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Review

# On hydrodynamic methods for the analysis of the sizes and shapes of polysaccharides in dilute solution: A short review

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ABSTRACT

*Background:* Polysaccharides and their derivatives are increasingly being used by the food, cosmetic and pharmaceutical industries: physical properties like size and conformation are important contributors to their performance.
*Discussion:* Here the use of hydrodynamic tools such as sedimentation velocity, sedimentation equilibrium, size exclusion chromatography – multi-angle light scattering (SEC-MALS), and viscometry are considered highlighting some recent developments in methodology and the application of these to help better understand polysaccharide structure–function relationships.
*Conclusions:* The size and shape of polysaccharides in solution can be estimated in a variety of ways. Molar masses and heterogeneities can be estimated to a good precision by sedimentation velocity, sedimentation equilibrium and SEC-MALS. An approximate idea of conformation and flexibility can be obtained from power-law coefficients and the Wales van Holde parameter. More sophisticated estimates can be obtained by combining methods together to yield the persistence length.


**Contents**



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## 1. Introduction

The last two decades has seen considerable advances in hydrodynamic methodology for the analysis of the dilute solution properties of polysaccharides. Advances include improved ways in which we can ascertain the molar mass (molecular weight) or molar mass distribution of polysaccharide systems using size exclusion chromatography coupled to multi angle light scattering (Wyatt, 1993) and sedimentation based techniques using the analytical ultracentrifuge (Harding, Abdelhameed, & Morris, 2010; Schuck et al., 2014). There have also been important advances in the way we can use these techniques in combination – and with other techniques like viscometry to characterise the shape and flexibility of polysaccharides in the environment in which many occur naturally – in solution. The focus of this article is to highlight some of the recent advances in hydrodynamic methodologies for estimating the size and conformation of some industrially important polysaccharides (Table 1).

## 2. Estimation of size

### 2.1. Sedimentation velocity (SV)

In a centrifugal field solute molecules will sediment towards the cell base, therefore the region near the meniscus will be depleted of solute and there will be a region nearer the cell base where the solute concentration is uniform and a transitional region is created (the "boundary region") where the solute concentration varies with distance from the axis of rotation. It is the rate of movement of the concentration distribution with time which allows the calculation of sedimentation coefficients and distribution of sedimentation coefficients (see, *e.g.*, Dam & Schuck, 2004; van Holde, 1985; Ralston, 1993; Schuck, 1998; Stafford, 1992). The progression of the concentration distribution with time is recorded by an optical system. Since polysaccharides are not usually absorbing in the visible or (near) ultraviolet region, the refractometric or Rayleigh interference optical system is the most useful, using a laser light source. Double-sector cells are employed with solution and reference solvent (dialysate) in each channel. A series of parallel Rayleigh interference fringes are captured on a CCD camera. These register the concentration distribution at regular time intervals throughout the experiment. The change in the distribution with time yields both the weight average sedimentation coefficient ($s$) measured in seconds (s) or Svedberg units (S) in which $1\ \mathrm{S} = 10^{-13}$ s and the distribution of sedimentation distribution $g(s)$.

(i) To facilitate comparisons, the $s$ value (a measure of the size and shape of the polysaccharide) is usually corrected to standard conditions (density and viscosity of water at 20.0 °C), to give $s_{20,w}$, and this is done using a database algorithm known as SEDNTERP (Laue, Shah, Ridgeway, & Pelletier, 1992; Laue & Stafford, 1999).

(ii) To correct for non-ideality the $s$ (or $s_{20,w}$) value is extrapolated to zero concentration to give $s^0_{20,w}$, using for example the Gralén relation (Gralén, 1944):

$$\frac{1}{s_{20,w}} = \frac{1}{s^0_{20,w}}(1 + k_s c) \tag{1}$$

where $k_s$ (mL g$^{-1}$) is the concentration dependence regression coefficient. For more severely concentration dependent systems other relations such as the equation of Rowe (1977, 1992) can be used. Alternatively low loading concentrations can be employed (it is possible to make measurements below 0.1 mg mL$^{-1}$), when $s_{20,w} \sim s^0_{20,w}$ is a reasonable approximation.

(iii) Besides non-ideality which needs to be accounted for as described above, the distribution $g(s)$ vs. $s$ will be affected by diffusion broadening (although polysaccharides are usually much slower diffusing compared to proteins). Dam and Schuck (2004) have described a procedure for making an approximate correction based on the assumption that all the species can be represented by an average frictional ratio. The diffusion corrected distribution is known as a $c(s)$ vs. $s$ plot.

(iv) Plots of $g(s)$ and $c(s)$ plots by themselves can provide a useful measure of heterogeneity (*e.g.* in mixed polysaccharide systems such as starch).

(v) Plots of $g(s)$ vs. $s$ (or $c(s)$ vs. $s$) plots can be converted into molar mass distributions provided the conformation/conformation type (sphere, rod, coil etc) of the polysaccharide is known or can be reasonably assumed. The procedure is known as the "Extended Fujita" method (Harding et al., 2011) and has recently been incorporated into the highly popular SEDFIT platform of algorithms to estimate the molar mass distribution (Fig. 1a and b) of heterogeneous systems including polysaccharides and mucins (Gillis et al., 2013; Harding et al., 2011).

One limitation is that this *Extended Fujita* (Fujita, 1962) method does need calibrating for each particular conformational system. The conformation coefficient $b$ and constant $\kappa_s$ in the transformations:

$$M = (s/\kappa_s)^{1/b} \tag{2}$$

and

$$f(M) = \mathrm{d}s/\mathrm{d}M \cdot g(s) \tag{3}$$

where

$$\mathrm{d}s/\mathrm{d}M = b \cdot \kappa_s^{1/b} \cdot s^{(b-1)/b} \tag{4}$$

are needed; if the conformation is known then this will define b: random coils $b \sim 0.4$–$0.5$; spheres, $b \sim 0.67$, rod shaped molecules $b \sim 0.2$). Knowledge of the both the weight average sedimentation coefficient and corresponding weight average molar mass from a sedimentation equilibrium experiment or SEC-MALS (Size Exclusion Chromatography coupled to Multi-Angle Light Scattering) can then be used to define $\kappa_s$, using equation (2).

If $b$ is also unknown then a number of pairs of $s$–$M$ values are required (see Section 3a and Fig. 1b).

### 2.2. Sedimentation equilibrium (SE)

In contrast to sedimentation velocity, sedimentation equilibrium requires lower angular velocities depending on the size of the macromolecule (van Holde, 1985). As the solute sediments towards the cell base the concentration therefore increases at base, this sets up a diffusion gradient, which opposes that of sedimentation. After a certain amount of time the two processes reach dynamic equilibrium leading to a steady state pattern of solute concentration increasing towards the cell base. As there is no net movement of solute at equilibrium the final pattern is not affected by frictional/conformation properties and is an absolute function of molar mass and polydispersity. For thermodynamically non-ideal and polydisperse systems such as polysaccharides, solute distributions at sedimentation equilibrium can be analysed using the MSTAR

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