

Size and shape of inulin in dimethyl sulphoxide solution

B.H. Azis^a, B. Chin^a, M.P. Deacon^a, S.E. Harding^a, G.M. Pavlov^{b,*}

^aNational Centre for Macromolecular Hydrodynamics, University of Nottingham, School of Biology, Sutton Bonington, LE12 5RD UK

^bInstitute of Physics, St. Petersburg University, Ul'anovskaya ul. 1, 19890 St. Petersburg, Russia

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Abstract

A comparative hydrodynamic characterization of the solution properties of the fructan polysaccharide inulin extracted from two different sources and solubilized in dimethyl sulphoxide is described. For Jerusalem artichoke inulin a weight average molecular weight M_w of 3400 ± 150 Da from sedimentation equilibrium in the analytical ultracentrifuge is obtained, together with an intrinsic viscosity $[\eta]$ of 9.1 ± 0.2 ml g⁻¹ and a sedimentation coefficient (corrected to a solvent density and viscosity of that at water at 20°C) $s_{20,w}$ of ~ 0.4 S. Chicory root inulin had somewhat similar properties, with an M_w of 6200 ± 200 g mol⁻¹, $[\eta]$ of 10.7 ± 0.2 ml g⁻¹ and $s_{20,w}$ also of ~ 0.7 S. These results appear reasonably consistent with a rather compact model with a relatively large degree of solvent association. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Inulins are members of the fructan class of storage polysaccharides and are found in large amounts in only a small variety of plants, but are widely distributed. Inulins, in common with the other fructans have considerable biotechnological importance (see Fuchs (1987), Silver (1996), Schacht (1985)) and for two reasons. The first is that they are a potential source of fructose, for which there is growing demand in the food industry as a sweetener; the second is they are the only major class of polysaccharide based on the furanose structure (Haworth et al., 1932). Fructans were first isolated from *Inula helenium*, hence the name 'inulin' and many other Compositae have since been found to make them. They occur in all parts of the plant, notably, from the biotechnology point of view, in the seeds of the Gramineae though only to the extent of 1% or 2% (Meier and Reid, 1982). The best known cultivated dicotyledon is the Jerusalem artichoke (*Helianthus tuberosus*), the tubers of which, together with cereals, are the major source of inulin used as a special food for diabetics. Apart from use in diabetic diets some herbal extracts which are probably high in inulin were advocated as diuretics, while inulin itself is used in kidney clearance tests since it remains intact in the blood after injection for long periods.

Fructans are synthesized by the transfer of a fructose residue from sucrose onto another sucrose receptor molecules (Pollock and Chatterton, 1988). Enzyme systems capable of doing this in three different ways are known to exist leading to the main three types of fructans: isokestose fructans or 'inulins', kestose fructans and neokestose fructan or 'levans', although the terms 'inulin' is sometimes used to describe all these fructans. The isokestose inulins are linear chains with $\beta(2 \rightarrow 1)$ links of the general form

$$\text{Glc} - 1 \rightarrow 2 - \text{Fru} - 1 \rightarrow (2 - \text{Fru} - 1)_n \rightarrow 2 - \text{Fru}$$

with a maximum degree of polymerization (DP) claimed to be in the region of 30 to 40 (Praznik and Beck, 1987).

In terms of physical properties early work on Jerusalem artichoke inulin by Phelps (1965) suggested a weight average molecular weight, M_w of ~ 7000 Da (light scattering data of P. Johnson, not ultracentrifugation as reported in their abstract) and an intrinsic viscosity $[\eta]$ of ~ 7.1 ml g⁻¹. The problems of solubility in aqueous solvents was illustrated. Eigner et al. (1988) obtained a similar result for M_w on heated solutions of 7200 ± 100 Da using size-exclusion chromatography coupled to fixed low-angle light scattering measurements. Despite the small molecular size these workers were also able to obtain an estimate for the diffusion coefficient and this together with a radius of gyration R_g from small angle x-ray scattering suggested a rather elongated helical structure in solution. French (1988) on the basis of computer modelling suggested possible conformations

* Corresponding author

Table 1
Properties of Jerusalem artichoke and chicory root inulin in DMSO as a solvent

Property	Jerusalem artichoke	Chicory root
Partial specific volume (ml g ⁻¹)	0.601 ± 0.021	N.D.
Molecular Weight (Da)	3400 ± 150	6200 ± 200
Intrinsic viscosity [η] (ml g ⁻¹)	9.1 ± 0.2	10.7 ± 0.2
Sedimentation coefficient, <i>s</i> _{20,w} (S)	~0.4	~0.7

N.D.: not determined.

of the inulin polymer, ranging from compact to more extended structures.

In this paper we report a complementary hydrodynamic study on inulins from two different sources: Jerusalem artichoke and chicory root. To avoid problems of poor solubility in aqueous solvent media we follow the practice successfully used by workers on starch (see Dickinson et al., 1984) of using dimethyl sulphoxide (DMSO) as the solvent (Table 1).

2. Materials and methods

2.1. Materials

The inulins were supplied by Sigma (Poole, Dorset, UK) (lot no. I2880, I2255) using an alcohol recrystallization purification procedure. Pure-grade dimethyl sulphoxide (DMSO) was supplied by Fisons Scientific equipment Ltd (Loughborough, UK).

2.2. Densimetry

An Anton Paar (Graz, Austria) digital precision density meter of the mechanical oscillator type (Kratky et al., 1973) was used. Constant temperature was maintained using a Hetrofrig water bath and recorded using a Comar platinum resistance thermometer. A plot of solution density ρ (g ml⁻¹) vs concentration c (g ml⁻¹) yielded the density increment $\delta\rho/\delta c$ from which the partial specific volume v was evaluated according to (Kratky et al., 1973):

$$v = \frac{1}{\rho_o} \left(1 - \frac{\delta\rho}{\delta c} \right) \quad (1)$$

where ρ_o is the solvent density.

2.3. Viscometry

An Ostwald type viscometer of 2 ml capacity was used to determine the intrinsic viscosity of the inulins. The viscometer was suspended in a thermostatically controlled water bath (Schott-Geräte, FRG) maintained at 25.00 ± 0.01°C and flow times recorded automatically. Relative viscosities were obtained from the ratios of flow times of solution to solvent. Because of the low concentrations used (< 0.5 mg ml⁻¹) density corrections to convert kinetic to

dynamic viscosities (Tanford, 1955) were not significant. Intrinsic viscosities [η] were obtained from a Huggins (1942) plot of reduced viscosity η_{red} vs concentration c :

$$\eta_{red} = [\eta](1 + K_H[\eta]c) \quad (2)$$

where K_H is the Huggins constant.

2.4. Sedimentation velocity

An MSE (Crawley, UK) Centriscan ultracentrifuge was employed equipped with scanning Schlieren optics. The top rotor speed of 50000 rpm was chosen because of the small size and expected small sedimentation coefficient for the inulin. Sedimentation coefficients, s , obtained at 25.0°C in the DMSO solvent were corrected to standard conditions of solvent density and viscosity (that of water at 20°C) according to (Van Holde, 1985):

$$s_{20,w} = \left\{ \frac{1 - v\rho_{20,w}}{1 - v\rho_o} \right\} \cdot \left\{ \frac{\eta_o}{\eta_{20,w}} \right\} \cdot s \quad (3)$$

where ρ_o and η_o are the solvent density and viscosity at 25.0°C and $\rho_{20,w}$ and $\eta_{20,w}$ are the corresponding values for water at 20.0°C, the assumption is made that v is the same.

2.5. Sedimentation equilibrium

A Beckman (Palo Alto, USA) Model E ultracentrifuge was used equipped with an RTIC temperature measurement system and a 5 mW He–Ne laser light source. A rotor speed of 40000 rpm was employed at a rotor temperature of 21.0°C. Equilibrium solute distributions were recorded using Rayleigh Interference Optics and data capture semi-automatically from photographic film read into an LKB Ultrosan densitometer. Fringe distributions were converted into accurate records of relative concentration vs radial displacement using the Fourier cosine series algorithm ANALYSER (see Rowe et al., 1992). Apparent weight average molecular weights $M_{w,app}$ were obtained using the MSTARI routine (Colfen and Harding, 1997) using the M^* function and the identity $M^*(cell\ base) = M_{w,app}$ for the whole distribution of solute in the ultracentrifuge cell.

3. Results and discussion

A partial specific volume of 0.601 ± 0.021 ml g⁻¹ was

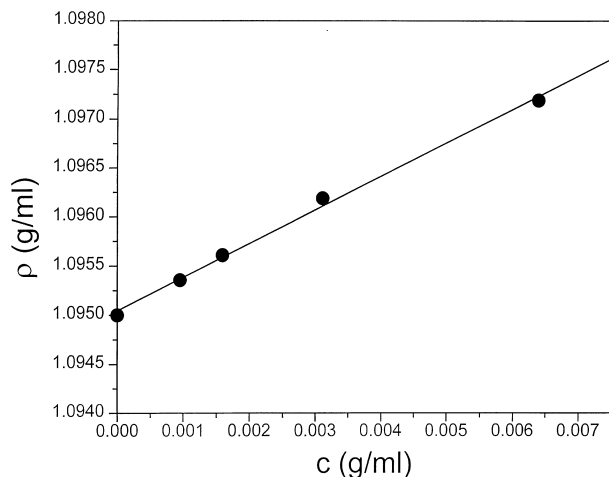


Fig. 1. Solution density vs concentration of Jerusalem artichoke inulin in DMSO obtained at a temperature of 25.00°C?. $\delta\rho/\delta c = (0.342 \pm 0.012) \cdot v = 0.601 \text{ ml g}^{-1}$.

obtained from the density increment plot (Fig. 1) for the inulin from Jerusalem artichoke. Since the saccharide composition of the two inulins was the same this value was taken as the same for the chicory root sample. The value obtained is in exact agreement with that of Phelps (1965) whose result was obtained in aqueous solvent.

Sedimentation velocity in the ultracentrifuge yielded sedimentation diagrams indicative of homogeneous preparations (see Fig. 2). However because of the very small size and rapid diffusion of the boundaries only an estimate for the sedimentation coefficient was possible for both species, with $s_{20,w}$ values of $\sim 0.4 \text{ S}$ (at 2.5, 3.4 and 5.2 mg ml^{-1}) for Jerusalem artichoke inulin and $\sim 0.7 \text{ S}$ (at 3.1 and 6.0 mg ml^{-1}) for chicory root inulin, values near the lowest limit measurable using current analytical ultracentrifuge technology.

It was considerably easier however obtaining quantitative information from sedimentation equilibrium. The higher equilibrium speeds (40000 rpm) were indicative of relatively small polysaccharides and Fig. 3 shows the extraction of $M_{w,app}$ from extrapolation of the M^* function to the cell base for both inulins. Values of $3400 \pm 150 \text{ Da}$ and $6200 \pm 200 \text{ Da}$ were obtained for the Jerusalem artichoke and chicory root inulins, respectively. Because of the low concentrations employed and the small size of these molecules, it is

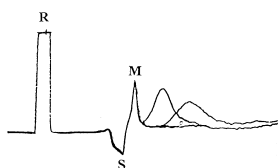


Fig. 2. Sedimentation diagrams taken at 20 min intervals of chicory root inulin in DMSO from a sedimentation velocity experiment. An MSE Centriscan ultracentrifuge was employed using scanning Schlieren optics at a rotor speed of 50000 rpm and temperature of 25.0°C.

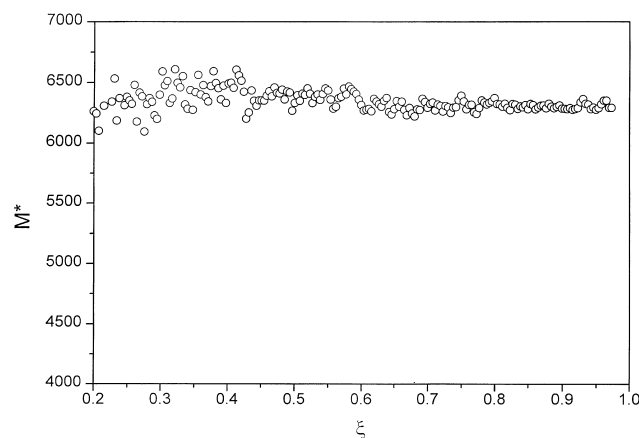
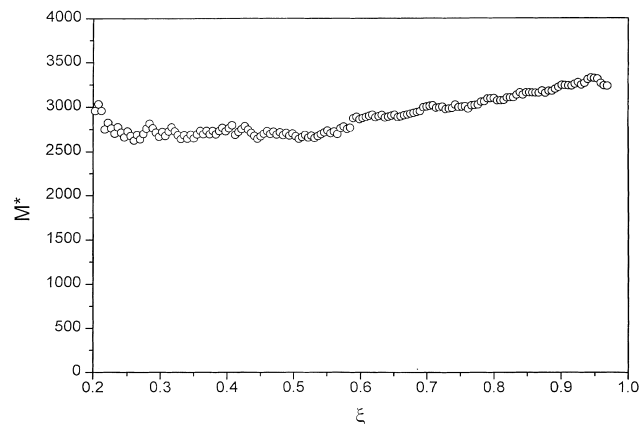


Fig. 3. Plot of M^* vs the radial displacement squared function ξ for: (a) Jerusalem artichoke inulin; and (b) chicory root inulin. Loading concentration $c \sim 0.7 \text{ mg ml}^{-1}$. Rotor speed = 40000 rpm. Solvent = DMSO. Temperature = 21.0°C. $\xi = (r^2 - a^2)/(b^2 - a^2)$ where r is the radial displacement at a given point in the solution distribution and a , b the radial displacement values at the meniscus and cell base, respectively. $M^*(\xi \rightarrow 1) = M_{w,app}$ (Creeth and Harding, 1982).

reasonable to assume that thermodynamic non-ideality is not significant and that $M_{w,app} \sim M_w$.

Fig. 4 shows the reduced viscosity vs concentration plots for both inulins, with Chicory root showing a slightly higher intrinsic viscosity $[\eta]$, $10.7 \pm 0.2 \text{ ml g}^{-1}$ compared with $9.1 \pm 0.2 \text{ ml g}^{-1}$. A non-linear fit to the latter data may be more appropriate and is indicative of a value for $[\eta]$ closer to 10 ml g^{-1} . The relatively small difference in intrinsic viscosities relative to the difference in molecular weights between the two species (MHKS a coefficient of ~ 0.3) is suggestive of an extended spheroidal conformation as opposed to a completely random coil ($a \sim 0.5\text{--}0.8$) or a linear rod ($a \sim 1.0\text{--}1.8$) and could well be consistent with the modest asymmetry (axial ratio ~ 3.8) proposed by Eigner et al. (1988) on the basis of x-ray scattering and dynamic light scattering measurements on heat-dissolved Jerusalem artichoke inulin in aqueous solvent. It is interesting to speculate on the extent of solvent association for such a structure. A

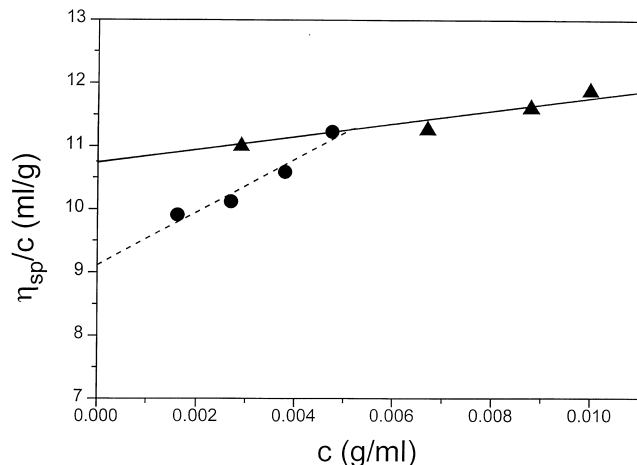


Fig. 4. Plot of reduced viscosity η_{sp}/c vs concentration c for Jerusalem artichoke (○) and chicory root inulins (△). Solvent = DMSO. Temperature = $(25.00 \pm 0.01)^\circ\text{C}$.

prolate ellipsoid of revolution of axial ratio 3.8 would have a value for the viscosity increment ν of 4.45 (using the routine ELLIPS2 of Harding et al., 1997). Substitution in the Simha–Saito equation

$$\nu = [\eta]/\nu_s \quad (4)$$

yields a value of $\sim 2.2 \text{ ml g}^{-1}$ for the swollen specific volume ν_s suggesting an expansion ratio (volume of solvated macromolecule/volume of anhydrous molecule) of $(2.2/0.601) \sim 4$. Of course if the molecule is more compact (as allowed by French, 1988 who presented a series of possible conformations of inulin based on energy minimization/steric hindrance considerations) the extent of solvent association would be greater.

Using the hydrodynamic values $[\eta]$, $s_{20,w}$ and molecular weight we can also estimate the value of the hydrodynamic invariant A_o (related to the Flory parameter Φ_o , see Tsvetkov et al., 1970).

$$A_o = [R\eta_o/(1 - \nu\rho_o)] \cdot s_{20,w} \cdot [\eta]^{1/3} \cdot M^{-2/3} \quad (5)$$

where R is the molar gas constant we obtain a value of $\sim 2 \times 10^{-10}$, within reasonable agreement of the theoretical value of 3.84×10^{-10} (Tsvetkov et al., 1970). If we take for the length of the repeat unit = 2.16 \AA (Marchessault et al., 1980) the estimate leads to the conclusion that the Kuhn segment length $\lambda^{-1} > 50 \text{ \AA}$ (persistence length $L_p = \lambda^{-1}/2 > 25 \text{ \AA}$) is consistent with a reasonably flexible molecule.

References

- Colfen, H., & Harding, S. E. (1997). MSTARA and MSTARI: interactive PC algorithms for simple, model independent evaluation of sedimentation equilibrium data. *Eur. Biophys. J.*, *25*, 333–346.
- Creeth, J. M., & Harding, S. E. (1982). Some observations on a new type of point average molecular weight. *J. Biochem. Biophys. Methods*, *7*, 25–32.
- Dickinson, E., Lelievre, J., Stainsby G., & Waight, S. (1984). The polymers in large and small wheat granules. In *Applications of Hydrocolloids II* (pp. 277–284). New York: Pergamon Press.
- Eigner, W., Abuja, P., Beck, H. F., & Praznik, W. (1988). Physico-chemical characterisation of inulin and sinistrin. *Carbohydr. Res.*, *180*, 87–95.
- French, A. D. (1988). Accessible conformation of the β -D-(2 \rightarrow 1) linked D-fructans inulin and levan. *Carbohydr. Res.*, *176*, 17–29.
- Fuchs, A. (1987). Potential of non-food utilization of fructose and inulin. *Starch*, *39*, 335–343.
- Harding, S. E., Horton, J. C., & Colfen, H. (1997). The ELLIPS suite of macromolecular conformation algorithms. *Eur. Biophys. J.*, *25*, 347–360.
- Haworth, W. N., Hirst, E. L., & Percival, E. G. V. (1932). Polysaccharides. Part XV. The molecular structure of inulin. *Journal of the Chemical Society, Part 2*, pp. 2384–2388.
- Huggins, M. L. (1942). The viscosity of dilute solutions of long-chain molecules IV. Dependence on concentration. *J. Am. Chem. Soc.*, *64*, 2716.
- Kratky, O., Leopold, H., & Stabinger, H. (1973). The determination of partial specific volume of proteins by the mechanical oscillation technique. *Meth. Enzymol.*, *27D*, 98–110.
- Marchessault, R. H., Bleha, T., Deslandes, Y., & Revol, J. F. (1980). Conformation and crystalline structure of (2 \rightarrow 1)- β -D-fructofurana (inulin). *Can. J. Chem.*, *58*, 2415–2422.
- Meier, H., & Reid, J. S. G. (1982). Reserve polysaccharides other than starch in higher plants. In F. A. Loewus, & W. Tanner (Eds.), *Plant carbohydrates* (chap. 11). New York: Springer.
- Phelps, C. F. (1965). The physical properties of inulin solutions. *Biochem. J.*, *95*, 41–47.
- Pollock, C. J., & Chatterton N. J. (1988). Fructans. In P. K. Stumpf, & E. E. Cohn (Eds.), *The biochemistry of plants. A comprehensive treatise* (vol. 14, chap. 4). New York: Academic press.
- Praznik, W., & Beck, R. H. F. (1987). Inulin composition during growth of tubers of *Helianthus tuberosus*. *J. Ag. Biol. Chem.*, *51*, 1593–1599.
- Rowe A. J., Wynne-Jones S., Thomas D. G., & Harding S. E. (1992). Methods for off-line analysis of sedimentation velocity and sedimentation equilibrium patterns. In S. E. Harding, A. J. Rowe & J. C. Horton (Eds.), *Analytical ultracentrifugation in biochemistry and polymer science* (chap. 5). Cambridge, UK: Royal Society of Chemistry.
- Schacht, E. (1985). Synthesis of succinoylated inulin and application as a carrier for procainamide. *Ann N.Y. Acad. Sci.*, *446*, 199–212.
- Silver, R. F. (1996). Use of inulin as a natural texture modifier. *Cereal Foods Wld*, *41*, 792–794.
- Tanford, C. (1955). Intrinsic viscosity and kinematic viscosity. *J. Phys. Chem.*, *59*, 798–799.
- Van Holde, K. E. (1985). *Physical biochemistry*. Englewood Cliffs, NJ: Prentice Hall.
- Tsvetkov, V. N., Eskin, V. E., & Frenkel, S. Ya. (1970). *Structure of macromolecules in solutions*. London: Butterworths.