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Some recent developments in the size and shape analysis of industrial polysaccharides in solution using sedimentation analysis in the analytical ultracentrifuge

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1. There is now a renaissance of interest in the application of sedimentation methods to the study of food macromolecules and follows some significant developments in instrumentation. 2. These methods are particularly relevant to our understanding of the properties of industrial or 'commercial' polysaccharides, molecules which are inherently polydisperse and thermodynamically non-ideal. 3. This short review article attempts to establish the contribution that sedimentation velocity and sedimentation equilibrium are making to our understanding of the sizes, shapes and heterogeneity of industrially important polysaccharides in dilute solution and also indicates recent developments in the analysis of gels, diffusion through matrices and interactions involving these molecules. 4. The importance of combining information from sedimentation analysis with that from complementary techniques such as viscometry, light scattering and electron microscopy is stressed. 5. Comments about the potential impact of a new commercially available ultracentrifuge are given.

INTRODUCTION

A knowledge of the fundamental behaviour of industrial or 'commercial' polysaccharides on a 'microscopic' scale (in terms of their sizes, shapes, molar mass distribution and interaction parameters) in dilute and concentrated solution form — both as homogeneous preparations and in mixtures with other macromolecular solutes — is important for the proper understanding of the behaviour of these molecules in industrial applications on a 'macroscopic' scale. For example, in the food industry this could be as gelling or thickening agents or as agents for phase separation; in the pharmaceutical industry, this could be as bioadhesives or in the design of slow release forms; in the oil industry this could be as an agent assisting oil recovery or as a lubricant. This information can help with the selection of the best polysaccharide for a particular application.

There are several techniques currently in popular use for obtaining this fundamental information (see e.g. Harding *et al.*, 1991b), but, as is well known, food polysaccharides are by no means simple to characterize because of their highly expanded nature in solution, their polydispersity, their variable conformations and in some cases their high charge and ability to self-associate. This article will describe some recent developments and applications with an old (much of the technology dates back to the 1920s) technique of sedimentation analysis in the analytical ultracentrifuge. Although some would say this technique is dated, there has been over the last few years an undoubted revival in interest including the launch of a new instrument (Giebeler, 1992). This article will describe how the ultracentracentrifuge's absolute nature and inherent fractionation property is providing useful information — especially when used in combination with other techniques — on the nature and behaviour of industrial polysaccharides, focussing on two areas — shape and size analysis. The purpose of this article is to give the general reader an overall picture of recent developments, but without going into any great depth. Examples quoted are largely drawn from our own laboratory. The interested reader if he/she so desires can obtain more information in a book that has appeared recently (Harding *et al.*, 1992b) of which a considerable part is devoted to polysaccharides and related glycopolymers.

TYPES OF ULTRACENTRIFUGE ANALYSIS AND INSTRUMENTATION

The sort of information we can obtain from sedimentation analysis in the analytical ultracentrifuge depends on the particular technique we apply — all possible with the same instrumentation (Fig. 1). *Sedimentation velocity* can provide us with information on the sample homogeneity, shape information — in some cases to surprising detail — and also interaction information by for example assaying for what we call 'co-sedimentation' phenomena (i.e. species sedimenting at the same rate). At lower rotor speeds, *sedimentation equilibrium* can provide absolute size and size distribution information — in terms of molar mass averages and molar mass distributions. There are two other important types of analytical ultracentrifuge measurement — namely isopycnic (='constant density') *density gradient analysis*, important for assaying the purity of a polysaccharide preparation (i.e. freedom from unconjugated protein, lipid or nucleic acid) and finally *diffusion analysis*, particularly in terms of matrix diffusion phenomena.

The technique of analytical ultracentrifugation is not new — Svedberg's inception of the technique dates from the early 1920s (Svedberg & Nichols, 1923; Svedberg & Rinde, 1923; Svedberg, 1927). Svedberg himself published several papers on polysaccharides — mainly in the 1940s and mostly on cellulose and cellulose derivatives. This work has been reviewed relatively recently by Jullander (1985). The period of maximum popularity and use of the technique was between 1940 and 1970 with several types of analytical ultracentrifuge commercially available, most notably the famous Beckman Model E (then present in virtually every Biochemistry and Chemistry department). The growing preference for biomolecular scientists for alternative technologies, such as gel filtration, electrophoresis and light scattering then led to a dramatic decline in use, until relatively recently, when, largely driven by a need from molecular biologists for precision physico-chemical measurements on newly engineered macromolecules (Schachman, 1989), Beckman Instruments (Palo Alto, USA) produced the successor to the Model E, the Optima XL-A (Giebler, 1992). Although since its introduction in 1991 (with our laboratory as one of the four test sites) it has been an invaluable tool for studying macromolecular systems involving proteins and nucleic acids, its lack of a refractive index based optical system (Schlieren and Rayleigh interference) has largely excluded its application to other classes of macromolecule, such as polysaccharides, since these macromolecules generally lack a suitable chromophore to enable use of the absorption optical system unless the polysaccharide is chemically modified (Errington *et al.*, 1992; Cölfen *et al.*, 1995). This has been unfortunate since there has also been a growing demand from the industrial polysaccharide community for precision measurements on polysaccharide sizes, shapes and interactions and the desire to make use of the analytical ultracentrifuge's absolute nature (i.e. not requiring calibration by macromolecular standards) and inherent fractionation ability (unlike light scattering) and without the need for a third 'inert' medium (unlike gel filtration). As a result we have maintained and developed older equipment (such as Beckman Model E's, now equipped with laser light sources and off-line automatic data capture systems, and an MSE (Crawley, UK) Centriscan equipped with scanning Schlieren and absorption optics, currently under re-construction and modernisation by the MOM company (Hungarian Optical Works, Budapest)). The other instrument that has been popular with workers in the polysaccharide field is the MOM ultracentrifuge (Görnitz & Linow, 1992; Seifert *et al.*, 1995). A new version of the XL-A is planned equipped with on-line Rayleigh interference optics, but its design excludes the use of long path length cells, necessary for the sedimentation equilibrium of polysaccharides (as will be evident from what is written below). The older instruments will therefore continue to be useful.

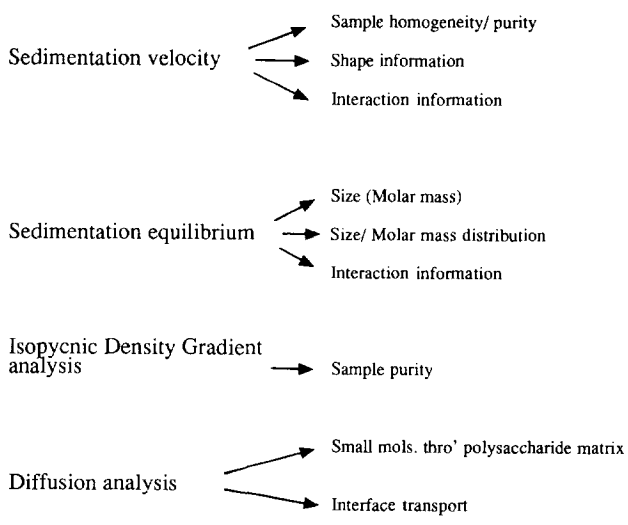

Fig. 1. Ultracentrifuge methods and the potential information available.

SHAPE ANALYSIS BY SEDIMENTATION VELOCITY

A typical analytical ultracentrifuge cell contains one or two sector shape channels which can take between 0.1 and 0.8 ml of solvent or solution, and with an *analytical* ultracentrifuge, using the appropriate optical system, one can record the position and rate of movement of the sedimenting boundary within the solution channel. With a polysaccharide, we use refractive index *gradient* or Schlieren optics which records the boundary on photographic film or chart paper as a peak. The rate of

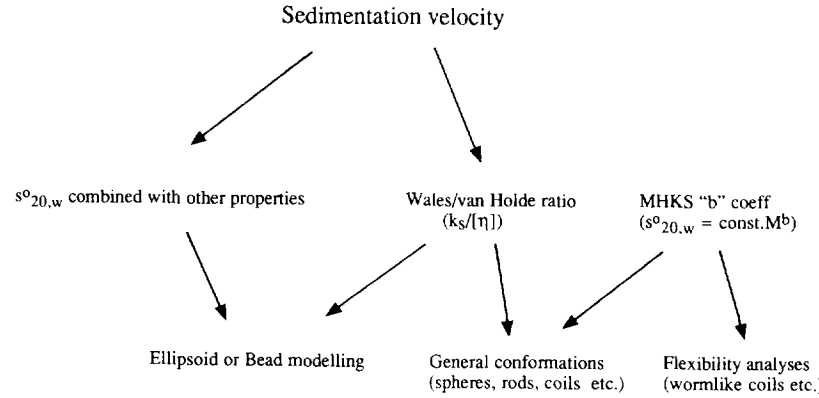

Fig. 2. Approaches, using sedimenatation velocity, to macromolecular conformation analysis.

movement of the boundary per unit centrifugal field gives the sedimentation coefficient, 's', which is one of our shape parameters. By correcting this using simple formulae to standard conditions — water as solvent at 20°C — and extrapolating this (or the reciprocal thereof) to zero concentration, we can get from the intercept the corrected sedimentation coefficient $s^{\circ}_{20,w}$ (unit = seconds or Svedbergs, S, where $1S = 10^{-13}$ s) and from the slope we can get the sedimentation concentration regression coefficient k_s (unit = ml/g) and then from *both parameters*, our shape information.

For shape analysis there are three approaches (Fig. 2). If the macromolecule is *fairly* rigid, we can combine the sedimentation coefficient with other techniques such as intrinsic viscosity, rotational diffusion (from fluorescence depolarisation or electric birefringence measurements), or the radius of gyration (from 'static' light scattering or low angle X-ray scattering) to model the conformation in terms of simple ellipsoids of revolution, general triaxial ellipsoids (Harding, 1987, 1989) or sophisticated, but beautiful bead models (Garcia de la Torre, 1989) — but this approach is really more for fairly rigid protein structures such as seed globulins. For not-so-rigid macromolecules like polysaccharides, we have to use more general shapes using the Wales/van Holde (Wales & van Holde, 1954) ratio of k_s to the intrinsic viscosity, $[\eta]$ or the "Mark–Houwink–Kuhn–Sakurada" (MHKS) (Mark, 1938; Houwink, 1941; Kuhn & Kuhn, 1943; Sakurada, 1941; Sakurada, 1943) 'b' coefficient which comes from the relation between $s^{\circ}_{20,w}$ and the molar mass, M {$s^{\circ}_{20,w}$ = const.M^b}. Similar coefficients exist for the intrinsic viscosity, the diffusion coefficient, and radius of gyration (a,-ε and c, respectively) — see for example Smidsrød & Andresen (1979) and Harding (1995) — to permit the modelling of the conformation in terms of general shapes, between the three extremes of compact sphere, rigid rod and random coil. For representing models from this general type of approach, a construction known as the Haug triangle has proved useful (Smidsrød & Andresen, 1979). Each of the three extremes of conformation has specific values for $k_s/[\eta]$ and the MHKS b coefficient, and from sedimentation velocity analysis, we can easily confirm observations made by other techniques that most industrial polysaccharides have conformations ranging from rigid rod to random coil, as can be observed by the collection of examples shown in Table 1.

More refined analyses are possible: (i) if, for example, the polysaccharide is fairly rigid then it is possible to represent the conformation in terms of ellipsoids: for example using this approach and the shape functions Π (Harding, 1981) and $k_s/[\eta]$ (both of which do not require knowledge or assumptions concerning the degree of hydration) an axial ratio for the xanthan rod of ≈70:1 was found (Dhami *et al.*, 1995b) and using Π an axial ratio of ≈15:1 found for arabinoxylan (Dhami *et al.*, 1995a); (ii) double logarithmic representations of $s^{\circ}_{20,w}$ against M which are used to obtain the MHKS ("Mark–Houwink–Kuhn–Sakurada") b coefficient can also be used to model the flexibility of a polysaccharide in terms of wormlike-coil and related models from the ratio of the contour length L to the persistence length, a: this, and related analyses, has been applied extensively to xanthan by T. Sato, T. Norisuye and coworkers (Sato *et al.*, 1984a, b, c; Sho *et al.*, 1986; Kawakami & Norisuye, 1991; Inatomi *et al.*, 1992). G. Pavlov and coworkers have applied similar types of analyses to celluloses (Pavlov & Shildiaeva, 1988) bacterial mannans (Pavlov *et al.*, 1992) and pullulans (Pavlov *et al.*, 1994). Another example, involving the application of the latest wormlike coil theory of J. Garcia de la Torre and coworkers to a pectin can be found in Harding *et al.* (1991a).

SIZE AND SIZE DISTRIBUTION ANALYSIS BY SEDIMENTATION EQUILIBRIUM

Whereas in a sedimentation velocity experiment at relatively high rotor speeds — for a polysaccharide say 40,000–50,000 rev/min — the sedimentation rate and hence sedimentation coefficient is a measure of the size and shape of the molecule, at much lower speeds, say 10,000 rev/min or less, in a sedimentation *equilibrium*

Table 1. Polysaccharide shapes from sedimentation velocity

	$k_s/[\eta]$	b	Conformation	Reference
Neutral:				
Dextran fractions		0.44	Random coil	Senti *et al.*, 1955
DIT-dextrans		0.56	Semi-flexible coil	Errington *et al.*, 1992
Pullulans	1.4	0.45	Random coil	Kawahawa *et al.*, 1984
Yeast mannan	1.3	0.43	Random coil	Pavlov *et al.*, 1994
β-Glucans	0.4		Extended	Woodward *et al.*, 1983
Amylopectin*	1.45		Spheroidal	Fronimos, 1991
Polyanionic:				
Alginates	0.6		Extended	Ball, 1989
Citrus pectins	0.2	0.17	Rigid rod	Harding *et al.*, 1991
Xanthan (keltrol)	0.28		Rigid rod	Dhami *et al.*, 1995b
κ-Carageenan	0.94		Extended coil	Day & Harding, 1995
Mucopolysaccharide	1.5	0.4	Random coil	Sheehan & Carlstedt, 1984
Polycationic:				
Chitosan ($DA^+ = 11\%$)	0.16		Rigid rod	Errington *et al.*, 1993

+ Degree of acetylation.
All in aqueous solvents apart from *(90% DMSO, 10% H_2O).

experiment the forces of sedimentation and diffusion on the macromolecule become comparable and instead of getting a sedimenting boundary you get a steady-state equilibrium distribution of macromolecule with a low concentration at the meniscus building up to a high concentration at the cell base. This final steady-state pattern is a function *only* of molar mass and related parameters (virial coefficients and association constants where appropriate) and not of molecular shape since at equilibrium there is no further movement of the macromolecule hence frictional effects through shape variation do not come into play — so like 'static' (as opposed to dynamic) light scattering, sedimentation equilibrium is an absolute way of obtaining the molar mass. Since industrial polysaccharides are by their very nature polydisperse, this will principally be either a 'weight-average' or a 'z-average' depending on the optical system used to record the equilibrium distribution. There are three types of sedimentation equilibrium distribution depending on the conditions chosen (speed, solution column length in the centrifuge cell): (i) the 'low' speed method (Van Holde & Baldwin, 1958) in which the solute concentration at the meniscus is finite; (ii) the 'meniscus depletion' (or 'high speed') method (Yphantis, 1964) in which the concentration at the meniscus is effectively zero; and (iii) the 'intermediate speed' method (Creeth & Harding, 1982) in which the concentration at the meniscus remains small but finite. Extraction of molar mass data from the optical records is considerably easier for the meniscus depletion method, but sadly this method is not usually applicable for industrial polysaccharides because of their polydispersity: a polydisperse solution will tend to redistribute its components so there is a greater proportion of higher molar mass end of the distribution near the cell base. Any attempt to deplete the meniscus (rich in the lower molar mass part of the distribution) from the macromolecular solute is likely to result in loss of optical registration of the higher molar mass components at the bottom of the cell, leading to underestimates for M. Methods (ii) or (iii) are usually required.

Equilibrium solute concentration distributions are recorded mainly, but not exclusively, using a special type of refractive index optics known as Rayleigh interference, which is the most sensitive type of optical system available (suitable for cell loading concentrations down to ~0.2 mg/ml if long (30 mm in a Beckman Model E) optical path length cells are used. In our laboratory, these patterns are digitised automatically using a commercially available laser densitometer (such as of the popular type used primarily to scan electrophoresis gels), and from a simple Fourier cosine series analysis of the digitised data we can accurately record our concentration distribution. Whereas for well behaved proteins, extraction of molar masses from simple linear regression of plots of the log of the concentration of solute as a function of the square of the radial displacement can suffice, for polysaccharides, where these plots can curve because of polydispersity, association and/or non-ideality, and where the base can be poorly defined, more sophisticated methods of data extraction are required. The M* function (Creeth & Harding, 1982) is particularly suited for this purpose and a routine is available known as MSTARI (Harding *et al.*, 1992). This was orginally written in FORTRAN 77 for a mainframe computer, but has been recently downloaded onto QUICKBASIC for PC (Cölfen & Harding, 1996). From the M* we can get the apparent weight average molar mass, $M_{w,app}$ which after an extrapolation (of $M_{w,app}$ or $1/M_{w,app}$) to zero concentration — to eliminate thermodynamic non-ideality effects — provides us with the weight average molar mass. This method has been used to obtain the weight average molar masses for a range of polysaccharides —

neutrals, polycationic and polyanionic — and some examples are given in Table 2. From this table it is worth pointing out that the molar mass of pullulans — widely used in the polysaccharide chromatography field as standards — were all worked out not by static light scattering, as many people think, but by sedimentation equilibrium (Kawahawa *et al.*, 1984). The second comment is if you compare in particular the values for chitosan KN50-4 and the xanthan, one can appreciate the range of sizes that can be comfortably handled by this technique (in fact the range is much wider; the technique has been used to characterise the molar mass of sucrose (342 g/mol) and tobacco mosaic virus (50×10^6 g/mol)). Finally with regard to the tomato pectin data of Table 2, it has been known for quite a while that when tomatoes ripen, the pectin components of the cell wall become degraded; in some recent work with D. Grierson's group at Sutton Bonington, if 'gene therapy' is used to eliminate the production of the key enzyme responsible, the pectins remain more or less intact — as confirmed by their molar masses — to result in the breed '*Craig ailsa*' or '*supertomato*'.

Sedimentation-diffusion method

Although sedimentation equilibrium is arguably the method of choice, an alternative sedimentation method for molar mass measurement (which, like sedimentation equilibrium is also absolute, i.e. not requiring assumptions about conformation) is to combine the sedimentation coefficient with the diffusion coefficient, the latter either determined from boundary spreading measurements in the ultracentrifuge (Creeth & Pain, 1967) or by dynamic light scattering measurements (see Pusey, 1974). This will tend to yield the weight average molar mass, even if the z-average diffusion coefficient (from dynamic light scattering) is employed (Pusey, 1974). For example, Pavlov *et al.* (1992; 1994) have combined the sedimentation coefficient with boundary spreading diffusion coefficients to measure the molar masses, respectively, of pullulans (value included in Table 2 for comparative purposes) and yeast mannans, whereas Wedlock *et al.* (1986) have combined the sedimentation coefficient with dynamic light scattering diffusion coefficients to measure the molar masses of alginates.

Table 2. Molar masses of polysaccharides (weight averages from sedimentation equilibrium analysis). These values are normally precise to ± 5–15%

Polysaccharide	M_w	Reference
Neutral:		
Pullulan P100	95,000	Kawahawa *et al.*, 1984
Pullulan P800	760,000	Kawahawa *et al.*, 1984
Pullulan P800	644,000 +	Pavlov *et al.*, 1992
Pullulan P1200	1,240,000	Kawahawa *et al.*, 1984
Dextran T500	500,000	Edmond *et al.*, 1968
Guar	800,000*	Gaisford *et al.*, 1986
Locust bean gum (Cold water soluble fraction)	340,000*	Gaisford *et al.*, 1986
Locust bean gum (Hot water soluble fration)	330,000*	Gaisford *et al.*, 1986
Glucomannan	280,000*	Winwood, 1986
Beta-Glucan (Barley)	160,000	Woodward *et al.*, 1983
Beta-Glucan (Wheat)	230,000	Harding *et al.*, 1991b
Polycationic:		
Chitosan (Protan 'Sea Cure')	162,000	Errington *et al.*, 1993
Chitosan (Trondheim 'KN50-1')	64,100	Errington *et al.*, 1993
Chitosan (Trondheim 'KN50-4')	4300	Errington *et al.*, 1993
DEAE-Dextran	530,000	Anderson, 1991
Polyanionic:		
Arabinoxylan (ws-AGX)	150,000	Dhami *et al.*, 1995a
Xanthan (keltrol)	5,900,000	Dhami *et al.*, 1995b
Alginate (Kelco 'Pro-nova')	210,000	Horton *et al.*, 1991
Pectin (Koch-Light, Citrus)	90,000	Harding *et al.*, 1991a
Pectin (Green Tomato)	160,000	Seymour & Harding, 1987
Pectin (Red Tomato)	96,000	Seymour & Harding, 1987
Pectin (Red Tomato — antisense polygalacturonase)	135,000	Smith *et al.*, 1990
κ-Carageenan	284,000	Day & Harding, 1995

Normally these values are obtained by extrapolation of $M_{w,app}$ values for a given cell loading concentration to zero concentration. Values indicated by * have been obtained from a single sedimentation equilibrium experiment at a single loading concentration by plotting 'point' apparent weight averages against local concentration in the solute distribution to zero concentration (see text).

+ Not from sedimentation equilibrium, but from combination of the sedimentation and diffusion coefficients. Included for comparative purposes.

Notes of caution

Three notes of caution are necessary for the application of sedimentation equilibrium to solutions of industrial polysaccharides:

1. Because optical records from Rayleigh optics are one of concentration relative to the meniscus, and since the meniscus depletion method is usually inapplicable because of the polydispersity of polysaccharide preparations, some procedure is necessary to obtain the meniscus conncentration (either in g/ml or in equivalent fringe displacement units). A useful procedure has been given by Creeth & Harding (1982) using mathematical manipulation of the basic fringe data. A similar method has been given by Teller *et al.* (1969); a review of other methods has been given by Creeth & Pain (1967).
2. For some polysaccharides, it is possible to make a single measurement at low concentration (~0.2–0.5 mg/ml) and approximate $M_w \approx M_{w,app}$. Unfortunately, many polysaccharides are extremely non-ideal (when compared with proteins). This derives from their high exclusion volumes (a polysaccharide molecule in solution can have a swollen volume in solution ~100× its volume in the dry state) and also high charge. For the polycationic and polyanionic in particular, measurements should be made in a buffer of ionic strength at least 0.1 (we usually employ 0.3), but even here non-ideality can be very high. Normally it is necessary to measure the $M_{w,app}$ for a few cell loading concentrations, c, and extrapolate to zero concentration. For proteins the conventional way is to plot $1/M_{w,app}$ versus c and obtain M_w (and the second virial coefficient B) by linear regression to $(1/M_{w,app}) = 1/M_w + Bc$. Unfortunately, although this usually yields a reasonable estimate for B, the concentration dependence is so severe that it is safer to extrapolate graphically a plot of $M_{w,app}$ versus c to $c = 0$. In performing these extrapolations, care must be made that the critical overlap concentration ('c*'), repesenting the transition from dilute to semi-dilute solution behaviour is not exceeded (this can be as low as 0.5 mg/ml, such as for xanthan (Dhami *et al.*, 1995b)). Therein lies a problem with the new (XL-A) ultracentrifuge from Beckman. Design restrictions means that the version equipped with interference optics can only accommodate centrifuge cells with a maximum optical path length of 12mm. With polysaccharide refractive increments generally in the range of 0.14–0.16 ml/g, this means that the minimum concentration that can be used to obtain a sufficient fringe increment for molar mass analysis is ~0.5–0.6 mg/ml. This is currently too high. The older Beckman Model E instrument permits the use of 30 mm optical path length cells (minimum loading concentration 0.2–0.3 mg/ml).
3. It is possible to obtain, from local slopes (by sliding-strip types of analyses) local or 'point' weight average molar masses $M_{w,app}$ (r) as a function of various radial positions, r along the concentration distribution in the ultracentrifuge cell. These are useful in giving an idea of the distribution of molar masses and/or the extent of thermodynamic non-ideality. A plot of $M_{w,app}$ versus local concentration c(r) can in principle be used to obtain M_w, for systems which are not too polydisperse (i.e. where redistribution throughout the cell is not significant).

Molar mass distribution analysis

It is therefore possible, after some care, to obtain reliable molar masses for food polysaccharides and with no problems of dust or supramolecular contamination, which conversely cause considerable problems with light scattering procedures. We can also obtain reliable molar mass distribution information out: this is probably of more direct interest since it is the extent of low molar mass material present in a saccharide preparation, which tends to compromise the gelling and thickening properties in food applications, or the hydration properties in slow release forms in pharmaceutical applications. It is worth stressing that with the advent of (multi-angle) light scattering procedures coupled to gel permeation/size exclusion chromatography (and other forms of separation) (Wyatt, 1992) for many polysaccharides and other glycopolymer systems, these latter methods, which have adopted the pseudonyms "GPC/MALLS" or "SEC/MALLS" usually give much more rapid results and are now the method of choice: the 'dust problem' is no longer a severe restriction since the gel columns tend to filter out any dust or other supramolecular contaminants. However, uncertainty can remain in terms of, for example, the 'inertness' of the column packing materials and in the extrapolation of the angular intensity functions to zero angle; sedimentation equilibrium procedures are still highly useful in providing, at the very least, a reliable independent quality check on distributions obtained. There are four clear approaches for obtaining this information (Table 3). The simplest procedure ('Method I') is to use polydispersity indices or ratios of the weight average to number average molar mass, or, as more appropriate to sedimentation equilibrium analysis, the ratio of the z-average to weight average molar mass. We have just considered above how we can get reliable weight average molar masses from Rayleigh interference optical records of the distribution of solute at sedimentation equilibrium. What is perhaps not widely appreciated is that one can get reliable z-average information directly if equilibrium solute patterns are recorded using the Schlieren optical system. This latter system records the concentration gradient against distance as opposed to concentration against distance which we get from

Table 3. Molar mass distributions by sedimentation equilibrium analysis

Method	Type of analysis	Reference
I	Polydispersity indices (M_z/M_w etc)	Herdan (1949)
II	Modelling the concentration distribution: Non-ideal polydisperse fit	Harding (1985); Lechner (1992)
III	Modelling the concentration distribution: Equivalent self association fit	Creeth & Cooper (1984)
IV	Coupling to gel permeation chromatography	Ball *et al.* (1988)

Rayleigh optics, and in our laboratory, we have a Beckman Model E ultracentrifuge dedicated to this optical system. Recent applications have been to xanthan (Dhami *et al.*, 1995b) and arabinoxylan (Dhami *et al.*, 1995a). Again, a further limitation of the new Beckman ultracentrifuge (besides the cell path length restriction noted above) is that it lacks the Schlieren system so these measurements currently have to be performed on the older ultracentrifuges. It is possible to obtain z-average molar mass data from the Rayleigh interference optical records, by various manipulations of the data (see e.g. Harding, 1992), but the precision is not high.

With Method II (Table 3), it is possible to model the log concentration versus distance squared plot directly, but this method which has been available for a decade (Harding, 1985) has only been applied to relatively simple two- or three-component systems because of the severe demands this method puts on computational resources. A much easier method — so-called Method III — is to model the concentration distribution as if the system were self-associating (even though it may not be). This approach is not disallowed, but uses the principle of indistinguishability in a single sedimentation equilibrium experiment between the solute distribution for a system where the species of different molar mass are in chemical equilibrium (i.e. a 'self-associating' system) from a system in which they are not (i.e. a 'polydisperse' one). Using this principle it is possible to use the easier to handle equations for a (non-ideal) association (as opposed to a non-ideal polydisperse distribution) to work out an *effective* association constant which then defines the distribution of molar mass at zero centrifugal field, no matter what the cause of that distribution is (by a self-association, or in our case, by straight polydispersity). Although this has been successfully applied to mucopolysaccharides (Creeth & Cooper, 1984), giving a distribution in agreement with a distribution evaluated from electron microscopy (Sheehan *et al.*, 1986) and appears promising it has not yet been applied to industrial polysaccharides.

The final method — so-called Method IV (Ball *et al.*, 1988), and which is currently our method of choice — is to use sedimentation equilibrium in conjunction with gel permeation chromatography, giving the latter an absolute basis, and this has been applied to alginate (Ball *et al.*, 1988), dextran (Ball *et al.*, 1990), guar (Ball, 1989) and citrus pectin (Harding *et al.*, 1991a), and good agreement with light scattering-based procedures have been obtained.

APPLICATIONS OF THE ANALYTICAL ULTRACENTRIFUGE TO INDUSTRIAL POLYSACCHARIDES: HETEROGENEITY, MATRIX DIFFUSION, GEL STRUCTURE AND MACROMOLECULAR INTERACTIONS

Although size and shape analysis has been the main thrust of this short review, mention should be made of other important applications of the analytical ultracentrifuge to systems involving industrial polysaccharides.

Density gradient assays of composition and purity

One such application, which is not widely appreciated, is that the purity of a macromolecular preparation (from protein, glycoconjugates and other contaminants) can be assayed on the basis of the density of its components using the technique of analytical density gradient equilibrium. It involves the sedimentation to equilibrium of the preparation in a solution of a dense salt (usually CsCl or Cs_2SO_4), and a component will 'band' at its isodensity point — the method is therefore called "*isopycnic density gradient equilibrium*", and was the classical method used by Meselson *et al.* (1957) to show that the replication of DNA is semi-conservative. Proteins, polysaccharides and nucleic acids have their isodensity points at ~1.3 g/ml, ~1.6 g/ml and ~1.7 g/ml, respectively, whereas caseium salts can be chosen to give a density increment at some interval in the range 1.2–1.7 g/ml. If there is protein covalently bound to the polysaccharide, it will lower the isodensity of the polysaccharide in the region 1.6–1.3 g/ml, depending on the extent of protein binding. Although the main application of this method has been in the nucleic acid and glycoprotein/mucopolysaccharide (Harding, 1992) fields, a recent example of its application on a preparation of arabinoxylan has been given (Dhami *et al.*, 1995a). Two other applications can be thought of as 'density'-based methods: interface diffusion through incompatible phases (involving polysaccharides) of different density, and the analysis of gels (in equilibrium with solution):

Matrix diffusion

Although dynamic light scattering is now the method of choice for the measurement of translational diffusion coefficients, the optical system on the analytical ultracentrifuge is proving very useful for investigating the *diffusion of molecules through matrices* and towards and through interfaces between two phase systems (including aqueous two-phase polysaccharide systems (Harding & Tombs, 1989; Comper & Preston, 1992)).

Gels and gel structure

The analytical ultracentrifuge can also be used to look at the diffusion of molecules through polysaccharide gels and also both velocity and equilibrium centrifugation can be used to investigate the structure of the gels themselves (in terms of swelling pressures etc.): the interested reader should consult a classical paper on agar gels by Johnson (1964) and recent work of Borchard, Cölfen and coworkers (Borchard, 1991; Cölfen & Borchard, 1991; Holtus *et al.*, 1991; Cölfen, 1994).

Macromolecular interactions

Another important application, which we will only touch upon, is in the investigation of macromolecular interactions involving polysaccharides. Three recent examples involving polysaccharide interactions with proteins have been a study of mixtures of alginate with bovine serum albumin (Kelly *et al.*, 1994), a study of galactomannan incubated with gliadin (as part of an investigation into the possible use of galactomannans to help intestinal problems) (Seifert *et al.*, 1995) and chitosan with lysozyme (Cölfen *et al.*, 1996). The latter uses a procedure of directly modelling the concentration distributions at sedimentation equilibrium known as the "omega analysis" (Milthorpe *et al.*, 1976), particularly useful for the analysis of interacting systems. There are various other sophisticated types of analysis (e.g., for distinguishing (non-interacting) polydispersity from reversible self-associations/interactions (Roark & Yphantis, 1969), or for the evaluation of interaction constants (Kim *et al.*, 1977)) and these vary in the requirements on the precision of the data.

An example of the use of the ultracentrifuge to study synergistic interactions involving polysaccharides can be found in Mannion *et al.* (1992) and of interactions involving chitosans with mucus glycoproteins as part of a general study involving the use of chitosans as pharmaceutical bioadhesives can be found in articles of Fiebrig *et al.* (1994; 1995). The latter articles also give a further illustration of the virtue of combining a variety of not only different sedimentation techniques together (sedimentation velocity, equilibrium and density gradient methods) but also in combining sedimentation methodologies with other methodologies such as light scattering, rheological, electron microscopy and other imaging techniques.

COMMENT

It is impossible to assess how much the expansion of interest in analytical ultracentrifugation currently being observed in the protein/molecular biological field with the first version of the new ultracentrifuge from Beckman (based on absorption optics) will be followed by the industrial polysaccharide field with the launch of the new model, the "XL-I", with an interference-based refractometric system. Certainly the lower limit of sample concentration (≈0.5–0.6 mg/ml) for sedimentation equilibrium is a restriction which could be removed if the design was modified so that longer optical path length centrifuge cells (and hence lower concentrations) could be accommodated. A further modification to permit the use of Schlieren measurements (for sedimentation velocity and for facilitating the extraction of z-average molar masses) would also be of considerable use. Until this happens, the older instruments with their 'in-house modifications' (laser light sources, off-line data capture and analysis attachments etc.) will still be useful.

For sedimentation velocity analyses, however, this path length restriction is not a problem and the new model will be of direct use. This is because identification of the position of boundary with sedimentation velocity is considerably easier — especially with modern methods of analysis (Stafford, 1992) — than providing a sufficient concentration distribution for sedimentation equilibrium. It is likely therefore that, for studies on industrial polysaccharides, although the new ultracentrifuge will have limited possibilities for molar mass analyses, for conformation analyses it will have a considerable impact.

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