value ( $\sim 0.5$  mg/ml) it was still possible to obtain meaningful physical measurements in the dilute solution regime with a weight average molecular weight  $M_w = (5.9 \pm 1.1) \times 10^6$  g mol<sup>-1</sup>, the second thermodynamic ('osmotic pressure') virial coefficient of  $(3.4 \pm 0.3) \times 10^{-4}$  ml mol<sup>-2</sup>, a sedimentation coefficient  $s_{20,w}^0 = (12.97 \pm 0.26)$  S, a sedimentation concentration regression parameter  $k_s$  of  $(2127 \pm 85)$  ml/g and an intrinsic viscosity  $[\eta] = (7534 \pm 2717)$  ml/g. From these data it is also possible to estimate the two 'hydration independent' shape parameters  $\Pi$  and the Wales van Holde ratio  $k_s/[\eta]$ ; the values obtained ( $\sim 0.53 \pm 0.20$  and  $\sim 0.28 \pm 0.10$ , respectively) are consistent only with a stiff, elongated molecule of aspect ratio  $\sim 70:1$ .

## INTRODUCTION

The exopolysaccharide xanthan is manufactured by the bacterium *Xanthomonas campestris*. Xanthan is a high molar mass, double stranded anionic polysaccharide which forms solutions that are both highly viscous and very stable under conditions of extreme temperature and salinity. As a consequence of these properties and its ability to be manufactured cheaply in large quantities, xanthan has gained widespread use as a viscosifier in a number of different commercial applications ranging from a gelling agent and thickener in the food industry (Pettit, 1978), to a hydrophilic matrix for controlled release of drugs in the pharmaceutical industry (Waler *et al.*, 1992; Talukdar & Plaizier-Vercammen, 1993) and as a viscosifier for controlling the rheology of oil-well drilling and for enhancing well-bore

recovery in the oil industry (Holzwarth, 1976). For all these applications knowledge of its fundamental solution properties is critical to a proper understanding of its function. These fundamental solution properties include: (i) molecular weight and polydispersity; (ii) viscosity; (iii) sedimentation behaviour; (iv) gross conformation; and (v) critical overlap concentration (i.e. the concentration at which dilute solution behaviour ceases), and the extraction of this information using hydrodynamic techniques (see, e.g., Kawakami & Norisuye, 1991; Inatomi *et al.*, 1992), for a popular commercially available purified xanthan (Keltrol), is the subject of the present study. In the first paper of this series, application of a similar approach to the study of xanthan interactions in dilute solution with galacto- and glucomannans will be described.

Xanthan solutions have been the subject of several previously detailed physico-chemical investigations, mostly, but not exclusively, centred around light scat-

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tering procedures. Paradossi and Brant (1982) and Coviello *et al.* (1986) used static light scattering to examine the molecular weight and conformation of xanthan fractions. Others studies have involved the coupling of low-angle light scattering to gel filtration (Lambert *et al.*, 1982) or multi-angle laser light scattering coupled to gel filtration (Muller & Lecourtier, 1988). In a series of papers, Sato and colleagues (Sato *et al.*, 1984a, 1984b, 1984c; Sho *et al.*, 1986; Inatomi *et al.*, 1992) used a combination of intrinsic viscosity, sedimentation velocity, sedimentation equilibrium and static light scattering on a series of Keltrol xanthan fractions and from worm-like coil type treatments an extended rod conformation with large persistence length was found, which was almost independent of ionic strength,  $I$ , above  $I = 0.01 \text{ mol l}^{-1}$ . In a related study on (ultrasonically produced) fractions of Keltrol-xanthan, Kawakami and Norisuye (1991) used a combination of static light scattering and sedimentation equilibrium to investigate the effect of ionic strength on the second thermodynamic virial coefficient, and demonstrated the dependence was consistent with a rod conformation. This rod-concept for xanthan has been used for other applications (see Sasaki, 1992). Christensen and Smidsrød (1991) have produced fractions of Keltrol-xanthan by acid hydrolysis using intrinsic viscosity as a monitor for the state of depolymerisation and optical rotatory dispersion to monitor the state of the so-called 'order-disorder transition' (double stranded single stranded transition). These data have been further studied using Monte-Carlo simulation models (Stokke *et al.*, 1992; Christensen *et al.*, 1993).

In this present study we examine whole, unfractionated Keltrol-xanthan, to investigate whether the extended model is supported on data obtained without fractionating the sample, and to establish its unfractionated molecular weight, and its critical overlap concentration.

## MATERIALS AND METHODS

### Xanthan

The commercial food grade xanthan (Keltrol, grade 'RD' — namely 'readily dispersable') was kindly provided by Kelco International (London, UK). Absorption spectra showed it to be free of maxima at 256 nm or 278 nm and hence free of nucleotide and protein, respectively. An average moisture content of  $(14.0 \pm 0.5)\%$  was found by vacuum drying in an oven.

### Solutions

A phosphate chloride buffer was used ( $\text{pH} = 6.5$ ,  $I = 0.30$ ) with the following composition (Green, 1933): 4.595 g  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , 1.561 g  $\text{KH}_2\text{PO}_4$ , 14.615 g

$\text{NaCl}$  per litre of deionised distilled water. Polysaccharide solutions were prepared by allowing the polymer to hydrate in deionised distilled water overnight at  $4^\circ\text{C}$  with gentle stirring, followed by exhaustive dialysis into the buffer.

### Sedimentation velocity

This was used to obtain a measure of the physical homogeneity of the preparation, the sedimentation coefficient,  $s_{20,w}^0$ , and the sedimentation concentration regression parameter  $k_s$ . A Beckman Model E analytical ultracentrifuge was employed, using the photographic Schlieren system. 0.6 ml of solution was loaded into single sector, 30 mm path length cells. A rotor speed of 30 000 r.p.m. at a rotor temperature of  $20.0^\circ\text{C}$  was employed. The following method of semi-automatic data capture was employed. Photographs were taken at 16 min intervals, and negatives enlarged directly onto a graphics tablet interfaced to an IBM-PC. The resulting records of the position of the sedimenting boundaries could then be analysed using the QUICKBASIC algorithm MOD\_EVEL (H. Cölfen, pers. commun.) to yield the (apparent) sedimentation coefficient  $s_{T,b}$ , corresponding to a temperature  $T$ , solvent  $b$ , and a solution concentration  $c$  (after correction for radial dilution effects (Schachman, 1959, pp. 70–73)).  $s_{T,b}$  values were corrected to  $s_{20,w}$  values (where the subscripts 20,  $w$  respectively mean a temperature of  $20^\circ\text{C}$  with water as a solvent) using the formula (Tanford, 1961):

$$s_{20,w} = \frac{(1 - \bar{v}\rho_{20,w}) \eta_{T,b}}{(1 - \bar{v}\rho_{T,b}) \eta_{20,w}} \cdot s_{T,b}, \quad (1)$$

where  $\rho_{T,b}$  and  $\rho_{20,w}$  are the densities of the solution and water, respectively,  $\eta_{T,b}$  and  $\eta_{20,w}$  the viscosities of the solvent and water, and  $\bar{v}$  the partial specific volume ( $0.602 \pm 0.010$ ) ml/g; Anderson 1994). Apparent  $s_{20,w}$  values were measured at a series of concentrations and extrapolated to zero concentration to give the infinite dilution sedimentation coefficient  $s_{20,w}^0$  by fitting to the equation

$$1/s_{20,w} = (1/s_{20,w}^0) \cdot (1 + k_s c), \quad (2)$$

where  $k_s$  (ml/g) is the sedimentation concentration regression coefficient (Schachman, 1959, p. 91; Creeth & Knight, 1965).

### Viscometry

This was used to obtain the intrinsic viscosity,  $[\eta]$  (ml/g) and an estimate for the Huggins constant,  $K_\eta$  and an estimate for the critical overlap concentration ( $c^*$ ). Because of the previously reported serious dependence of the reduced viscosities on shear rates for xanthan (Milas *et al.*, 1985), a Bohlin CS (Lund, Sweden) rheometer, allowing measurements at several shear rates

and extrapolation to zero shear, as opposed to a capillary viscometer was used. Measurements were made at the following shear rates: 0.01–100 s<sup>-1</sup>, and in the concentration range 0.1–1.0 mg/ml.

The reduced viscosity,  $\eta_{red}$  data were fitted to the Huggins (1942) equation:

$$\eta_{red} = [\eta](1 + K_{\eta}[\eta]c), \quad (3)$$

where  $K_{\eta}$  is the Huggins (1942) constant.

### Sedimentation equilibrium

This was used to obtain estimates for the weight average molecular weight,  $M_w$  and the second thermodynamic (or 'osmotic pressure') virial coefficient  $B$  (or ' $A_2$ ').

For the  $M_w$  and  $B$  estimations, a Model E analytical ultracentrifuge was used similar to the one described above, except it had a dedicated Rayleigh interference optical system equipped with a 5 mW He-Ne laser light source. Double sector cells were used with optical path lengths of 30 mm, with the dialysate used in the reference channel in each case. Short solution columns (~2 mm) and low rotor speeds (3000 r.p.m.) were used, at a rotor temperature of 20.0 °C. As with other polysaccharides, because of the quasi-continuous polydispersity of the sample, the low-(or 'intermediate') speed (as opposed to the 'meniscus depletion') (Yphantis, 1964) method was followed to minimise loss of optical registration of the fringes at the cell base (Creeth & Harding, 1982). In this method the concentration at the meniscus remains finite and is found by mathematical manipulation of the fringe data (Creeth & Harding, 1982; Harding *et al.*, 1992).

Optical records of solute distributions at sedimentation equilibrium were recorded on photographic film and then captured automatically using an LKB Laser Densitometer using the TURBO-PASCAL routine ANALYSER (Harding & Rowe, 1987; Rowe, 1992) which gives an accurate record of  $j$ , the solute concentration (expressed in fringe numbers) relative to the meniscus as a function of radial position ( $r$ ) in the centrifuge cell. These data were then transferred to the FORTRAN routine MSTARI (Harding *et al.*, 1992) which calculates amongst other things the whole distribution weight average molecular weight  $M_{w,app}$  using a function known as  $M^*$  defined by

$$M^*(r) = \left\{ \frac{j(r)}{\left[ J(a)(r^2 - a^2) + 2 \int_a^r rj dr \right]} \right\} \left\{ \frac{2RT}{[\omega^2(1 - v\rho)]} \right\}, \quad (4)$$

where  $r$  is the radial position in the ultracentrifuge cell,  $a$  is the radial position at the cell meniscus,  $J(a)$  the solute concentration (expressed in fringe displacement numbers) at the cell meniscus,  $\omega$  the rotor speed, and  $\bar{v}$  the partial specific volume, (0.602 ± 0.010) ml/g; Anderson (1991): this value appears to be in good

agreement with values given elsewhere. For example, Dintzis *et al.* (1970) gave a value of 0.59 ml/g, and both values are in very good agreement with the value for the density increment  $\frac{\partial \rho}{\partial c}$  of 0.401 given by Sato *et al.* (1984b). The limiting value at the cell base of  $M^* = M_{w,app}$  (Creeth & Harding, 1982). This is only an apparent value because of the non-ideality of the xanthan. Estimates of the 'ideal' value,  $M_w$  were obtained by measuring  $M_{w,app}$  at a range of cell loading concentrations,  $c$  (g/ml) and either: (i) graphical extrapolation to zero concentration; (ii) fitting to (Tanford, 1961)

$$(1/M_{w,app}) = (1/M_w) + 2Bc, \quad (5)$$

the latter extrapolation also yielding an estimate for the second thermodynamic (or 'osmotic pressure') virial coefficient  $B$  (ml mol g<sup>-2</sup>).

## RESULTS AND DISCUSSION

A summary of the hydrodynamic properties discovered in this study is given in Table 1.

### Homogeneity

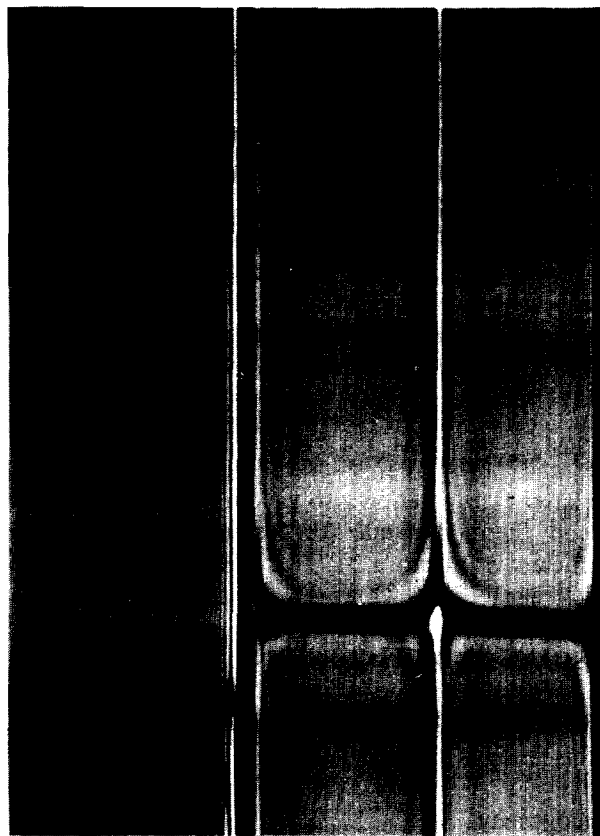
Only single (Schlieren) peaks were observed from the sedimentation velocity records (Fig. 1) supporting the view that the Keltrol-xanthan is homogeneous. The peaks are hypersharp, characteristic of a highly concentration dependent system: similar features have been observed for DNA (Peacocke & Schachman, 1954).

### Viscosity parameters

From the zero-shear reduced viscosity vs concentration plot (Fig. 2a) a clear discontinuity is seen in the region of ~0.5 mg/ml. Only those data points below this concentration were used in the linear fit (Fig. 2b) to estimate  $[\eta]$  via eqn (3), but a reasonable estimate was still possible ((7534 ± 2717) ml/g). Only an order of magnitude estimates for  $K_{\eta}$  (~2.5) is possible, but this is still consistent with a very large, non-ideal molecule.

**Table 1. Hydrodynamic (and thermodynamic) parameters for (unfractionated) Keltrol-xanthan**

Parameter	
$M_w$ (g/mol)	$(5.9 \pm 1.1) \times 10^6$
$B$ (ml <sup>2</sup> mol/g)	$(3.4 \pm 0.3) \times 10^{-4}$
$s_{20,w}^0$ (S)	$12.97 \pm 0.26$
$k_s$ (ml/g)	$2127 \pm 85$
$[\eta]$ (ml/g)	$7534 \pm 2717$
Derived shape parameters:	
$k_{s,0}[\eta]$	$0.28 \pm 0.10$
$\Pi (=2BM_w/[\eta])$	$0.53 \pm 0.21$



**Fig. 1.** Sedimentation velocity Schlieren diagram for unfractionated Keltrol xanthan. Rotor speed, 30 000 r.p.m.; temperature, 20.0 C; solvent pH, 6.5,  $I$ , 0.30. Note the single hypersharp boundary, indicative of (i) sample purity (ii) high thermodynamic non-ideality. Direction of sedimentation is from left to right.

Our value for  $[\eta]$  for Keltrol xanthan is in good agreement with previously reported values for xanthan preparations, which range from 3400 ml/g to 10 500 ml/g (Chauveteau & Lecourtier, 1982; Jamieson *et al.*, 1983; Milas & Rinaudo, 1984; Sato *et al.*, 1984a; Lund *et al.*, 1988; Lecourtier *et al.*, 1986; Cuvelier, 1988; Cuvelier & Launay, 1986). Milas and Rinaudo (1984) discuss the importance of zero-shear measurements on xanthan solutions, and this is also borne out from our data.

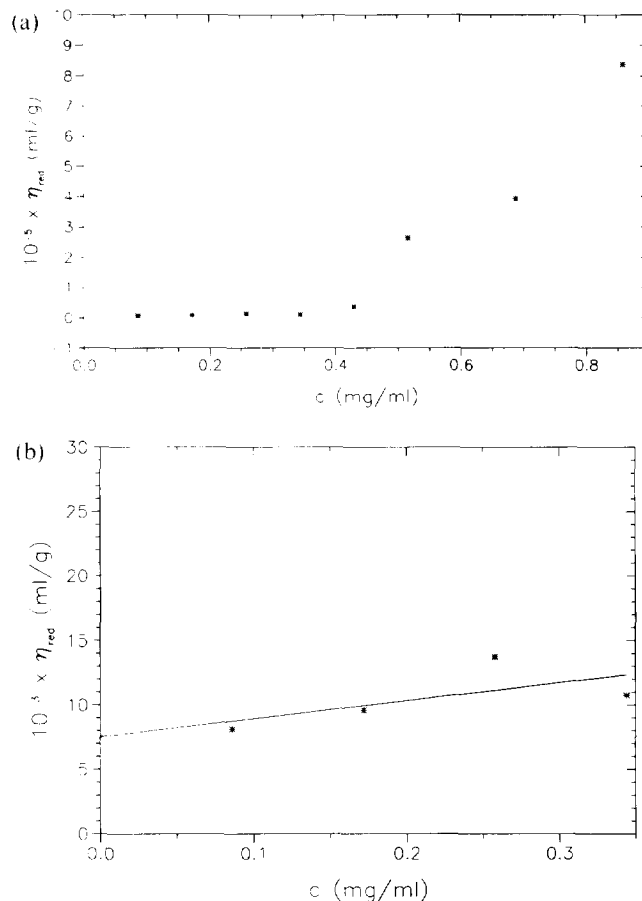
From our intrinsic viscosity and using the simple empirical approximation (Launay *et al.*, 1986)

$$c^* \sim 3.3/[\eta]. \quad (6)$$

we can predict the critical overlap concentration,  $c^*$ , to be  $\sim 0.5$  mg/ml, a value consistent with the discontinuity observed in Fig. 2a. This value was borne in mind for the subsequent evaluation of sedimentation and molecular weight parameters.

#### Sedimentation coefficient and $K_s$

Even though the highest concentration studied was only  $\sim 0.9$  mg/ml, the data for both  $s_{20,w}$  vs concen-

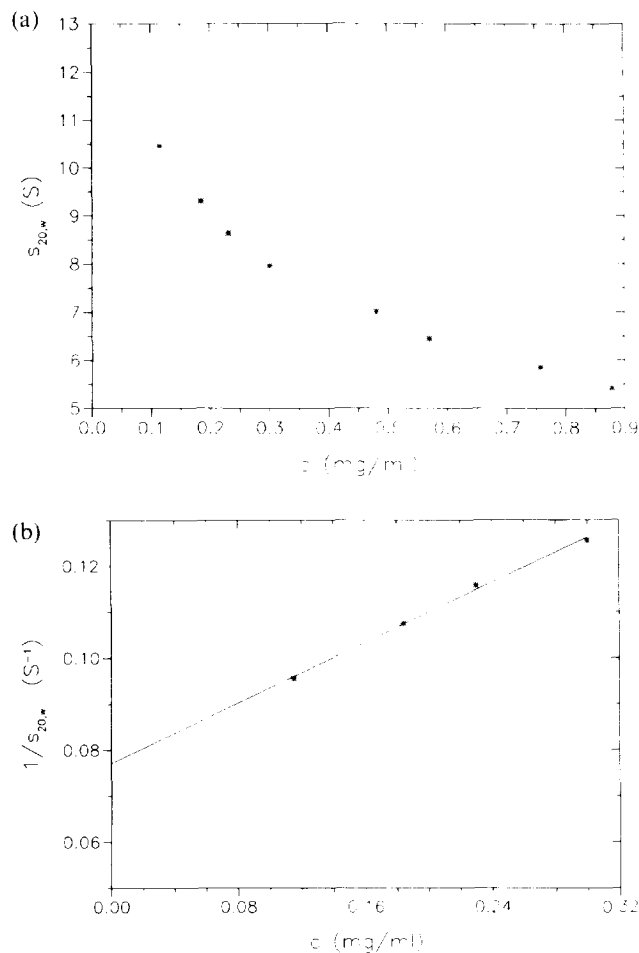


**Fig. 2.** Concentration dependence of the reduced (dynamic) viscosity  $\eta_{red}$  for Keltrol-xanthan. (a)  $\eta_{red}$  vs concentration,  $c$ , in the range 0.0–0.9 mg/ml. (b)  $\eta_{red}$  vs  $c$  in the range 0.0–0.35 mg/ml. The line fitted is  $\eta_{red} = [\eta](1 + K_s[\eta]c)$ .

tration (corrected for radial dilution),  $c$  (Fig. 3a) and  $1/s_{20,w}$  vs  $c$  showed significant curvature: this behaviour would appear to be consistent with the value for  $c^*$  observed in the viscosity experiments (Fig. 2a). It was possible however to reasonably fit the data below 0.4 mg/ml (Fig. 3b) and values for  $s_{20,w}^0 = (12.97 \pm 0.26)S$  and  $k_s = (2127 \pm 85)$  ml/g were obtained, again consistent with a very large, highly non-ideal molecule, the high non-ideality confirmed by the hypersharp nature of the sedimenting boundaries noted above (Fig. 1).

#### Weight average molecular weight and $B$

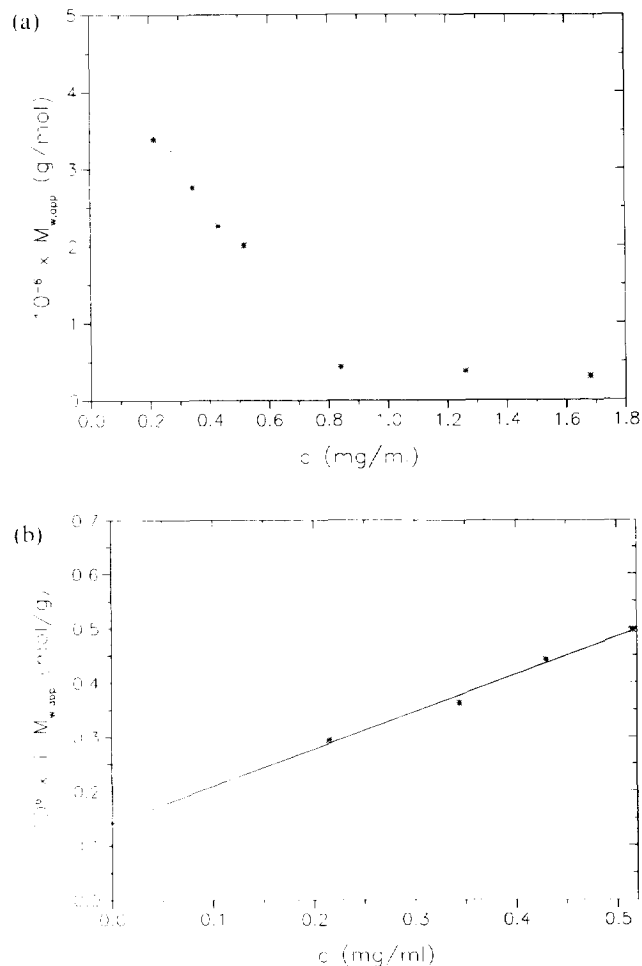
A very large concentration dependence of  $M_{w,app}$  was seen in the range  $c = 0.0$ –0.6 mg/ml, with a noticeable discontinuity above this concentration and loss of concentration dependence (Fig. 4a). Again, only those data points below this  $c^*$  discontinuity ( $< 0.6$  mg/ml in this case) were used for molecular weight and second virial coefficient evaluations. Further, because of the large concentration dependence, it was deemed unsafe to extrapolate alone the reciprocal to zero concentration



**Fig. 3.** Concentration dependence of the (apparent) sedimentation coefficient (a)  $s_{20,w}$  vs concentration,  $c$ , in the range 0.0–0.9 mg/ml. (b)  $1/s_{20,w}$  vs  $c$  in the range 0.0–0.32 mg/ml. Concentrations are true sedimenting concentrations (i.e. corrected for moisture content and radial dilution). The line fitted is  $1/s_{20,w} = (1/s_{20,w}^0)(1 + k_s c)$ .

via eqn (5), (see Harding, 1982). Indeed, a measure of the uncertainty in the extrapolated  $M_w$  can be obtained by comparing (i) a simple graphical extrapolation of the data ( $<0.6$  mg/ml) of Fig. 4a with (ii) the extrapolation from the conventional  $(1/M_{w,app})$  vs  $c$  plot (Fig. 4b) fitted to eqn (5). Method (i) yields an estimate for  $M_w$  of  $\sim (4.8 \pm 0.8) \times 10^6$  whereas (ii) yields  $(7.0 \pm 1.0) \times 10^6$ . Realistically, therefore, the 'safest' estimate for the molecular weight is probably  $\sim 5.9 \times 10^6$  but with a large ( $\sim 20\%$ ) uncertainty:  $M_w \sim (5.9 \pm 1.1) \times 10^6$  g/mol. Despite the large, but we feel realistic, error, this value is similar to values given for other unfractionated xanthans (Milas & Rinaudo, 1979; Milas *et al.*, 1990).

Assuming that a single non-ideal virial coefficient ( $B$ ) is sufficient to account for all the non-ideality of the system in the concentration range 0.0–0.52 mg/ml, we can also use the fit of eqn (5) to the data of Fig. 4b to estimate  $B$ : a value of  $(3.4 \pm 0.3) \times 10^{-4}$  ml mol $^{-2}$  is obtained.



**Fig. 4.** Concentration dependence of the (whole-distribution) apparent weight average molecular weight from low speed sedimentation equilibrium. (a) Plot of  $M_{w,app}$  vs cell loading concentration  $c$  in the range 0.0–1.7 mg/ml. (b) Plot of  $1/M_{w,app}$  vs  $c$  in the range 0.0–0.52 mg/ml. The line fitted is to  $(1/M_{w,app}) = (1/M_w^0) + 2Bc$  where  $B$  is the second thermodynamic or 'osmotic pressure' virial coefficient.

### Excluded volume/molar covolume

From  $B$  and  $M_w$  we can further estimate the excluded volume,  $u$  (ml/g) =  $2BM_w$  and the molar covolume  $U$  (ml/mol) =  $2BM_w^2$ , assuming that (i) a single non-ideal virial coefficient ( $B$ ) is sufficient to account for all the non-ideality of the system; and (ii) at  $I = 0.30$  there are no charge contributions to  $B$ . Huge values are obtained:  $u \sim 4010$  ml/g and  $U \sim 2.4 \times 10^{10}$  ml/mol, consistent with the large values for  $k_s$  and  $K_\eta$  which represent similar, but not identical phenomena. The value for the excluded volume is considerably in excess of values found for other polysaccharides (see Table 3 of Harding *et al.*, 1991).

### Shape of unfractionated Keltrol-xanthan

Previous attempts at representing the gross conformation or 'shape' of xanthan have revolved around Mark-

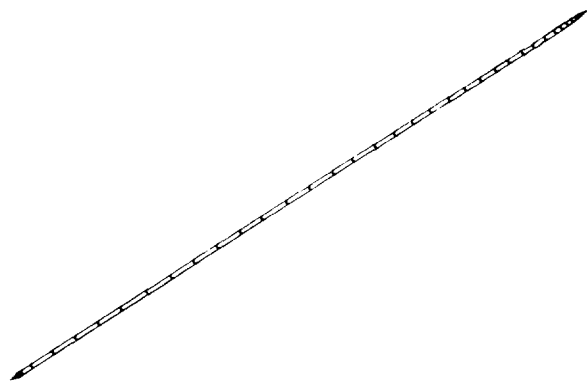


Fig. 5. Hydrodynamically equivalent ellipsoid model (prolate of average axial ratio  $\sim 70:1$ ) for Keltrol xanthan based on the hydration independent shape parameter  $\Pi$ .

Houwink–Kuhn–Sakurada (MHKS, see Smidsrød and Andresen, 1979; Harding *et al.*, 1991) or worm-like coil representations of either  $[\eta]$  as a function of  $M_w$  or  $s_{20,w}^0$  as a function of  $M_w$  for a 'homologous' series of the xanthan polymer obtained by fractionation of the sample by gel filtration or sonication (Sato *et al.*, 1984a, 1984b, 1984c) Sho *et al.*, 1986; Inatomi *et al.*, 1992) and an extended conformation was found (stiff coil/flexible rod).

For the unfractionated material under study here, two approaches are possible.

(i) *Wales van Holde ratio  $k_s/[\eta]$  approach*

An idea of the gross conformation of the xanthan molecule can be obtained from this ratio, which has a value of  $\sim 1.6$  for spheres and random coils and  $< 1$  for rigid, extended structures. Thus from our estimated value of  $0.28 \pm 0.10$  we conclude that the molecules are extended rods.

(ii) *Equivalent hydrodynamic ellipsoid approach*

The  $s_{20,w}^0$  values can be combined in the standard way to obtain an estimate for the frictional ratio  $f/f_0$ , (where  $f$  is the frictional coefficient and  $f_0$  the corresponding coefficient for a rigid sphere of the same mass and anhydrous volume as the xanthan) and, if the molecule is assumed reasonably rigid, as would seem likely from (i) above, an estimate for the axial ratio  $a/b$  of the hydrodynamically equivalent ellipsoid of revolution of semi-axes  $a > b$  can, in principle, be obtained.  $f/f_0$  is obtained from  $s_{20,w}^0$  and  $M_w$  via the equation (Tanford, 1961):

$$\left( \frac{f}{f_0} \right) = \left\{ \frac{M(1 - \bar{v}\rho)}{[N_A \cdot (6\pi\eta_0 s_{20,w}^0)]} \right\} \left[ \frac{4\pi N_A}{3vM} \right]^{1.3} \quad (7)$$

and using this we obtain an estimate for  $f/f_0$  of  $(14.2 \pm 0.4)$ . The 'Perrin' shape parameter  $P$  (also known as the 'frictional ratio due to shape',  $f/f_{0(a,b)}$  (Squire & Himmel, 1979)) is obtained from

$$P = (f/f_0) \cdot (\bar{v}/v_s) \quad (8)$$

$v_s$  is a composite factor, the 'swollen specific volume' including not only the partial specific volume, namely the volume actually occupied per gram by the dry molecule, but also the volume occupied by the solvent associated with each gram of dry molecule, namely the volume occupied by the molecule + associated solvent per gram of dry molecule. Although for globular proteins it is possible to make a fair estimate for this ( $\sim 1$  ml/g), for highly solvated polysaccharides this is not so. Similar problems exist with attempts to use the viscosity increment. For a rigid macromolecule, the viscosity increment  $v$  is obtained from the intrinsic viscosity via

$$v = [\eta]/v_s \quad (9)$$

Again, although an exact relation exists between  $v$  and  $a/b$ , (Simha, 1940; Saito, 1951) its application relies on an accurate estimate for  $v_s$ .  $v_s$ , however, can, in principle, be eliminated from eqns (8) and (9) to yield the well-known solvation-independent Scheraga–Mandelkern (1953)  $\beta$ -function: the value obtained ( $\sim 2.6 \times 10^6$ ) is consistent with an extended molecule, but the large error in this ( $\pm 1.2 \times 10^6$ ) largely deriving from the uncertainties in  $[\eta]$  and  $M$ , compounded by the well reported insensitivity of this function to axial ratio, makes it impossible to predict an axial ratio.

Fortunately there are other solvation independent parameters we can use (Harding, 1995). One is the  $\Pi$  function defined by (Harding, 1981):

$$\Pi = \{2BM/[\eta]\} + f(Z, I)/([\eta]M) \quad (10)$$

If we assume the ionic strength ( $I = 0.30$ ) is sufficiently high enough to suppress charge ( $Z$ ) effects then the term on the right of eqn (10) goes to zero, and so for Keltrol-xanthan we can estimate  $\Pi$  to be  $(0.53 \pm 0.21)$ . The hydrodynamically equivalent ellipsoid of revolution for Keltrol-xanthan is a prolate ellipsoid (the minimum value of  $\Pi$  for an oblate ellipsoid is 3.17) with an axial ratio of  $(70 \pm 20):1$ . Further, if it is again assumed that charge effects are negligible at  $I = 0.30$ , we can use the Wales-van Holde ratio,  $k_s/[\eta]$ , directly to predict the axial ratio using an approximate formula given by Rowe (1977, 1992): the value of  $k_s/[\eta] = (0.28 \pm 0.10)$  suggests again an extended molecule of axial ratio  $(60 \pm 20):1$ . It has to be stressed that although the hydration problem has been compromised, these values are still only approximate estimates because of the large degree of polydispersity of the unfractionated material, and an average somewhere between a weight and so-called 'viscosity average' (see Tanford, 1961). However, what we can reasonably say is that, although our approach is quite different, what is true in terms of the gross conformation for the fractionated molecule from previous studies, appears to be true for the whole unfractionated molecule.

Finally, although the findings of this study have been largely confirmatory in nature, underpinning what is largely already known about the solution properties of xanthan, the techniques/approaches have been somewhat different. We have applied similar techniques and approaches to study the behaviour of mixtures of xanthans with galacto- and glucomannans in the hope of enhancing our understanding of possible synergistic interaction phenomena in these mixtures; this will be the subject of the second paper in this series.

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