Table 3 Dielectric constant of solvents at 25°C

Solvent	*Dielectric constant*
Water	78
Nitrobenzene	35
Methanol	33
Ethanol	24
Ammonia	17
Hydrogen sulfide	9 (at − 85°C)
Benzene	2
Carbon tetrachloride	2
Cyclohexane	2

and agglomerating before dissolving, then it is likely that the dissolution will be fast. This is an area which requires further investigation.

MAE extractions of polymers have not been reported as much as SFE and PFE extractions, but those that have indicate very rapid extractions. In some cases, the most time-consuming part is the cooling of the vessels before opening. The advantages over PFE are more rapid heating and no possibility of blocking the transfer lines. The disadvantages are a more limited choice of solvents and longer cooling down times. SFE, PFE and MAE offer distinct advantages over conventional extraction methods, but at higher initial cost. The particular method chosen will depend on the exact requirements for extractions in the individual laboratory.

See also: **II/Extraction:** Microwave-Assisted Extraction; Supercritical Fluid Extraction. **III/Microwave-Assisted Extraction: Environmental Applications. Pressurised Fluid Extraction: Non-Environmental Applications.**

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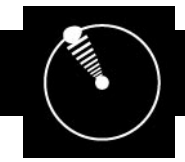
POLYSACCHARIDES



Centrifugation

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Polysaccharides are, as their name implies, polymers of saccharide residues. The general formula of saccharide or carbohydrate residues is often quoted as $(CH_2O)_n$, although this is an oversimplification which needs to be modified in many cases to take into account, e.g. amino, sulfate and phosphate groups. Most saccharide residues are five or six membered ring structures with one member of the ring being oxygen.

Polysaccharides are becoming increasingly important in biomedical, pharmaceutical food and health products. The role of preparative centrifugation in polysaccharide development has not been significant compared to other classes of compounds such as nucleic acid and proteins. In part, this may be due to the considerable heterogeneity of polysaccharides, and techniques such as chromatography and precipitation-based methods have been more commonly applied. However, *analytical* centrifugation techniques are becoming increasingly used as a means of characterizing the size and shape of polysacchrides in solution as well as for investigating their interaction with each other and with other biopolymers such as pro-

teins. Analytical centrifugation is also being used as an alternative to rheological methods for investigating the structure of polysaccharide gels. Analytical centrifugation is well suited for the analysis of polydisperse materials and (apart from density gradient methods) does not involve another separation medium.

The aim of this article is threefold:

1. to survey briefly the types of information that can be obtained about polysaccharide systems by analytical centrifugation;
2. to describe those aspects of the equipment that are of particular relevance for obtaining this information;
3. to describe the types of experiments used to obtain this information.

The centrifugation methods applied to macromolecules are termed *ultra*centrifugation, because of the high rotor speeds (up to 60 000 rpm) required to produce sedimentation or a measurable concentration redistribution. At these high speeds the rotor needs to be in a vacuum chamber to avoid friction-heating effects. An analytical ultracentrifuge is an ultracentrifuge equipped with a special optical system for monitoring the sedimentation process.

Despite the fact that Svedberg, who won the Nobel Prize in 1926 for inventing the analytical ultracentrifuge, and his PhD student Gralen both conducted extensive work on the ultracentrifugation of polysaccharides, it is only relatively recently that the technique has been more widely utilized for the study of these substances.

Measurable Parameters

Molecular Weights and Heterogeneity

One of the most fundamental pieces of information describing a macromolecule is its molecular weight, M. M values for polypeptides can usually be evaluated without difficulty from chemical sequence information. For polysaccharides this is not the case, primarily because of their heterogeneity due to polydispersity and also, in some cases, self-association phenomena. An average molecular weight, usually the weight average, M_w, is normally specified. M_w is usually available to better than $\pm 10\%$ from sedimentation equilibrium in the ultracentrifuge, and M_z to somewhat less precision depending on the optical detection system employed. In some circumstances M_n can also be measured, although osmotic pressure is a more appropriate technique. The ratios of the z average molecular weight, M_z to M_w or M_w to the number-average, M_n are used as indices for polydispersity, popular with commercial manufacturers of polysaccharides as a measure of the narrowness of a molecular weight distribution.

Direct molecular weight distributions are more difficult to obtain with centrifugation procedures and depend on assumed models (Gaussian, log-normal etc.): nowadays size exclusion chromatography systems coupled online to multi-angle light-scattering systems, or SEC-MALLS are much more common, but the ultracentrifuge still provides a valuable check on the M_w or M_z.

A good indication of chemical heterogeneity (e.g. purity) of a saccharide system can be provided by analytical isopycnic density gradient ultracentrifugation.

Molecular Shape or Gross Conformation

In general two levels of information are sought:

1. Delineation between the conformation type or zone of a polysaccharide, as represented by the Zonal diagram of **Figure 1**: extra rigid rod, rigid rod, semi-flexible coil, random coil and globular/highly branched.
2. Having established the conformation type, more quantitative information can be sought. For example, for approximately rigid structures this can be given in terms of the triaxial dimensions or shape of the molecule: for more flexible chain-like structures the chain diameter, d, contour length L and the flexibility can be described in terms of the persistence length, L_p (**Figure 2**).

Water Binding or Hydration

The sedimentation coefficient from sedimentation velocity experiments in the ultracentrifuge depends not only on molecular conformation but also the extent of solvent binding (chemical interactions via, for example, hydrogen bonds or just physical entrainment). Water binding is an important functional property of polysaccharides (for example, in foods, or as hydrogel drug delivery forms). Unfortunately, the conformation has to be known or assumed to obtain the extent of swelling through water binding: conversely, assumptions of water binding extent are often required to obtain quantitative conformation information.

Analytical Ultracentrifuges and Polysaccharides

Analytical ultracentrifuges have already been described by Lewis elsewhere in this encyclopedia. The traditional ultracentrifuges, such as the classical Model E analytical ultracentrifuge from Beckman

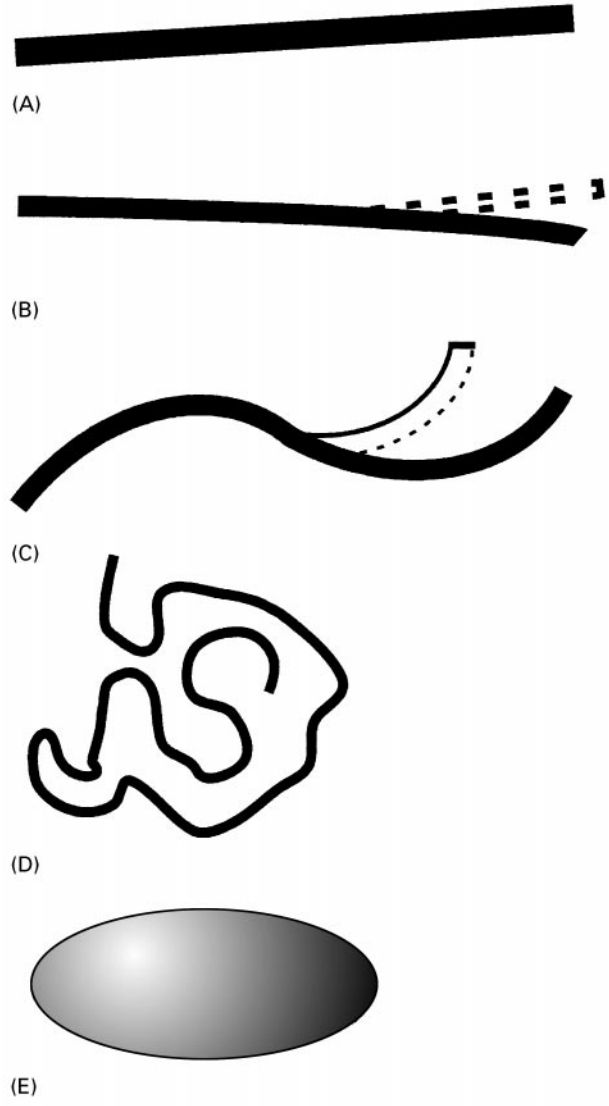

Figure 1 Conformation zoning of polysaccharides: (A) extra rigid rod; (B) rigid rod; (C) semi-flexible coil; (D) random coil; (E) globular or highly branched. (Reproduced from Pavlov GM, Rowe AJ and Harding SE (1997) Conformation zoning of large molecules using the analytical ultracentrifuge. *Trends in Analytical Chemistry* 16: 401, with permission from Elsevier Science.)

Instruments (Palo Alto, USA), have now largely been replaced by the new generation Optima XL-A and XL-I, also from Beckman. Both have full online computer data capture and analysis facilities.

Optima XL-A Ultracentrifuge

This model appeared in *c.* 1990 and is equipped with a UV/visible absorption optical detection system. Unfortunately, polysaccharides are generally transparent in the visible and also, unlike proteins and nucleic acids, in the near UV (wavelength $\lambda > 240$ nm). In the far UV (200–220 nm), they absorb and can therefore in principle be detected. Unfortunately other materials such as buffer salts also absorb strongly in this region and hence for many applications an absorbing chromophore must be attached to the polysaccharide. Some success has been achieved with this latter approach, although possible alterations to the structure and molecular weight (e.g. a state of self-association) caused by the incorporation of the chromophore can result in measurement errors. Other polysaccharides, such as xylans and pectins, have some inherent absorbance at $\sim$240–260 nm and this can be taken advantage of.

Optima XL-I Ultracentrifuge

This model appeared in *c.* 1996 and, in addition to the UV/visible absorption optics of the XL-A, has a refractometric optical system known as Rayleigh interference optics. This optical system can be applied to proteins, nucleic acids and polysaccharides. One drawback for sedimentation equilibrium (molecular weight) work is that, because of the upper limit of $\sim$ 12 mm of the optical path length of the centrifuge cell, the lower limit for the concentration of the polysaccharide solution loaded into the cell is ~ 0.8 mg mL^{-1}. This is a major limitation for polysaccharides since low concentrations are necessary to minimize the effects of the large thermodynamic nonideality of these substances, compared to proteins.

Model E Ultracentrifuge

These have not been commercially available for 20 years but there are a number still in active use, although only a few are being applied to the study of polysaccharides. Besides Rayleigh interference, they also have another type of optical system, not current-

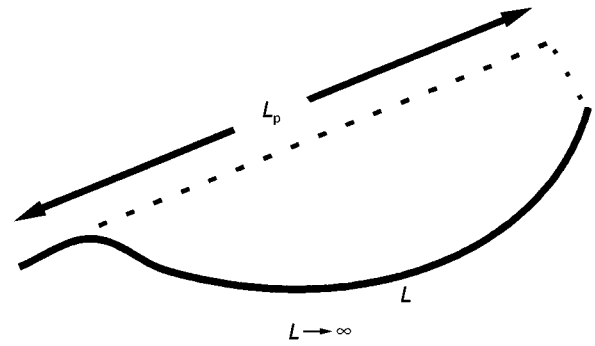

Figure 2 More detailed conformation representations of polysaccharides in solution: length and flexibility parameters for a linear polysaccharide: L, Contour length; L_p, persistence length, defined as the projection length along the initial direction of chain of length L and in the limit of $L \rightarrow$ infinity. (Reproduced with permission from Tombs and Harding 1998.)

ly available on the Optima machines, the Schlieren or refractive index gradient system, permitting high concentrations and facilitating gel work. For molecular weight analysis, Rayleigh optics give primarily M_w, whereas Schlieren optics yield M_z. Thus the model E is ideal for obtaining polydispersity indices (M_z/M_w, see above). However, because of the lack of availability of this instrument, people requiring this type of measurement need to consult the handful of laboratories still running these instruments.

Types of Ultracentrifuge Experiment for Polysaccharide Analysis

The principal types of measurement using the analytical ultracentrifuge on solutions of polysaccharide are:

- Sedimentation equilibrium: for obtaining the weight average molecular weight, M_w, and the z average molecular weight, M_z. Molecular weights are conventionally expressed in daltons or in molar mass units (g mol^{-1}). The thermodynamic second virial coefficient, B (mL mol^{-1} g^{-2}) can also be measured.
- Sedimentation velocity: for obtaining the sedimentation coefficient, s (measured in seconds (s) or svedbergs (S), where 1 S = 10^{-13} s) and the concentration dependence or Gralen parameter k_s (mL g^{-1}) of the sedimentation coefficient. Both s and k_s can be used to obtain conformation, water binding and molecular weight information.
- Boundary spreading experiments: for obtaining the translational diffusion coefficient, D (cm^2 s^{-1}) and its concentration dependence parameter, k_D (mL g^{-1}). The polydispersity of polysaccharides is however a major complicating factor.
- Analytical isopycnic density gradient ultracentrifugation: primarily used for assaying the purity of a polysaccharide preparation on the basis of density.

In addition, sedimentation velocity and equilibrium can be used to probe the structure of gels, primarily through measurement of the swelling pressure of the gel, and also the mobility/diffusion of molecules through gels or incompatible mixed-phase systems.

Sedimentation Equilibrium of Polysaccharide Solutions

In this method the ultracentrifuge is spun at a speed sufficiently low (for example, ~10 000 rpm for a pectin polysaccharide of $M_w \sim 200$ kDa) that the centrifugal force is comparable to the back-force due to diffusion. The distribution of the polysaccharide in the centrifuge cell is recorded using Rayleigh optics and equilibrium is typically established between 24 and 48 h. Attainment of equilibrium is assessed by comparing optical records taken at 4–8 h intervals. The time required to attain equilibrium depends on the polysaccharide and the length of solution column in the centrifuge cell: a 3 mm column requires ~ 0.1 mL for a 12 mm optical path length cell (XL-I or Model E) or ~ 0.25 mL for a 30 mm cell (Model E only). Shorter columns take less time but yield less precise information. The length of time of an experiment can be compensated for by running samples in multiples using multi-hole centrifuge rotors and/or multi-channel centrifuge cells.

Molecular Weight Determination

The simplest interpretation of the optical records from a sedimentation equilibrium experiment is obtained from the average slope of a plot of the log of the concentration, $C(r)$ in the cell versus the square of the radial displacement, r, from the rotor centre: such a slope yields the apparent weight–average molecular weight, $M_{w,app}$. In evaluating the average slope, care must be taken to include as much of the distribution in the centrifuge cell as possible (from meniscus to cell base). Fortunately, software (MSTAR) is available to assist with this task. Rayleigh optics only record concentration of solute relative to the meniscus, thus requiring the concentration at the meniscus to be determined in order to convert relative concentrations to $C(r)$. After these factors have been taken into account, extrapolation of $1/M_{w,app}$ to $C = 0$ (where C is the cell loading concentration) yields the reciprocal of the weight average M_w from the intercept and, also the second thermodynamic virial coefficient, B, or as an alternative notation A_2 from the limiting slope. (This term also appears in osmotic pressure or static light-scattering measurements.) Correct to first order in concentration, this extrapolation is described by:

$$1/M_{w,app} = (1/M_w) \cdot (1 + 2BM_wC) \qquad [1]$$

Herein lies a difference between proteins and polysaccharides. The nonideality term $2BM_wC$, which can often be neglected at low concentration for proteins, is usually significant even at low concentration for polysaccharides because of their much greater nonideality: indeed, the term $1 + 2BM_wC$ represents the factor by which measurement of $M_{w,app}$ at a finite concentration C underestimates the true molecular weight (**Table 1**). This nonideality derives from the large exclusion volumes of these substances and from the fact that many are polyelectrolytes (highly negatively charged, such as pectins, alginates, xanthan, carrageenan, or highly positively charged, such as chitosans). However, although these polyelectrolyte

Table 1 Comparative molecular weights and nonidealities of polysaccharides from sedimentation equilibrium measurements

	$10^{-6} \times M_w$ (g mol^{-1})	$10^{4} \times B$ (mL mol^{-1} g^{-2})	BM_w (mL g^{-1})	$1 + 2BM_wC^a$
Pullulan P5	0.0053	10.3	5.5	1.002
Pullulan P50	0.047	5.5	25.9	1.010
Xanthan (fraction)	0.36	2.4	86	1.035
β-glucan	0.17	6.1	104	1.042
Dextran T500	0.42	3.4	143	1.057
Pullulan P800	0.76	2.3	175	1.070
Chitosan (Protan 203)	0.44	5.1	224	1.090
Pullulan P1200	1.24	2.2	273	1.109
Pectin (citrus, fraction)	0.045	50.0	450	1.180
Scleroglucan	5.7	0.50	570	1.228
Alginate	0.35	29.0	1015	1.406

[a]Based on the lowest possible loading concentration ($\sim$ 0.2 mg mL^{-1} in a cell with a 30 mm path length centrepiece).

effects can be largely suppressed by the inclusion of a low molecular weight electrolyte to increase the ionic strength of the solution, eqn [1] is usually only valid for dilute solutions of polysaccharides and at higher concentrations, extra terms (in C^2 or C^3) are necessary. In such cases direct nonlinear, extrapolation of $M_{w,app}$ to $C = 0$ usually gives a better estimate of M_w than the reciprocal ($1/M_{w,app}$) extrapolation procedures. The other big difference between proteins and polysaccharides is that the latter are usually very polydisperse.

It is also possible from a single experiment to obtain local or point average $M_{w,app}(r)$ at radial positions r, as a function of $C(r)$. Extrapolation of $M_{w,app}(r)$ to $C(r) = 0$ provides a further estimate for M_w, although because of redistribution of solute in the centrifuge cell this can yield and underestimate for M_w. From further manipulations, including concentration extrapolations to the radial positions of the solution meniscus and bottom of the centrifuge cell, the (apparent) z average molecular weigh $M_{z,app}$ can be obtained. Similar corrections to $C = 0$ are required to obtain M_z (although the factor in eqn [1] is 4, not 2). A more precise estimate for $M_{z,app}$ can be obtained if Schlieren optics are used instead of Rayleigh optics: this is because Schlieren optics record the concentration gradient, $dC(r)/dr$ (as opposed to $C(r)$) versus radial displacement, r.

In cases where there may be reversible self-association phenomena, the B term in eqn [1] must be modified to incorporate an association term.

Sedimentation Velocity of Polysaccharide Solutions

At higher speeds than used for sedimentation equilibrium, sedimentation forces become well in excess of diffusion forces and a macromolecular species will sediment. The sedimentation coefficient, s, of a macromolecular component is its sedimentation rate per unit centrifugal field. It will be a function of the size, shape and degree of water association or hydration of the biopolymer. Like the apparent molecular weight, $M_{w,app}$, it will be affected by the nonideality of the system, and will need correcting for these effects by measuring at a series of concentrations and extrapolating to zero concentration to yield the ideal or infinite dilution value s^o. For polysaccharides this is best accomplished with a reciprocal plot:

$$1/s_{20,w} = (1/s^o_{20,w}) \cdot (1 + k_s C) \qquad [2]$$

where C is the sedimenting concentration and k_s is the concentration dependence or Gralen parameter. The subscripts 20,w mean that, by convention, sedimentation coefficients measured at a given temperature are corrected using simple formulae to standard conditions of solvent viscosity and density: that of water at 20°C.

As with eqn [1] for M_w, eqn [2] for $s^o_{20,w}$ is generally only applicable for dilute solutions of polysaccharides: for higher concentrations, and even for dilute solutions of certain extremely nonideal polysaccharides like alginate and xanthan, higher order terms in C are needed to describe adequately the concentration dependence. As with M_w, where such higher terms are necessary, direct extrapolation of $s_{20,w}$ to $C = 0$ rather than $1/s_{20,w}$ is generally a more reliable procedure for estimating $s^o_{20,w}$. Both $s^o_{20,w}$ and k_s can be used for conformation and hydration analysis, and also as a less direct alternative to sedimentation equilibrium for molecular weight estimation.

Besides the use of higher rotor speeds (for example, $\sim$50 000 rpm for a pectin polysaccharide of $s^o_{20,w} \sim 2$ S), longer solution column lengths are gener-

ally used (~1 cm corresponding to ~0.4 mL of solution in a 12 mm path length Optima XL-I centrifuge cell), compared with sedimentation equilibrium experiments.

General Conformation Analysis of Polysaccharides

The Wales–van Holde ratio A useful guide to the gross conformation is the Wales–van Holde ratio. The Wales–van Holde ratio is defined as the ratio of the Gralen parameter k_s to the intrinsic viscosity $[\eta]$. The intrinsic viscosity can be measured relatively simply by viscometric or rheological methods, and both parameters are expressed in units of mL g^{-1}. It is known that this ratio has values of about 1.6 for random coils and spherical conformations and reduces to a limiting value of approximately 0.2 for rigid rod-shaped molecules. A proviso for the use of k_s in this way is that, for highly charged macromolecules, charge effects must be suppressed by using (aqueous) solvents of sufficient ionic strength (**Table 2**).

The Mark–Houwink–Kuhn–Sakurada Representation The Wales–van Holde ratio can be used to distinguish rods from either spheroidal or random coil conformations but cannot be used to distinguish between the latter two. However, advantage can be taken of the polydispersity of polysaccharides in that if the sedimentation coefficient and molecular weights are measured for a series of fractions (separated by column chromatographic procedures) of a polysaccharide preparation, then the exponent in the relation between $s^o_{20,w}$ and M weight or volume-average can be used to distinguish random coils from rods and spheroidal conformation types:

$$s^o_{20,w} = K'' M^b \qquad [3]$$

Table 2 Values of the Wales–van Holde ratio $k_s/[\eta]$ and the Mark–Houwink–Kuhn–Sakurada b coefficient for some polysaccharides

	$k_s/[\eta]$	b	*Conformation*
Dextran fractions		0.44	Random coil
DIT-dextrans		0.56	Semi-flexible coil
Pullulans	1.4	0.45	Random coil
Yeast mannan	1.3	0.43	Random coil
β-glucans	0.4		Extended
Alginates	0.6		Extended
Pectins (low methoxy)	0.2	0.17	Rigid rod
Xanthan (keltrol)	0.28		Rigid rod
Amylopectin*	1.45		Spheroidal/heavily branched

DIT, di-iodotyrosine dextran. All in aqueous solvent except* (90% dimethyl sulfoxide).

Eqn [3] is known as a Mark–Houwink–Kuhn–Sakurada (MHKS) or just Mark–Houwink relation. Other MHKS relations exist between intrinsic viscosity and M (exponent a or α), radius of gyration and M (exponent c or ν) and the diffusion coefficient and M (exponent-ε). The exponent b (sometimes given as 1-b), measured from double logarithmic plots of $s^o_{20,w}$ versus M, has characteristic values depending on the conformation type: these are 0.67, 0.4–0.5 and 0.15 for spheres, random coils and rigid/extra rigid rods respectively (**Table 2**).

If K'' and b have been measured for a series of polysaccharide fractions, the molecular weights of subsequent preparations of that polysaccharide can then be estimated from measurement of $s^o_{20,w}$.

Conformation zoning from combination of $s^o_{20,w}$, k_s and the mass per unit length An even better guide to the overall conformation type or zone (**Figure 1**) can be obtained by combining $s^o_{20,w}$, k_s and the mass per unit length, M_L. The latter parameter must be measured using a separate technique, but a number of M_L values for polysaccharides have been published on the basis of electron microscopy or X-ray diffraction studies. The concept of zoning in this manner was conceived in terms of empirical data of polysaccharides of known conformation (**Figure 3**A), supported by the theoretically known limits for spheres and extra-rigid rods, to produce the characteristic diagnostic plot of **Figure 3**B.

For a polysaccharide whose conformation type or zone is unknown, from measurement of $s^o_{20,w}$ and k_s from a sedimentation velocity experiment and knowledge of, or an assumption concerning, M_L, the conformation zone can be read directly from **Figure 3**B.

More Detailed Conformation Analysis of Polysaccharides

Once the conformation type or zone has been established, more detailed information about conformation can be sought. It is worth stressing that, because of the inherent polydispersity of polysaccharides, much of the information obtained can only represent average properties: claims of parameter measurements to a high precision should be treated with some caution.

$s^o_{20,w}$-M relations: worm-like coil modelling The simple MHKS dependence of $s^o_{20,w}$ on M described above has been extended for linear polymers (zone A–D molecules of Figure 1), including DNA and polysaccharides to include terms containing the mass per unit length M_L (or molecular weight M and the

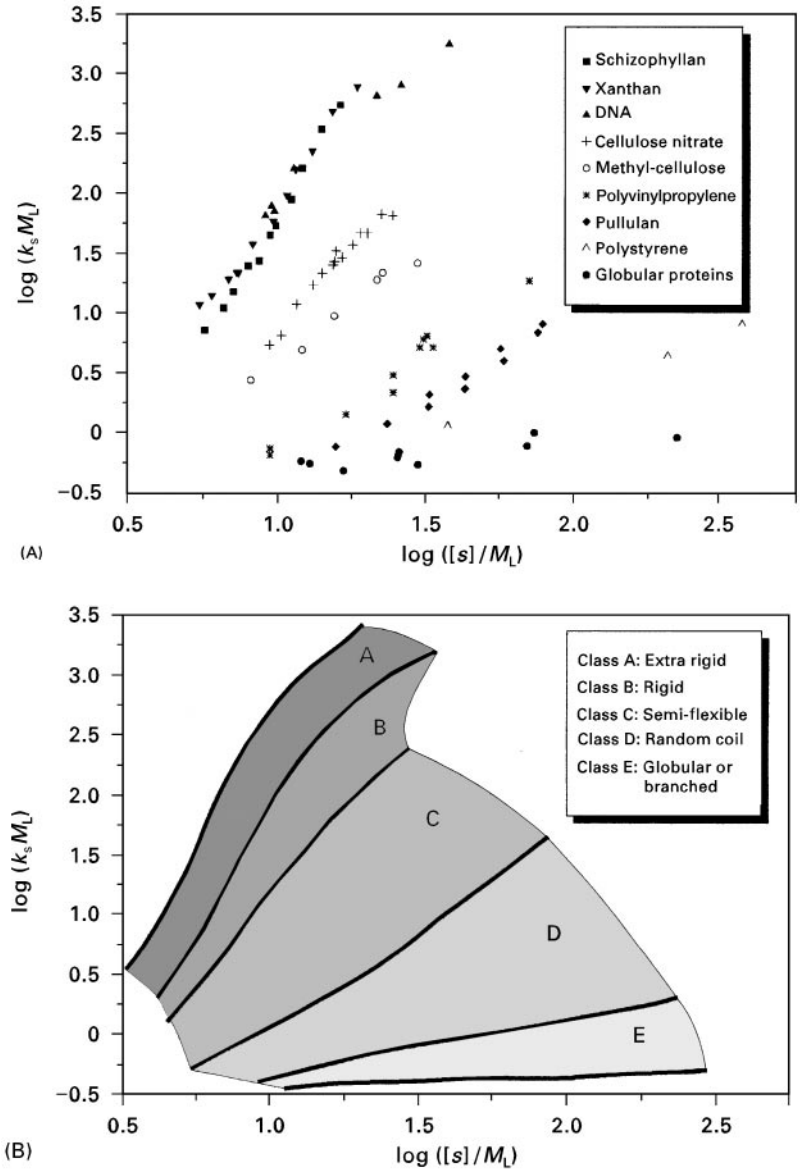

Figure 3 Sedimentation conformation zoning of polysaccharides. (A) Dependence of log ($k_s M_L$) versus log [s]/M_L for 82 macromolecules of known conformation type. (B) Corresponding diagnostic plot: for a measured [s], k_s and M_L, the conformation of a given polysaccharide can be zoned. $[s] = s^{\circ}_{20,w}\eta_0/(1 - \bar{v}\rho_0)\}$ where η_0 and ρ_0 are the viscosity of water at 20°C and v$ the partial specific volume. [s] can also be defined for other solvents, although k_s must then correspond to that solvent. (Reproduced from Pavlov GM, Rowe AJ and Harding SE (1997) Conformation zoning of large molecules using the analytical ultracentrifuge. *Trends in Analytical Chemistry* 16: 401, with permission from Elsevier Science.)

contour length, L), the chain diameter, d, and the persistence length, L_P. As with MHKS, worm-like coil modelling has not only been worked out for the sedimentation coefficient, but also for other hydrodynamic parameters.

The Wales–van Holde ratio, $k_s/[\eta]$, for rigid structures It has already been noted how this ratio can help to distinguish rod-shape polysaccharides from the more randomly coiled and spheroidal materials. For approximately rigid structures, more precise information about the molecular conformation, in terms or average ellipsoidal axial ratios, can be obtained without the complication of having to take into account molecular hydration, since this is essentially eliminated in the ratio. Detailed relations are available between $k_s/[\eta]$ (referred to for simplicity by the parameter R) and the axial ratio for ellipsoids of revolution, or the two axial ratios for general triaxial ellipsoids. We stress the phrase approximately rigid

structures. For polysaccharides this procedure would not be applicable to flexible coils, but may be applicable to rod-shaped or highly branched structures (zones A, B or E molecules of Figure 1): k_s has however been mainly applied in this way to protein systems, and not polysaccharides.

$s^o_{20,w}$ and the frictional ratio In addition to $k_s/[\eta]$, the sedimentation coefficient $s^o_{20,w}$, can also be used directly to extract the axial ratios of a quasi-rigid structure. Knowledge of $s^o_{20,w}$, together with the molecular weight and other parameters (density ρ_0 and viscosity η_0 of water at 20.0°C) yields the particle frictional parameter known as the translational frictional ratio f/f_0. This in turn can be related to a shape parameter known as the Perrin function, P, and swelling ratio, S_w, defined as the ratio of the volume of a macromolecule swollen through hydration to that of the anhydrous macromolecule:

$$f/f_0 = P \cdot S_w^{1/3} \qquad [4]$$

Thus, if S_w is known or assumed, the shape parameter P can be found and from this the axial ratios from rigorous hydrodynamic relations. Besides complications through molecular flexibility, the stumbling block is S_w. Whereas for proteins, S_w will be moderate ($\sim$1–2) and predictable to a certain degree, for polysaccharides this will not be so. In fact, eqn [4] is more useful from a polysaccharide context for predicting S_w, since, despite the presence of the cube root term, f/f_0 is still essentially a more sensitive function of swelling than shape.

Measurement of Water Binding or Swelling Ratios S_w

It has been shown how measurement of the frictional ratio (via $s^o_{20,w}$) can be used to estimate S_w, the swelling ratio. For a rigid structure, if the shape is established from measurement of $k_s/[\eta]$, then P can be defined and hence S_w found from eqn [4].

For more general structures, another route that has been suggested, applicable to both rigid and flexible structures, is to use the ratio of the concentration dependence term for viscosity (k_η) to k_s:

$$S_w \sim k_\eta/k_s \qquad [5]$$

k_η (mL g^{-1}) comes from the analogous relation of eqn [2] for viscosity:

$$\eta_{red} = [\eta](1 + k_\eta \cdot C)$$

where η_{red} (mL g^{-1}) is the reduced viscosity at concentration C.

Diffusion Measurements

One of the classical ways of measuring translational diffusion coefficients, D, is to use the optical system on the ultracentrifuge to follow the time-dependent spreading of a layered boundary between a macromolecular solution and its solvent: if a rotor speed is employed, large enough to stabilize the system but low enough so as to minimize appreciable sedimentation, measurement of boundary height and width changes with time can be used to measure D. The diffusion coefficient itself can be corrected to standard conditions of temperature and solvent viscosity (water at 20.0°C) to give $D_{20,w}$, and extrapolated to zero concentration to give $D^o_{20,w}$ and the concentration dependence parameter k_d (mL g^{-1}). $D^o_{20,w}$ combined with $s^o_{20,w}$ in the Svedberg equation can be used to estimate the molecular weight. Alternatively $D^o_{20,w}$ provides a different route to $s^o_{20,w}$ for obtaining the frictional ratio f/f_0.

Although measurement of $D^o_{20,w}$ in this way is highly reliable for many protein systems, there are problems with polysaccharides, again deriving from polydispersity: indeed, this application of the ultracentrifuge has now largely been superseded by dynamic light-scattering methodology.

Analytical Density Gradient Centrifugation

Density Gradient Sedimentation Equilibrium

By dispersing the polysaccharides in a dense salt solution, a density gradient can be created in an ultracentrifuge cell. For a mixed macromolecular solute a particular macromolecular species will move or band at that region in the density gradient which corresponds to it own density: this procedure is known as density gradient or isopycnic (isodensity) sedimentation equilibrium. Normally, caesium salts are used to provide this gradient for polysaccharides. Since most polysaccharides have similar densities (or partial specific volumes), this is not very good for separating mixed polysaccharide systems, but is, however, useful for separating polysaccharides from glycoproteins or proteins. It has, for example, been recently used to assay the purity of xylans from other molecular contaminants. The method assumes no significant interaction between the caesium salts and the polysaccharide.

Density Gradient Sedimentation Velocity

A less dense solute may be used (e.g. sucrose) to retard the sedimentation rate in sedimentation velocity (enhancing resolution) as opposed to inducing banding in sedimentation equilibium. The method

assumes no interaction between the solute and the macromolecule. However, since the sedimentation coefficients are usually very small (often between 1 and 2 S) – because of their large frictional ratios – and because of potential interactions with the separating medium, this method has not found major use for polysaccharide analysis or separation.

Polysaccharide Gels: Swelling Pressure

Sedimentation Equilibrium

If a gel is subjected to a centrifugal field, low enough to avoid sedimentation of the gel itself (typically $<$ 10 000 rpm), a concentration gradient will be established as in a conventional sedimentation equilibrium experiment on a solution. The gradient indicates the locally dependent de-swelling of the gel, which is caused by the swelling pressure generated by the centrifugal field. The concentration gradient will depend on the structure of the gel (number and strength of the cross-links) and whether the gel is reversible or not.

Sedimentation Velocity

At sufficiently high rotor speed ($>$ 10 000 rpm), the polymer concentration may drop to a sufficiently low level near the meniscus that a sol phase will appear: a conventional sedimentation velocity experiment can be performed, monitoring the movement of the boundary between gel and sol.

Data from sedimentation equilibrium and sedimentation velocity can be used to obtain the thermodynamic, elastic and structural parameters of the gel, complementing data from classical rheological approaches. Although gelatin has been the main focus of attention with this technique, several polysaccharide gels have been successfully characterized, such as carrageenan, pectin and alginate.

See also: **II / Particle Size Separation:** Theory and Instrumentation of Field Flow Fractionation. **III / Polysaccharides:** Liquid Chromatography.

Further Reading

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Liquid Chromatography

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Introduction

Separation, analysis and molecular weight distribution are very important for the characterization of polysaccharides in biochemistry, microbiology, agriculture and the food industry. During biosynthesis, a large range of components of different molecular weight are formed. Gas chromatography (GC) can be used for the analysis of very complex polysaccharide mixtures whereas high performance liquid chromatography (HPLC) is preferred for simple polysaccharide mixtures and for purification.

High Performance Liquid Chromatography

HPLC was introduced into the field of carbohydrate chemistry for the separation of mono- to tetrasaccharides of neutral sugars found in natural products