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A novel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan

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Abstract
Konjac glucomannans have been widely considered in health food products although their hydrodynamic properties have been poorly understood. The weight-average molecular weight ($M_w$); sedimentation coefficient ($s_{20,w}^0$) and intrinsic viscosities ($[\eta]$) have been estimated for five different preparations. The decrease in both intrinsic viscosity and sedimentation coefficient with molecular weight enables the estimation of molecular flexibility in terms of persistence length ($L_p$) using the traditional Bohdanecky-Bushin and Yamakawa-Fujii analyses for intrinsic viscosity and sedimentation data respectively. However, this requires an assumption of the mass per unit length $M_L$. Advantage can now be taken of a recent development in data interpretation which allows the estimation of $L_p$ from combined intrinsic viscosity and sedimentation coefficient data and also an estimate for $M_L$. Using this “global” procedure an estimate of $(13 \pm 1) \text{ nm}$ is found for $L_p$ and a value of $(330 \pm 10) \text{ g mol}^{-1} \text{ nm}^{-1}$ for $M_L$.

The value for $L_p$ suggests a molecule of considerable flexibility, comparable to galactomannans ($L_p \sim 8 \text{ - 10 nm}$) but not as flexible as pullulan ($L_p \sim 1 \text{ - 2 nm}$).

Keyword: Konjac glucomannan, molar mass, intrinsic viscosity, persistence length, semi-flexible coil
1. Introduction
Dietary fibre polysaccharides are of considerable physiological importance. They influence the digestion of food in general and in particular reduce the insulin needs of people with diabetes, influence bile acid metabolism, alter lipid digestion, cholesterol absorption and protect against colonic cancer (Sonnichsen & Apostoloff, 1992; Marsh, 1992). They can also screen against wheat protein allergy. These materials are essentially all polysaccharides and associated lignins in the diet that are not digested by the endogenous secretions of the human digestive tract (Trowell, Southgate, Wolever, Leeds, Gassull & Jenkins, 1976). Due to the great importance of food proteins in human nutrition the subject of the interactions of such polysaccharides with food proteins is of particular interest. For example there is evidence to suggest that such interactions could protect sensitive persons from harmful allergic reactions involving wheat, soya and milk proteins (Yamauchi & Suetsuna, 1993; Konig, 1993). Recently some researchers also have shown that proteins and polysaccharides can actually form conjugates (Tolstoguzov, 1993; Harding, Jumel, Kelly, Gudo, Horton & Mitchell, 1993; Dickinson, 1993). Proteins can also self-associate strongly and weakly (van der Merwe & Barclay, 1994) and polysaccharides can form strong self-aggregation complexes. A recent study by Patel et al. (2007) showed that they can even interact with each other weakly with interaction strengths resembling those of molecules involved with cell-cell recognition and signalling, with molar dissociation constant $K_d \approx 100 \mu M$.

Konjac glucomannan (KGM) is a neutral glucomannan heteropolysaccharide extracted from the tubers of *Amorphopallas Konjac* (Figure 1). It is composed of a backbone chain of $\beta$-1,4 linked D-mannose and D-glucose with a low degree of acetyl groups related to its gel formation properties (Maeda, Shimahara & Sugiyama, 1980; Nishinari, Williams & Phillips, 1992; Takigami, 2000; Williams, Foster, Martin, Norton, Yoshimura & Nishinari, 2000; Katsuraya, Okuyama, Hatanaka, Oshima, Sato & Matsuzaki, 2003; Gao & Nishinari, 2004).

<Figure 1 here>
Typically reported M:G ratio is approximately 1.6:1 (Maeda et al., 1980; Shimahara, Suzuki, Sugiyama & Nishizawa, 1975; Cescutti, Campa, Delben and Rizzo, 2002). Smith and Srivastava (1959) proposed that the glucomannan has $\beta$-1,4 linked D-glucose and D-mannose residues as the main chain with branches joined through C-3 carbon of D-glucosyl and D-mannosyl residues. Degree of branching is reported to be about 8% and the ratio of terminal glucosyl units to mannosyl units is calculated to be approximately 2 by $^{13}$C NMR studies (Katsuraya et al., 2003). While some authors reported a random distribution of these residues (Williams et al., 2000), other researchers prefer a complex non-random distribution as the basic polymeric repeating unit has the patterns of:

$\text{--G--M--M--M--G--M--}$ or $\text{--G--G--M--M--M--M--}$


$\text{--G--G--M--M--G--M--M--M--}$

Maeda et al. (1980)

$\text{M--M--M--M--G--G--M--G--M--}$

Shimahara, Suzuki, Sugiyama, and Nishizawa (1975); Takahashi et al. (1984)

KGM contains some acetyl groups in the main chain. In the presence of alkali, deacetylation occurs and a thermally stable gel is formed (Maeda et al., 1980; Maekaji, 1974). The gel is the traditional Japanese food Konjac, and high molar mass KGM is essential for the preparation of high-quality Konjac. Maeda et al. (1980) reported that, short side chains of 11-16 monosaccharides occur at intervals of 50-60 units of the main chain attached by $\beta$-1,3 linkages. Also, acetate groups on carbon 6 occur at every 9-19 units of the main chain. Unsubstituted linear $\beta$-1,4 mannans and glucans (cellulose) are both insoluble in water owing primarily to interchain association through hydrogen bonding yet KGM is water soluble. This solubility may partly be attributed to the long side chains of the glucomannan (Wen, Wang, Wang, Li & Zhou, 2008; Hwang & Kokini, 1991) which serve to hinder intermolecular
association and enhance solvation (Wen et al., 2008; Dea, Morris, Rees, Welsh, Barnes & Price, 1977) however it is predominantly believed to be associated with the presence of the acetyl substituents. Although the removal of these groups facilitates gelation, the precise role of the acetyl groups in promoting solubility is still a matter of controversy (Maeda et al., 1980; Maekaji, 1974; Wen et al., 2008; Dea et al., 1977).

Chemical modification of KGM has been reported including methylation, nitration and oxidation. However such procedures are time consuming and are likely to result in the degradation of the polymer (Wen et al., 2008). Owing to its poor solubility, even in 70 % aqueous cadoxen, KGM aggregates have always been a problem affecting the determination of the true molar mass (Wang, Wood, Cui & Ross-Murphy, 2000). However, considerable success in producing homogeneous solutions of KGM and similar materials has been reported using “physical” methods whereby supra-molecular aggregates are dispersed by increasing the energy of the component polymer chains. Such techniques include; sonication, irradiation and the application of heat at elevated pressures. The need to overcome such aggregation was recognised by Clegg and co-workers (Clegg, Phillips & Williams, 1990) who employed a sonication technique. Other methods of dispersing aggregates have also been possible including treatment with heat under increased pressure e.g. heating in a sealed vessel (microwave bomb) in a microwave oven, which was applied in this research.

The KGM flour is used in the production of Japanese shirataki noodles, which are very low in calories, and jellies. It has been cultivated for centuries in Japan and KGM was known to be used as a food storage polysaccharide. It is also known as a hunger suppressant because it produces a feeling of fullness by creating very viscous solutions that retard absorption of the nutrients in food. It is commonly applied in absorbent material such as disposable diapers and sanitary towels because, it is reported that, this soluble fibre has an extraordinarily high water-holding capacity, forming highly viscous solutions when dissolved in water. Reportedly, one gram of KGM can absorb up to 200 ml of water (Maeda, et al., 1980; Wen et al., 2008). It has been suggested that KGM has the highest viscosity at lowest concentration of any known dietary fibre (Ozu, Baianu & Wei, 1993; Yaseen, Herald, Aramouni & Alavi, 2005).
Recently KGM has been also found to have many different uses, for example in food production as a texture modifier and thickener, and especially in pharmaceutical industry. For example, KGM is involved in the production of DNA-advanced controlled release hydrogels (Wen et al., 2008) adjunctive therapeutic agent in the treatment of thyrotoxicosis (Hopman, Houben, Speth & Lamers, 1988). Prevention of postprandial hypoglycaemia has been reported without the disadvantage of unpalatability and carbohydrate malabsorption (Vuksan et al., 1999). It has been used to improve glycaemia and other associated risk factors for coronary heart disease in type II diabetes (Katsuraya et al., 2003). Currently, there are increasing demands for biopharmaceutical products such as polysaccharide vaccines and the detailed characterisation of these products are necessary. Producing a successful and safe polysaccharide vaccine not only depends on its carbohydrate sequence, but also its molecular weight, conformation and any self-associative behaviour. Poorly characterised materials may lead to dangerous side effects or the production of less immunogenic materials (Jódar, Feavers, Salisbury & Granoff, 2002).

Locust bean and guar galactomannans, like the wheat proteins, have presented problems in the past, but recently Harding and co-workers (Patel, Picout, Ross-Murphy & Harding, 2006) have shown that after solubilisation with pressure-temperature treatment, full characterisations are possible. Arabinoxylans (Patel, et al., 2007) too have recently been characterised, but konjac glucomannan (KGM) has not been so well described. This conducted study aims to fill these gaps in our knowledge before the behaviour of the protein-polysaccharide mixtures are properly investigated.

2. Materials and Methods

Materials

Two konjac glucomannan samples KGM-1 and KGM-4 were obtained from FMC BioPolymer (Philadelphia, U.S.A.) and Dr. Robert Winwood, University of Nottingham, U.K., respectively. Both samples were used as supplied without any further purification and assumed to be of similar composition.

Both KGM-1 and KGM-4 (3.0 g) were dissolved 50.0 ml in 0.1 M pH 6.8 phosphate buffer (Green, 1933) at 20.0°C and mixed by magnetic stirring for 24 hours. Konjac samples of different molar masses were then prepared by heating 15 ml of the native
konjac solutions (KGM-1 and KGM-4) in a sealed vessel (microwave bomb) in an 800 W microwave oven (Panasonic UK Ltd. Bracknell, UK) for different time periods (Table 1). The temperature and pressure within the “bomb” were not measured.

Viscometry
The densities and viscosities of the reference solvent (0.1 M pH 6.8 phosphate buffer) and of the sample dispersions were analysed using an AMVn Automated Micro Viscometer and DMA 5000 Density Meter (both Anton Paar, Graz, Austria) under precise temperature control (20.00 ± 0.01 °C). The relative, \( \eta_{rel} \) and specific viscosities, \( \eta_{sp} \) were calculated as follows:

\[
\eta_{rel} = \left( \frac{\eta}{\eta_0} \right)
\]

\[
\eta_{sp} = \eta_{rel} - 1
\]

where \( \eta \) is the dynamic viscosity (i.e. corrected for density) of a konjac dispersion and \( \eta_0 \) is the dynamic viscosity of buffer (1.0032 mPas).

Measurements were made at different concentrations and extrapolated to infinite dilution using both the Huggins (1942) and Kraemer (1938) approaches (Figure 2):

\[
\frac{\eta_{sp}}{c} = [\eta](1 + K_H[\eta]c)
\]

\[
\frac{\ln(\eta_{rel})}{c} = [\eta](1 - K_K[\eta]c)
\]

where the intrinsic viscosity \([\eta]\) is taken as the mean of the intercepts from equations (3) and (4) and \( K_H \) and \( K_K \) are the Huggins and Kraemer constants respectively.
Sedimentation velocity experiments were performed using a Beckman Instruments (Palo Alto, U.S.A.) Optima XLI Analytical Ultracentrifuge. Konjac dispersions (380 µl) of various concentrations (0.25 – 1.5 x 10^{-3} g ml^{-1}) and 0.1 M pH 6.8 phosphate buffer (400 µl) were injected into the sample and reference channels respectively of a double sector 12 mm optical path length cell. Samples were centrifuged at 45000 rpm (~150000g) at a temperature of 20.0 °C. Concentration profiles and the movement of the sedimenting boundary in the analytical ultracentrifuge cell were recorded using the Rayleigh interference optical system and converted to concentration (in units of fringe displacement relative to the meniscus, \( j \)) versus radial position, \( r \) (Harding, 2005). The data was then analysed using the “least squares, ls-g(s) model” incorporated into the SEDFIT (Version 9.4b) program (Schuck, 1998; 2005). This software based on the numerical solutions to the Lamm equation follows the changes in the concentration profiles with radial position and time and generates an apparent distribution of sedimentation coefficients in the form of \( g^*(s) \) versus \( s_{T,b} \), where the * indicates that the distribution of sedimentation coefficients has not been corrected for diffusion effects (Harding, 2005).

As sedimentation coefficients are temperature and solvent dependent it is conventional to convert sedimentation coefficients (or their distributions) to the standard conditions of 20.0 °C and water using the following equation (Ralston, 1993):

\[
 s_{20,w} = s_{T,b} \left[ \frac{(1 - \bar{\nu} \rho_{20,w}) \eta_{T,b}}{(1 - \bar{\nu} \rho_{T,b}) \eta_{20,w}} \right] \tag{5}
\]

where \( \bar{\nu} = 0.63 \text{ ml g}^{-1} \) is the partial specific volume of konjac, \( \eta_{T,b} \) and \( \rho_{T,b} \) are the viscosity and density of the experimental solvent (0.1 M pH 6.8 phosphate buffer) at the experimental temperature (20.0 °C) and \( \eta_{20,w} \) and \( \rho_{20,w} \) are the viscosity and density of water at 20.0 °C.

To account for hydrodynamic non-ideality (co-exclusion and backflow effects), the apparent sedimentation coefficients (\( s_{20,w} \)) were calculated at each concentration and
extrapolated to infinite dilution using the following equation (Gralén, 1944; Rowe, 1977; Ralston, 1993).

\[
\frac{1}{s_{20,w}} = \frac{1}{s_{0,20,w}^0} (1 + k_s c)
\]  

(6)

where \(k_s\) (ml g\(^{-1}\)) is the sedimentation concentration dependence or “Gralén” coefficient (Gralén, 1944).

**Size Exclusion Chromatography coupled to Multi-Angle Laser Light Scattering (SEC-MALLS)**

Analytical fractionation was carried out using a series of SEC columns TSK G6000PW, TSK G5000PW and TSK G4000PW protected by a similarly packed guard column (Tosoh Bioscience, Tokyo, Japan) with on-line MALLS (Dawn DSP, Wyatt Technology, Santa Barbara, U.S.A.) and refractive index (Optilab rEX, Wyatt Technology, Santa Barbara, U.S.A.) detectors. The eluent (0.1 M pH 6.8 phosphate buffer) was pumped at 0.8 ml min\(^{-1}\) (PU-1580, Jasco Corporation, Great Dunmow, U.K.) and the injected volume was 100 µl (~1.5 x 10\(^{-3}\) g ml\(^{-1}\)) for each sample. Absolute weight-average molar masses (\(M_w\)) were calculated using the ASTRA® (Version 5.1.9.1) software (Wyatt Technology, Santa Barbara, U.S.A.), using the refractive index increment, \(dn/dc = 0.150\) ml g\(^{-1}\).

3. Results

Viscometry

In all cases we have good linear extrapolations for both the Huggins and Kraemer plots (Figure 2). We can see from Table 1 that heating konjac glucomannan samples for 30 and 45 seconds results in a reduced intrinsic viscosity. For example the native sample KGM-1 has an intrinsic viscosity of (1300 ± 15) ml g\(^{-1}\) whilst after heating for 45 seconds in a microwave bomb intrinsic viscosity is reduced to (475 ± 5) ml g\(^{-1}\) (KGM-3). This would appear to indicate depolymerisation of the konjac chains.

<Table 1 and Figure 2 here>
Sedimentation Velocity in the Analytical Ultracentrifuge

As with intrinsic viscosity we see a decrease in weight average sedimentation coefficient with increased heating time in the microwave bomb (Table 1). This is again consistent with depolymerisation upon heating. The good solubility of konjac under these conditions (buffer, temperature and concentration) is demonstrated by the areas under the Is-g(s) curves (Figure 3). The concentration in can be estimated from the following relationship:

\[
\text{concentration (g ml}^{-1}\text{)} = 3.8 \times 10^{-4} \times \text{Area (fringes)}
\]  

< Figure 3 here >

Size Exclusion Chromatography coupled to Multi-Angle Laser Light Scattering (SEC-MALLS)

Depolymerisation is confirmed by a decrease in weight average molar mass with increased heating time (Table 1). It would seem that heating for 30 seconds only mildly depolymerises the konjac chain whereas a prolonged exposure of 45 seconds has a pronounced affect. N.B. When KGM-1 and KGM-4 were heated for 60 and 45 seconds, respectively, in the microwave bomb this resulted in brown slurries which were not characterised further.

4. Discussion: conformational analysis

The translational frictional ratio, \( ff_0 \)

The translational frictional ratio (Tanford, 1961), \( ff_0 \) is a parameter which depends on molecular weight, conformation and molecular expansion through hydration effects. It can be measured experimentally from the sedimentation coefficient and molecular weight:

\[
\frac{f}{f_0} = \frac{M_w (1 - \bar{v} \rho_{20,w})}{(N_A 6 \pi \eta_{20,w} s_{20,w}^2)} \left( \frac{4 \pi N_A}{3 \sqrt[3]{M_w}} \right)^{\frac{1}{2}}
\]  

where the partial specific volume for konjac, \( \bar{v} = 0.63 \text{ mL g}^{-1} \).
The mean translational frictional ratio, \( f/f_0 = (11 \pm 2) \) (Table 2) is similar to that of methylcellulose of similar molar mass (Patel, Morris, García de la Torre, Ortega, Mischnik & Harding, 2008). \( f/f_0 \) has contributions from asymmetry and solvation and has a minimum value of 1.0 so these molecules are either considerably extended, considerably hydrated, or perhaps a contribution from both.

To be more specific we need to consider the change of the sedimentation coefficient or intrinsic viscosity with molecular weight.

**Mark-Houwink-Kuhn-Sakurada power law relationships**

We can take advantage of the fact that heating for different times resulted in different weight average molar masses, \( M_w \), facilitating the use of the “Mark-Houwink-Kuhn-Sakurada”- (MHKS) power law relation linking both \([\eta]\) and \( s^0_{20,w} \) with \( M_w \):

\[
[\eta] \propto M_w^a \tag{9}
\]

\[
s^0_{20,w} \propto M_w^b \tag{10}
\]

The MHKS exponents \( a \) and \( b \) are derived using double logarithmic plots of intrinsic viscosity and sedimentation coefficient versus molecular weight respectively (Harding, Vårum, Stokke & Smidsrød, 1991) (Figures 4 and 5). In this case we find \( a = (0.74 \pm 0.01) \) which is indicative of a semi-flexible coil type molecule and is in good agreement with the recent results of Prawitwong et al. (2007) of \( a = 0.78 \), whereas \( b = (0.32 \pm 0.01) \) which is also consistent with a semi-flexible coil conformation.

<Figures 4 and 5 here>

**Wales-van Holde ratio**, (Wales & van Holde, 1954) \( R \)

The mean Wales-van Holde ratio (equation 10), \( R = (0.4 \pm 0.1) \) (Table 2) which is again indicative of a semi-flexible structure and is similar to those of pectin (Morris, Foster & Harding, 2000; Morris, García de la Torre, Ortega, Castille, Smith & Harding, 2008) and methylcellulose (Patel et al., 2008).
The sedimentation conformation zoning (Pavlov, Rowe & Harding, 1997; Pavlov, Harding & Rowe, 1999) plot $k_s M_L$ versus $[s]/M_L$ enables an estimate of the “overall” solution conformation of a macromolecule in solution ranging from Zone A (extra rigid rod) to Zone E (globular or branched). The parameter $[s]$ related to the sedimentation coefficient by the relation:

$$[s] = \frac{s^{0,20w} \eta_{20,w}}{(1 - \bar{\rho}_{20,w})}$$

and $M_L$, the mass per unit length is just

$$M_L = \frac{m}{l}$$

The mass of glucose (or mannose) monomer, $m$ is 162 g mol$^{-1}$ and the average monomer mass is therefore approximately 166 g mol$^{-1}$ for a degree of acetylation of 10% where $l$ is the diameter of a monosaccharide $\sim 0.5$ nm. Therefore $M_L$ was fixed at 330 g mol$^{-1}$ nm$^{-1}$.

The sedimentation conformation zoning (Figure 6 and Table 2) places all five konjac samples in Zone C (semi-flexible coil).

Estimation of persistence length

The linear flexibility of polymer chains can also be represented quantitatively in terms of the persistence length, $L_p$ of equivalent worm-like chains (Kratky & Porod, 1949)
where the persistence length is defined as the average projection length along the initial direction of the polymer chain. In the case of a theoretical perfect random coil $L_p = 0$ and for the equivalent extra-rigid rod (Harding, 1997) $L_p = \infty$, although in practice limits of ~1 nm for random coils (e.g. pullulan) and 200 nm for a extra-rigid rod (e.g. DNA) are more appropriate (Tombs & Harding, 1998).

We have used three different approaches to measure chain flexibility in terms of persistence lengths:

1. **Bushin-Bohdanecky method** (Bushin, Tsvetkov, Lysenko & Emel'yanov, 1981; Bohdanecky, 1983)
2. **Yamakawa-Fujii method** (Yamakawa & Fujii, 1973) and
3. **Combined analysis – HYDFIT** (Ortega & García de la Torre, 2007)

1. **Bushin-Bohdanecky method** (Bushin et al., 1981; Bohdanecky, 1983)

This is a popular method for estimating chain persistence lengths particularly for semi-flexible polymers, and has been applied to range of polysaccharides. In its simplest form, the Bushin-Bohdanecky method involves plotting $\left(\frac{M_w^2}{[\eta]}\right)^{\frac{1}{3}}$ versus $M_w^{1/2}$ and from the slope $L_p$ can be calculated using the following relation and tabulated values (Bohdanecky, 1983) of the coefficient $B_0$:

$$
\left(\frac{M_w^2}{[\eta]}\right)^{\frac{1}{3}} = A_0 M_L \Phi^{-1/3} + B_0 \Phi^{-1/3} \left(\frac{2L_p}{M_L}\right)^{-1/2} M_w^{1/2}
$$

From a plot of $\left(\frac{M_w^2}{[\eta]}\right)^{\frac{1}{3}}$ versus $M_w^{0.5}$ (**Figure 7**) we obtain a slope of $(0.76 \pm 0.04)$. Taking $B_0$ as ~1.10 (Bohdanecky, 1983), $2.86 \times 10^{23}$ mol$^{-1}$ for the Flory-Fox ‘constant’ $\Phi$ and a (molar) mass per unit length $M_L$ of ~330 g mol$^{-1}$ nm$^{-1}$ the value obtained for $L_p$ is ~$(8 \pm 1)$ nm. Although $M_L$ may be found from the intercept of the Bohdanecky-Bushin plot this is very sensitive to estimation of $A_0$ (Bohdanecky, 1983).
2. Yamakawa-Fujii method (Yamakawa & Fujii, 1973)

Hearst and Stockmayer (1962) first reported the sedimentation coefficient in relation to wormlike chain parameters, later refined by Yamakawa and Fujii (1973). The original relation given by Yamakawa and Fujii relating the sedimentation coefficient with persistence length was unfortunately misprinted; the correction was given by Freire and García de la Torre (1992):

\[
\begin{align*}
0^0 &= \frac{M_L}{3\pi \eta_0 N_A} \times \left[ 1.843 \left( \frac{M_w}{2M_L L_p} \right)^{1/2} + A_2 + A_3 \left( \frac{M_w}{2M_L L_p} \right)^{-1/2} + \ldots \right] \\
\end{align*}
\]

(15)

Yamakawa and Fujii (1973) showed that \( A_2 = -\ln(d/2L_p) \) and \( A_3 = 0.1382 \) if the \( L_p \) is much higher than the chain diameter, \( d \). Using the Yamakawa-Fujii procedure a plot of \( s_{20, w}^0 \) versus \( M_w^{1/2} \) (Figure 8) yielded a slope of \( (2.66 \pm 0.06) \times 10^{-16} \), and using equation (14) and a fixed \( M_L \) of 330 g mol\(^{-1}\) nm\(^{-1}\) the \( L_p = (34 \pm 1) \) nm which is considerably higher than the value obtained from the Bushin-Bohdanecky analysis and more likely indicates a rigid rod type conformation.

3. Combined analysis – HYDFIT (Ortega & García de la Torre, 2007)

It can clearly be seen that different methods provide their own bias on results (Bohdanecky & Petrus, 1991; Picout, Ross-Murphy, Jumel & Harding, 2002; Patel et al., 2008) and in response to this problem Ortega and García de la Torre have created a new software package, HYDFIT (Ortega & García de la Torre, 2007) which considers data sets of both intrinsic viscosities for different molecular weights and sedimentation coefficients for different molecular weights.

\[
\begin{align*}
0^0 &= \frac{M_L}{3\pi \eta_0 N_A} \times \left[ 1.843 \left( \frac{M_w}{2M_L L_p} \right)^{1/2} + A_2 + A_3 \left( \frac{M_w}{2M_L L_p} \right)^{-1/2} + \ldots \right] \\
\end{align*}
\]

(15)
returned for $M_L$ and $L_p$ are insensitive to $d$ so this is usually fixed (Ortega & García de la Torre, 2007).

\[ d = \left( \frac{4M_L \bar{\nu}}{\pi N_A} \right)^{1/2} \]  

(16)

where $M_L = 330 \text{ g mol}^{-1} \text{ nm}^{-1}$ and the partial specific volume, $\bar{\nu} = 0.63 \text{ ml g}^{-1}$ and therefore $d \approx 0.7 \text{ nm}$.

The Multi-HYDFIT program then “floats” the variable parameters in order to find a minimum of the multi-sample target (error) function, $\Delta$ (Ortega & García de la Torre, 2007).

In this procedure as defined in Ortega and García de la Torre (2007), $\Delta$ is calculated using equivalent radii, where the equivalent radius ($a_e$) is defined as the radius of an equivalent sphere having the same value as the determined property. In the present study we are interested in the equivalent radii resulting from the sedimentation coefficient, i.e. translational frictional coefficient ($a_T$), and from the intrinsic viscosity ($a_I$).

\[ a_T = \frac{f}{6\pi \eta_0} \]  

(17)

where $\eta_0$ is the viscosity of water at 20.0 °C, and

\[ a_I = \left( \frac{3[\eta]M_w}{10\pi N_A} \right)^{1/3} \]  

(18)

where $N_A$ is Avogadro’s number.

The target function, $\Delta$ can be evaluated from the following relations:
\[ \Delta^2 = \frac{1}{N_s} \sum_{i=1}^{N_s} \left[ (\sum W_T)^{-1} \sum_T W_T \left( \frac{a_{T(\text{cal})} - a_{T(\text{exp})}}{a_{T(\text{exp})}} \right)^2 \right] \]

(19)

\[ \Delta^2 = \frac{1}{N_s} \sum_{i=1}^{N_s} \left[ (\sum W_I)^{-1} \sum_I W_I \left( \frac{a_{I(\text{cal})} - a_{I(\text{exp})}}{a_{I(\text{exp})}} \right)^2 \right] \]

(20)

where \( N_s \) is the number of samples in multi-sample analysis, \( W_T \) and \( W_I \) are the statistical weights for equivalent radii \( a_T \) and \( a_I \) (from translation frictional coefficient and intrinsic viscosity data respectively) and the subscripts cal and exp represent values from calculated and experimental values respectively.

\( \Delta \) is thus a dimensionless estimate of the agreement between the theoretical calculated values for the intrinsic viscosity for a particular molar mass, persistence length and mass per unit length and the experimentally measured parameters (Ortega & García de la Torre, 2007).

The minimum in the target function (\( \Delta = 0.220 \)) corresponds to a persistence length of \((13 \pm 1) \) nm and a mass per unit length of \((330 \pm 10) \) g mol\(^{-1}\) nm\(^{-1}\) (Figure 9). The persistence length is again in good agreement with other semi-flexible coil type polysaccharides, for example pectin (Morris et al., 2008), and methylcellulose (Patel et al., 2008) and is somewhat less flexible than for galactomannans (Patel et al., 2006; Morris et al., 2008). The estimation for the mass per unit length is in excellent agreement with the predicted value from the chemical structure.
5. Conclusions
In this paper we have shown, using three different approaches based on intrinsic viscosity \([\eta]\), sedimentation coefficient \((s_{20,w}^o)\) and weight average molar mass \((M_w)\), that microwave treated konjac glucomannan most likely adopts a semi-flexible coil conformation (Zone C) when dispersed in 0.1 M pH 6.8 phosphate buffer. The conformation may be very different in other dispersion media for example cadoxen as the solvent – solute interactions may be different.

The solution conformation plays an important role in the structure-function relationship of polysaccharides (Tombs & Harding, 1998), intra- and inter-chain entanglements (Cheng, Abd Karim & Seow, 2007) and in interactions with other biopolymers.

We have again demonstrated that different approaches (e.g. Bushin-Bohdanecky and Yamakawa-Fujii) used in the estimation of the persistence lengths can lead to a bias in the results (Table 2) and therefore it is more appropriate to characterise macromolecules using more than one hydrodynamic technique.
References


Legends to Figures

**Figure 1** - Chemical structure of konjac glucomannan (KGM).

**Figure 2** – The Huggins (!) and Kraemer (,) extrapolations for KGM-4 ([η] = 765 ± 10 ml g⁻¹).

**Figure 3** – The sedimentation coefficient distributions for KGM-2 at different concentrations as calculated from equation 7: 1.29 x 10⁻³ g ml⁻¹ (▼); 8.13 x 10⁻⁴ g ml⁻¹ (▲); 6.08 x 10⁻⁴ g ml⁻¹ (▲); 4.33 x 10⁻⁴ g ml⁻¹ (●) and 2.89 x 10⁻⁴ g ml⁻¹ (■).

**Figure 4** – Mark-Houwink-Kuhn-Sakurada viscosity power law double logarithmic plot for konjac glucomannan (KGM) where the slope, $a = 0.74 ± 0.01$.

**Figure 5** – Mark-Houwink-Kuhn-Sakurada sedimentation power law double logarithmic plot for konjac glucomannan (KGM) where the slope, $b = 0.32 ± 0.01$.

**Figure 6** - The sedimentation conformation zoning plot (adapted from Pavlov et al., 1997; Pavlov et al., 1999). Zone A: extra rigid rod; Zone B: rigid rod; Zone C: semi-flexible; Zone D: random coil and Zone E: globular or branched.

**Figure 7** - Bushin-Bohdanecky plot for konjac glucomannan (KGM) where $L_p = 8 ± 1$ nm from the slope.

**Figure 8** - Yamakawa-Fujii plot for konjac glucomannan (KGM) where $L_p = 34 ± 1$ nm from the slope.

**Figure 9** - Solutions to the Bushin-Bohdanecky and Yamakawa-Fujii equations for konjac glucomannan (KGM) using equivalent radii approach. The x-axis and y-axis represent $L_p$ (nm) and $M_L$ (g mol⁻¹ nm⁻¹) respectively. The target function, $\Delta$ is calculated over a range of values for $M_L$ and $L_p$. In these representations, the values of $\Delta$ function are represented by the full colour spectrum, from the minimum in the target function in blue ($\Delta = 0.220$) to red ($\Delta ≥ 1$). The calculated minimum ($L_p = 13 ± 1$ nm and $M_L = 330 ± 10$ g mol⁻¹ nm⁻¹) is indicated.
Tables

Table 1 – Hydrodynamic properties of konjac glucomannan (KGM)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Heating time (s)</th>
<th>$M_w$ (g mol$^{-1}$)</th>
<th>$[\eta]$ (ml g$^{-1}$)</th>
<th>$s_{20,w}^0$ (S)</th>
<th>$k_\eta$ (ml g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KGM-1</td>
<td>0</td>
<td>740000 ± 20000</td>
<td>1300 ± 15</td>
<td>3.40 ± 0.02</td>
<td>665 ± 20</td>
</tr>
<tr>
<td>KGM-2</td>
<td>30</td>
<td>695000 ± 20000</td>
<td>1190 ± 25</td>
<td>3.00 ± 0.03</td>
<td>455 ± 25</td>
</tr>
<tr>
<td>KGM-3</td>
<td>45</td>
<td>210000 ± 5000</td>
<td>475 ± 5</td>
<td>1.92 ± 0.10</td>
<td>160 ± 30</td>
</tr>
<tr>
<td>KGM-4</td>
<td>0</td>
<td>305000 ± 10000</td>
<td>765 ± 10</td>
<td>2.50 ± 0.10</td>
<td>275 ± 30</td>
</tr>
<tr>
<td>KGM-5</td>
<td>30</td>
<td>240000 ± 5000</td>
<td>565 ± 10</td>
<td>1.67 ± 0.20</td>
<td>115 ± 30</td>
</tr>
</tbody>
</table>

Table 2 – Conformational parameters for konjac glucomannan (KGM)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHKS exponent “a”</td>
<td>0.74 ± 0.01</td>
</tr>
<tr>
<td>MHKS exponent “b”</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>$\rho/\rho_o$</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>$k_\eta/[\eta]$</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Conformation Zone</td>
<td>C</td>
</tr>
<tr>
<td>$L_p$ (nm) from Bohdanecky-Bushin</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>$L_p$ (nm) from Yamakawa-Fujii</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>$L_p$ (nm) from HYDFIT</td>
<td>13 ± 1</td>
</tr>
</tbody>
</table>
Figures

Figure 1

Concentration (g ml\(^{-1}\))

\[ \eta_{\text{red}}, \ln(\eta_{\text{rel}})/c \text{ (ml g}^{-1} \text{)} \]

Figure 2
Figure 3

Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9