[40] I. Marle, C. Pettersson and T. Arvidsson, *J. Chromatogr.*, 456 (1988) 323.
[41] J. Ståhlberg, S. Jönsson and G. Pettersson, in P. Suominen and T. Reinikainen Editors), *Proceedings of the 2nd TRICEL symposium on Trichoderma cellulases and other hydrolases, Espoo, Finland 1993*, Foundation for Biotechnical and Industrial Fermentation Research, Helsinki, Vol. 8, 1993, pp. 273–280.

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The analytical ultracentrifuge spins again

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Since its inception by T. Svedberg and coworkers in the 1920's the technique of analytical ultracentrifugation has undergone an extraordinary evolution, peaking in popularity in the 1950's and 1960's followed by a steady decline until the last few years with a renewal of interest culminating in the launch of a new commercially available instrument. The technique is worthy of consideration by any analytical chemist interested in the characterisation of the physical behaviour of macromolecules in solution. This article attempts to indicate the breadth of the range of different types of measurements on both bio- and synthetic macromolecular systems that are now possible with the analytical ultracentrifuge

1. Introduction

About a decade ago a young post-doctoral scientist submitted a manuscript on a topic concerning the analytical ultracentrifugation of macromolecules to a leading biochemical journal. It was quickly returned by an Editor with the fatherly advice to this post-doc that he should seriously consider moving away from such an out-of-date technique and turn instead towards more modern and informative techniques — such as nuclear magnetic resonance (advice politely declined!). A decade is a long time in modern science and in this period there has been a quite remarkable turnabout in the fortunes of this technique which is now over 70 years old and whose climax of popularity was probably over three decades ago. After many years of decline since that time (largely because of the advance of gel electrophoresis and gel filtration methods for molecular weight determination and the advent of high resolution structural methods in protein biochemistry) it has now reemerged. There is a growing recognition across the chemical and biochemical communities that this technique still has a leading role to play in the characterisation of, and understanding the behaviour of, polymers, macromolecules and macromolecular assemblies — whether they be biological in origin or not — in terms of

- the size (either the molecular weight or dimensions);
- the size distribution (for a polydisperse solution of macromolecules);
- solution conformation (the overall or gross conformation);
- the purity of a macromolecular preparation (a sedimentation velocity diagram or a density spectrum);
- the thermodynamic non-ideality of a macromolecular solution (virial coefficients or activity coefficients);
- the interactions between macromolecules (including self-association behaviour, complex formation and ligand binding).

The technique is, however, most powerful when used with complementary techniques, such as: (for conformation analysis) X-ray scattering, viscometry, rotational diffusional techniques and electron microscopy, or (for e.g., protein subunit composition work) gel electrophoresis, or (for molecular weight distribution studies) gel filtration and light scattering.

This article is intended to give the reader an impression of the range of measurements and analyses that are possible with the modern analytical ultracentrifuge. For more details on any of the applications mentioned here, see Ref. [1], or the references cited therein.

2. Types of macromolecule or polymer

Although the main emphasis of the technique in the past has been in the fields of biochemistry and molecular biology (focusing on proteins and nucleic acids) it is of increasing importance in polysaccharide chemistry and the general field of polymer science (including the characterisation of synthetic macromolecules). Insofar as the range of macromolecular sizes that can be studied, it can be used to characterise the molecular weight of a sucrose molecule (MW = 342) right through to looking at the sedimentation coefficients of huge macromolecular complexes used for drug targeting [2].

3. Dilute solutions, concentrated solutions and gels

The analytical ultracentrifuge is primarily a technique for looking at the structure and behaviour of a macromolecule in dilute solution (i.e. < 10 mg/ml). It is however finding increasing use for the investigation of the behaviour of macromolecules in concentrated solution form, the diffusion of small molecules through concentrated phase systems and the thermodynamic and elastic properties of gels.

4. Types of analytical ultracentrifuge measurement

What sort of information can we get from sedimentation analysis in the analytical ultracentrifuge? It depends on the type of ultracentrifuge technique we apply — all possible with the same instrumentation. *Sedimentation velocity* can provide us with information on the sample heterogeneity, 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 molecular weight averages and molecular weight distributions. There are two other important types of analytical ultracentrifuge measurement — namely isopycnic (i.e. 'constant density') *density gradient analysis*, important for assaying the composition (and hence purity) of a macromolecular preparation and finally *diffusion analysis*: 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.

5. Instrumentation: what is an analytical ultracentrifuge?

An analytical ultracentrifuge is simply an ultracentrifuge (i.e., high speed centrifuge — up to 60 000 rev/min) with an appropriate optical system for observing and recording solute distributions during and at the end of the sedimentation process. The technique of analytical ultracentrifugation is not new — Svedberg's inception of the technique dates from the early 1920's. The boom period of the technique was in the period 1950–1970 with several commercially produced analytical ultracentrifuges available, most notably the famous Beckman Model E (then present in virtually every biochemistry and chemistry department) but for the last 20 years until very recently there has been no commercially available instrument, apart from second hand Model E's. Concentration distributions of the macromolecular solution during ultracentrifugation were recorded using either refractive index optics (Schlieren and interference) or absorption optics for those particular macromolecules such as proteins and nucleic acids possessing a suitable chromophore. Very recently the latter day successor to the Model E was launched by Beckman — the Optima XLA — with

full on-line computer data capture and analysis facilities, superb drive stability (even at 1000 rev/min) and absorption optics reliable even in the far ultraviolet (down to a wavelength of ca. 210 nm).

The aim of the rest of this article is to give a brief overview of the four types of ultracentrifuge techniques mentioned above and then very briefly indicate their potential for the characterisation of proteins, polysaccharides, nucleic acids and synthetic macromolecules.

6. Sedimentation velocity: shape analysis and homogeneity

A typical analytical ultracentrifuge cell contains two sector shape channels (one for the solution, one for the reference solvent) which can take between 0.1 to 0.8 ml. With an *analytical* ultracentrifuge, using the appropriate optical system you can record the position and rate of movement of the sedimenting boundary within the solution channel. For a macromolecule with an absorbing chromophore (such as a protein or nucleic acid) we can use the *absorption* optical system. Otherwise we can use a special type of refractive index (gradient) optics called *Schlieren optics* which records the boundary on photographic film or chart paper as a peak. Fig. 1 compares some of these patterns: Fig. 1a for a monodisperse protein preparation, Fig. 1b for a protein preparation containing two components and Fig. 1c for a polydisperse polysaccharide.

The rate of movement of a sedimenting boundary from these patterns per unit centrifugal field gives the sedimentation coefficient, s, which is one of our shape parameters. By adjusting 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 $1\ S = 10^{-13}$ 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

The usefulness of sedimentation velocity as a rapid check for sample homogeneity can also be seen directly from Fig. 1, from inspection of the number of boundaries and shape for each individual scan: (a) is a monodisperse protein preparation (one symmetrical boundary per scan), whereas (b) clearly shows two components, with about two

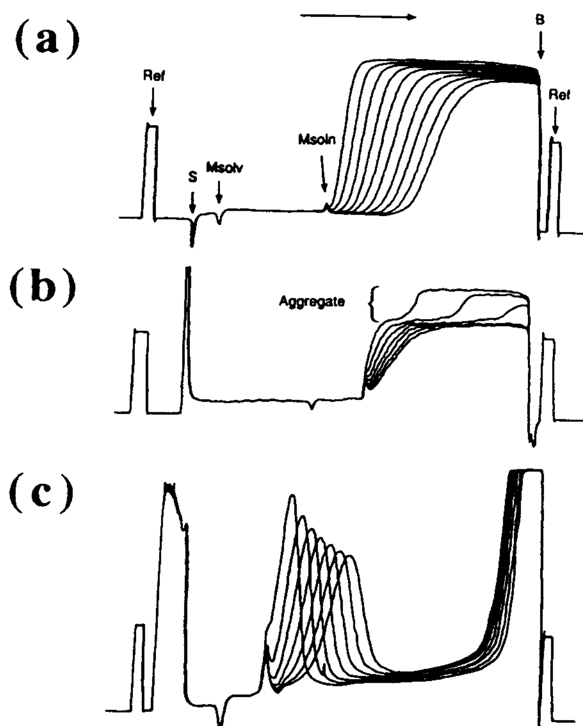

Fig. 1. Examples of analytical ultracentrifuge sedimentation velocity patterns (recorded using scanning optics). (a) Absorption optics scans (every 30 min at 49 000 rev/min) at a wavelength of 295 nm for the enzyme methylmalonyl mutase. Ref = reference mark allowing calibration of radial positions; S = start of cell; Msolv = solvent meniscus; Msoln = solution meniscus; B = cell base. Arrow indicates direction of sedimentation. (b) Absorption optics scans (every 8 min at 40 000 rev/min) at 278 nm for the "Gene 5" DNA binding protein. (c) Schlieren optics scans (30 min, 49 000 rev/min) for an alginate polysaccharide.

thirds of the material a macromolecular component sedimenting at 2.6 S, the rest a large aggregate sedimenting at 35.5 S. In Fig. 1c it is not quite so apparent that the sample is polydisperse. This is because the polysaccharide is polydisperse in a 'quasi-continuous' sense (i.e., many components with a quasi-continuous distribution of molecular weights) as opposed to the discrete 'two-components' case of Fig. 1b. Nonetheless from mathematical analysis of the boundary shape and its rate of movement it is possible to estimate a sedimentation coefficient distribution [3].

For shape analysis there are three lines of attack. 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 bire-

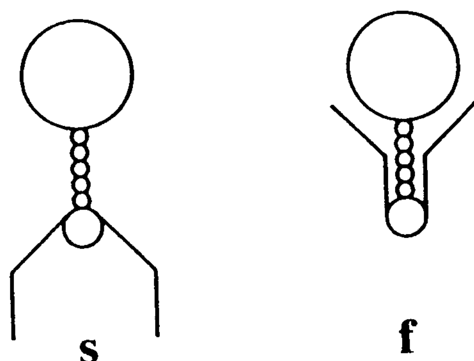

Fig. 2. Hydrodynamic bead model for a bacteriophage virus in 'slow' and 'fast' moving forms ($s^{\circ}_{20,w}$ 710 S and 1020 S, respectively) (Reproduced with permission from Ref. [6].)

fringence 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 [4] or sophisticated but beautiful bead models [5] (see Fig. 2), but this approach is really more for *fairly* rigid (in a time-averaged sense) macromolecules such as many proteins [6–8]. For not-so-rigid macromolecules such as synthetic polymers and polysaccharides we have to use more general shapes using the 'Wales/van Holde' ratio of k_s to the intrinsic viscosity, $[\eta]$ or the 'Mark-Houwink-Kuhn-Sakurada' (MHKS) b coefficient which comes from the relation between $s^{\circ}_{20,w}$ and the molecular weight, M:

$$s^{\circ}_{20,w} = \text{const. } M^b$$

(similar coefficients exist for the intrinsic viscosity, the diffusion coefficient, and radius of gyration with M, see Ref. [1]). The MHKS b coefficient is usually obtained by preparing a 'homologous' series (i.e., the same polymer but different molecular weights) of the polymer (by e.g., chromatographic separation or heat degradation) and then taking the slope of a double logarithmic plot of log $s^{\circ}_{20,w}$ versus log M [9].

Both $k_s/[\eta]$ and the MHKS b coefficient permit the modelling of the conformation in terms of general shapes, between the three extremes of compact sphere, rigid rod and random coil. For this general type of modelling we employ a very useful construction known as the Haug triangle (Fig. 3) [10]. Each of the three extremes of conformation has specific values for $k_s/[\eta]$ and the MHKS b coefficient and this type of analysis has been very useful in, for example, polysaccharide conformation analysis (Table 1)

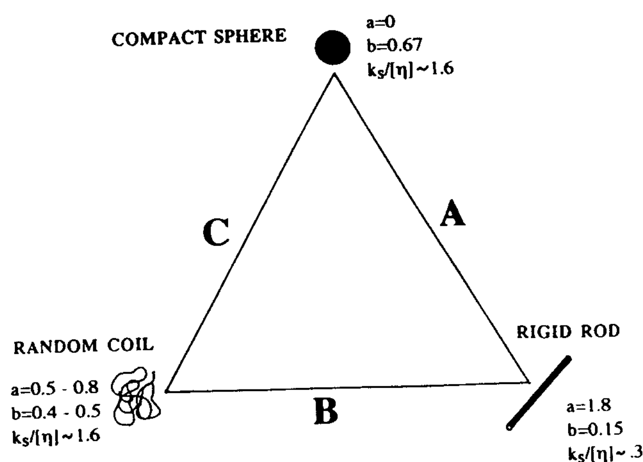

Fig. 3. The Haug triangle (see, e.g., Ref. [10]). The conformation of a given macromolecule can be represented by a locus along the sides of the triangle, e.g., globular proteins on side A, polysaccharides and linear polymers on side B, denatured proteins on side C. *a*,*b*: MHKS exponents from respectively, viscosity and sedimentation, other symbols as in the text.

Sedimentation velocity can also be used to assay for interaction in a mixed solute system, especially if the different species possess differing chromophores absorbing in the visible or useable part of the ultraviolet spectrum (for a modern instrument, wavelengths between 210 and 800 nm), by using the principle of co-sedimentation. Fig. 4 shows an example for the enzyme methylmalonyl mutase, an important enzyme in molecular biosynthesis, where from co-sedimentation experiments [11] the B_{12} co-factor was shown to be firmly bound to the protein.

7. Sedimentation equilibrium: molecular weight and molecular weight distribution analysis

In a sedimentation velocity experiment at relatively high rotor speeds (for a globular protein or

Table 1
Conformation types of three macromolecules (two polysaccharides and a mucin) from sedimentation velocity

	$k_s/[\eta]$	b	Conformation
Pullulans	1.4	0.45	Random coil
Citrus pectins	0.2	0.17	Rigid-rod
Bronchial mucin (Cystic fibrotic)	1.5	0.4	Random coil

The MHKS b coefficient can also be used to model the flexibility of a polymer in terms of 'wormlike coil' models from the ratio of the contour length L to the persistence length a (see, e.g. Ref. [9]).

Fig. 4. Co-sedimentation diagram (at 44 000 rev/min) for an enzyme (methyl-malonyl mutase), scanned at 295 nm and (top) its B12 co-factor, scanned 2 min later at 608 nm. The centre of the sedimenting boundary is virtually the same for both, with no residual absorbance, confirming complete binding of the co-factor to the enzyme.

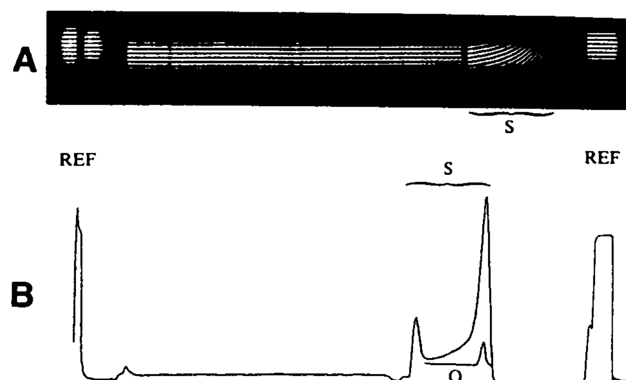

Fig. 5. Optical records of solute distributions at sedimentation equilibrium. The direction of the centrifugal field is from left to right. (A) Rayleigh interference profiles for human IgG; (B) absorption optical profile (at 280 nm) for the muscle protein titin. S = solution record; O = optical baseline.

linear 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* experiment the forces of sedimentation and diffusion on the macromolecule become comparable and instead of getting a sedimenting boundary you get, after a period of time (from a few hours to a few days depending on the nature of the solute), a steady-state equilibrium distribution of macromolecules 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 molecular weight 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 and hence frictional effects through shape variation don not come into play — so like 'static' (as opposed to 'dynamic') light scattering it is an absolute way of getting (weight-average) molecular weight.

The most accurate way of recording these final steady-state concentration distributions is using a special type of refractive index optics known as Rayleigh interference optics (Fig. 5A). For proteins, nucleic acids and other macromolecules with an absorbing chromophore we can use the more convenient (but less accurate) absorption optics (Fig. 5B) (down to a wavelength of 210 nm or possibly less with the Beckman XLA ultracentrifuge). When absorption optics are used patterns can be routinely captured automatically on-line and analysed, while with the interference system it is much more complicated and indeed expensive [12]. Whatever the system, these patterns are now usually captured and read automatically, either directly into a computer or via a bit of photography or chart recorder output first.

How do we get molecular weight information? The computer usually converts the digitised information of Fig. 5 into a record of log concentration (expressed in terms of absorbance units, A, or fringe displacement, J) versus radial distance squared. For fairly ideal monodisperse systems (e.g. dilute solutions of some small proteins) such plots are linear, but for non-ideal or heterogeneous systems they are not (Fig. 6). The average slope of these plots, linear or otherwise, gives the

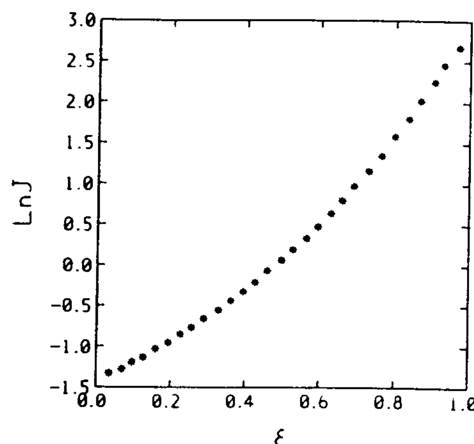

Fig. 6. Log concentration (expressed in Rayleigh fringe displacement units, J) versus distance squared (expressed in terms of the normalised parameter ξ), for a heterogeneous mucus glycoprotein.

(weight) average molecular weight. Local slopes along curves as in Fig. 6 can also be taken to give point or 'local' average molecular weights at a given radial position in the ultracentrifuge cell, and these can be particularly useful for assaying interacting systems. Further, a different type of average molecular weight, the *z-average* can be obtained either by further mathematical manipulation of the data or directly by using the Schlieren optical system mentioned earlier in the context of sedimentation velocity. (In some special cases the number average molecular weight can also be obtained) [1]. The ratio of the z-average to the weight average molecular weight is often used as an 'index of polydispersity' of polymeric samples, and can be related to the breadth of a distribution.

There are other ways of using sedimentation equilibrium data to get molecular weight distributions. Perhaps the simplest in principle and most useful is to combine with gel filtration, giving the latter an 'absolute' basis (i.e., not subject to assumptions concerning the conformation of calibration standards). Gel filtration is a very simple but powerful way of separating macromolecules of different sizes in a polydisperse solution. It can also give molecular weight distributions by comparing elution profiles with those of standard molecules of known molecular weight *and* the same conformation as the molecules being studied. Although the latter requirement is usually OK if globular proteins are being studied and globular protein standards are used, it fails for synthetic polymer and polysaccharide systems were so-called 'polystyrene' standards or 'pullulan' standards are often used. These problems can be avoided by taking fractions from the column and determining their molecular weights by sedimentation equilibrium [9].

[1] Normally, unless you are working with say a well-behaved solution of a macromolecule of low molecular weight (< 50000) at low loading concentration (< 1 mg/ml) the weight, z-average molecular weights obtained in this way (and the number average) will be *apparent* molecular weights due to solution non-ideality effects (real solutions of macromolecules differ from ideal solutions in much the same way as real gases differ from ideal gases). There are a number of ways of overcoming this, the most simple being to measure the apparent molecular weight at a series of concentrations and extrapolate back these values (or the reciprocals thereof) to zero concentration to give the "ideal" value. The non-ideality parameter itself (called 'B' or 'A_2') which comes from the slope can also give useful information about macromolecular conformation.

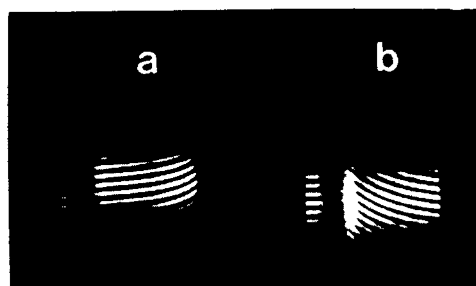

Fig. 7. (a) Rayleigh sedimentation equilibrium pattern for poly[(isoprene)-β-(ethylene oxide)] block copolymer micelles in water. (b) Rayleigh flotation equilibrium pattern, same polymer but in unimer form, in chloroform. Reproduced with permission from Ref. [13].)

8. Flotation velocity and equilibrium

Under the influence of a centrifugal field, conventional sedimentation takes place provided that the density of the sedimenting particle is larger than that of the solvent it is dispersed in. If the density is less, then the particle will move in the reverse direction. This situation happens with lipoproteins in aqueous solvents and some synthetic polymers in very dense solvents such as chloroform. The basic theory as for sedimentation velocity and equilibrium is the same, the only difference being the direction of movement. Conformations and homogeneity information can be deduced from flotation velocity, and molecular weights from flotation equilibrium experiments in much the same way [13,14]. An example is shown in Fig. 7 for a copolymer system (a model system for drug delivery), which in aqueous solvent gives a conventional sedimentation equilibrium pattern in which the polymer forms micelles, but in chloroform it gives a flotation equilibrium pattern corresponding to the polymer as discrete unimers. From the respective molecular weights for micelle and unimer it is possible to obtain an estimate for the association number of the micelle.

9. Analytical density gradient sedimentation equilibrium: composition analysis

Density gradient sedimentation equilibrium procedures using *preparative* ultracentrifuges are routinely used in biochemical science for purifying macromolecular systems on the basis of their density — the method is sometimes called *isopycnic*

density gradient equilibrium. The analogous situation in an *analytical* ultracentrifuge permits us to assay for purity. (This was the classical method used by Meselson, Stahl and Vinograd [15] to show that the replication of DNA was semi-conservative.) The idea is to have as a solvent a dense material which redistributes so that there is a distribution of density throughout the sedimentation cell. Any dissolved macromolecule will move until at equilibrium it reaches its isodensity point, i.e., the position in the cell where its own density matches that of the solvent. In aqueous solvents caesium salts are often used to produce the gradient (in the range 1.2 to 1.7 g/ml). Proteins, polysaccharides and nucleic acids have their isodensity points at approximately 1.3, 1.6 and 1.7 g/ml, respectively. For non-aqueous systems solvents like mixed diiodomethane–tetrahydrofuran systems can be used [16]. For a monodisperse system (such as a well fractionated polystyrene) density gradient analysis can also be used to estimate the diffusion coefficient [15].

10. Gels

The technique of sedimentation equilibrium can also be applied to the study of the structure of gels, especially if the Schlieren optical system is used [17], and can provide complementary information to conventional rheological methods. For example, because the network concentration will vary in the gel as a function of radial position it is possible to monitor the swelling pressure and other thermodynamic properties of the gel as a function of concentration [17]. Using the absorption optical system and after selection of an appropriate wavelength (i.e., in which the gel matrix is invisible) it is possible in principle to follow the diffusion of small molecules (including small proteins) through the gel, as a probe into gel structure.

11. Diffusion analysis

Historically the ultracentrifuge was used as a tool for measuring the diffusion coefficients of macromolecules in solution using a procedure known as 'boundary spreading'. How fast a sedimenting boundary broadened out was a function of the diffusion coefficient (then by combining the diffusion coefficient with the sedimentation coefficient the molecular weight could be obtained via a relation known as the 'Svedberg equation'). Nowadays the more rapid technique of dynamic light scattering is the method of choice for diffusion measurements, but for certain systems the analytical ultracentrifuge has clear advantages. One of these is for the analysis of the diffusion of molecules or macromolecules through concentrated media, including gels and two-phase systems. With the latter it is possible to model membrane phenomena. Applied in this way, the ultracentrifuge is not being used as a sedimentation tool as such, but rather the optical system alone is being used. A low rotor speed is chosen simply to minimise convection effects [18].

12. The future

As long as there is a need to measure the size, shape and interaction properties of macromolecules in solution there will always be a need for the analytical ultracentrifuge. True, for certain systems there are more powerful methods for weight, conformation or interaction analysis but each method has its own limitations, usually relating to maximum or minimum size of the macromolecule that can be studied, whether or not it can be crystallized, the minimum concentration required (a problem especially relevant to resonance methods), and the purity of the sample. The ultracentrifuge, with its versatility, will, besides remaining a powerful tool in its own right, become an increasingly important additional technique used in conjunction with some of these other methods, whether, for a particular macromolecular system, it be for example crystallography, NMR, light scattering, mass spectrometry, chromatography or electrophoresis. The virtue of combining measurements from independent techniques for confirmatory purposes or otherwise should never be ignored.

References

[1] S.E. Harding, A.J. Rowe and J.C. Horton (Editors), *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, Royal Society of Chemistry, Cambridge, 1992, 629 pp.
[2] I. Fiebrig, S.E. Harding and S.S. Davis, *Progr. Colloid Polym. Sci.*, 94 (1994) 66–73.
[3] W.F. Stafford, III, in S.E. Harding, A.J. Rowe and J.C. Horton (Editors), *Analytical Ultra-*

centrifugation in Biochemistry and Polymer Science, Royal Society of Chemistry, Cambridge, 1992, pp. 359–393.
[4] S.E. Harding, in S.E. Harding and A.J. Rowe (Editors), *Dynamic Properties of Biomolecular Assemblies*, Royal Society of Chemistry, Cambridge, 1989, pp. 32–56.
[5] J. Garcia de la Torre, (1989) in S.E. Harding and A.J. Rowe (Editors), *Dynamic Properties of Biomolecular Assemblies*, Royal Society of Chemistry, Cambridge, 1989, pp. 3–31.
[6] J. Garcia de la Torre and V.A. Bloomfield, *Biopolymers*, 16 (1977) 1779.
[7] S.J. Perkins, in S.E. Harding and A.J. Rowe (Editors), *Dynamic Properties of Biomolecular Assemblies*, Royal Society of Chemistry, Cambridge, 1989, pp. 226–245.
[8] J. Garcia de la Torre and V.A. Bloomfield, *Biochemistry*, 19 (1980) 5118.
[9] S.E. Harding, G. Berth, A. Ball, J.R. Mitchell and J. Garcia de la Torre, *Carbohydr. Polym.*, 16 (1991) 1.
[10] O. Smidsrød and I.-L. Andresen, *Biopolymerkjemi*, Tapir, Trondheim, 1979, 383 pp.
[11] E.N. Marsh and S.E. Harding, *Biochem. J.*, 290 (1993) 551.
[12] T.M. Laue, in S.E. Harding, A.J. Rowe and J.C. Horton (Editors), *Analytical Ultracentrifugation in Biochemistry and Polymer Science* Royal Society of Chemistry, Cambridge, 1992, pp. 63–89.
[13] S.E. Harding, P.J. Morgan and K. Petrak, *J. Phys. Chem.*, 94 (1990) 978.
[14] P.J. Morgan, S.E. Harding and K. Petrak, *Macromolecules*, 23 (1990) 4461.
[15] M. Meselson, F.W. Stahl and J. Vinograd, *Proc. Nat. Acad. Sci. U.S.A.*, 43 (1957) 581.
[16] W. Mächtle, in S.E. Harding, A.J. Rowe, and J.C. Horton (Editors), *Analytical Ultracentrifugation in Biochemistry and Polymer Science*, Royal Society of Chemistry, Cambridge, 1992, pp. 147–175.
[17] H. Cölfen and W. Borchard, *Prog. Colloid Polym. Sci.*, 86 (1989) 102.
[18] S.E. Harding and M.P. Tombs, in K.D. Schwenke and B. Raab (Editors), *Interactions in Protein Systems*, Abhandlungen der Akademie der Wissenschaften der DDR, Akademie-Verlag, Berlin, 1989, pp. 65–72,

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Interpretation and analysis of complex environmental data using chemometric methods

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An overview of the application of chemometric data analysis methods to complex chemical mixtures in various environmental media is presented. Reviews of selected research are given as examples of the application of principal components analysis and other statistical methods to identify contributions from multiple sources of contamination in air, water, sediments, and biota. Other examples are cited that illustrate how scientists have used classification and regression methods to model the distribution of anthropogenic contaminants and predict their environmental effects or fate.

1. Introduction

Environmental scientists are confronted with the daunting task of assessing ecosystem and human health impacts arising from a multitude of modern day pollutants. From the basic steps of data collection, data analysis/structure interpretation, and

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