Sedimentation velocity spins a new weave for an old fabric

Walter F Stafford III

Sedimentation velocity analysis is a powerful tool for the investigation of biological macromolecules under a wide range of solution conditions. If carefully applied, it can be an effective tool for the characterization of interacting systems in solution. It is rapidly becoming a method of choice among the biotechnology community. In recent years, there have been notable advances in the ease of acquisition and analysis of analytical ultracentrifugation data.

Addresses
Analytical Ultracentrifugation Research Laboratory, Boston Biomedical Research Institute, 20 Staniford Street, Boston, MA 02114, USA; e-mail: stafford@bbri.harvard.edu

Current Opinion in Biotechnology 1997, **8**:14–24

Electronic identifier: 0958-1669-008-00014

Abbreviations
FTP file transfer protocol
IL interleukin

Introduction

The analytical ultracentrifuge is a high-speed centrifuge equipped with an optical system that allows the observation of proteins, nucleic acids, carbohydrates, lipids and other macromolecules during sedimentation. The velocity, as well as the shape of the moving boundary formed in the centrifugal field, can give information about the size, shape and association properties of these kinds of molecules.

Recent notable advances in the ease of acquisition and analysis of analytical ultracentrifugation data have been largely due to the introduction of two modern computer-controlled analytical ultracentrifuges by Beckman Instruments: the Optima XL-A and XL-I. The XL-A has a double beam absorption scanning optical system allowing the user to observe the sedimenting sample at any wavelength in the range 190–650 nm. The Optima XL-I, in addition to having absorption optics, is also equipped with a differential Rayleigh interferometer, allowing the user to follow the refractive index of the sample as a function of position during sedimentation. The Rayleigh optical system is essential for the analysis of nonabsorbing or weakly absorbing materials like carbohydrates, lipids and peptides or proteins that lack an appropriate chromophore.

The online Rayleigh optical system was pioneered by Tom Laue and has become the basis for further developments [1–8]. In 1996, Beckman Instruments Inc introduced the Optima XL-I with Rayleigh optics based on his system. The availability of Rayleigh optics greatly increases the number and types of systems that can be analyzed with this instrument.

An important feature of sedimentation analysis is that it can be used to investigate systems under ordinary solution conditions. One often requires information about the behavior of a system under a specific set of conditions that might differ quite significantly from those required for high performance liquid chromatography (HPLC), size exclusion chromatography or nondenaturing gel electrophoresis. Sedimentation analysis is ideally suited to the study of large macromolecular assemblies that might be difficult or impossible to study by chromatography or gel electrophoresis. This applies especially to glycosylated proteins and polysaccharides, because they often tend to interact with the support materials used for chromatography and electrophoresis. Moreover, large particles like viruses, which may be too large to be accommodated by the support matrices used by those techniques, can be studied conveniently by sedimentation velocity analysis.

Both titration calorimetry and light scattering share with sedimentation analysis the ability to be applied under ordinary solution conditions; however, both of these techniques require separate measurements at each concentration. Sedimentation equilibrium, in particular, allows high-quality data to be acquired over a broad range of concentrations in a single experiment. Sedimentation velocity analysis, to a lesser extent, can be carried out to span a modest concentration range in a single experiment. Even though each cell loading constitutes a single concentration point when analyzing interacting or otherwise concentration-dependent systems, analysis of the shape of the boundary at each loading concentration can be a rich source of information about the system.

Sedimentation velocity analysis has seen a recent upsurge in use with the development of several new methods of boundary analysis used to obtain either diffusion-corrected sedimentation coefficient distribution functions or both sedimentation and diffusion coefficients. Recently, time-derivative methods have been applied to the analysis of both irreversible and rapidly reversible interacting systems by sedimentation velocity. Before the widespread use of computers and digital data acquisition systems, the main piece of information that one obtained from a sedimentation velocity experiment was a determination of the sedimentation coefficient, along with an estimate of the purity of a particular sample. Although it was widely known that useful information could be obtained from an analysis of boundary shape [9–12], and from an analysis of the rate of boundary spreading [13,14], detailed analysis was usually thwarted by the Herculean effort required to extract the parameters of interest. This situation has

changed dramatically with the introduction of desktop computers.

The reader is referred to an excellent review by Hansen *et al.* [15], covering the new developments up to 1994, and reviews by Hensley [16••] and by Schuster and Toedt [17••] that cover more recent developments. This review discusses some of the more recent developments as well as those we can expect to see in the near future.

Background

The ultracentrifuge can be operated in two different modes (Fig. 1). In the first mode, sedimentation velocity, the centrifuge is operated at relatively high speeds so that a well-defined boundary is formed between the sedimenting material and the solvent in which it is dissolved. In a sedimentation velocity experiment, one can measure the velocity as well as analyze the shape of the boundary. By careful analysis of boundary shape and movement, one can obtain estimates of stoichiometries and equilibrium constants for interacting systems in addition to sedimentation and diffusion coefficients of the macromolecular species involved. In the second mode, sedimentation equilibrium, the centrifuge is operated at relatively low speeds until a gradual concentration gradient is set up such that sedimentation and diffusion forces are balanced and the system is at mechanical and chemical equilibrium. Methods of analysis of the equilibrium concentration distribution are well grounded in thermodynamic theory [10] and, therefore, allow one to obtain an absolute determination of molecular mass as well as a determination of stoichiometries and equilibrium constants for interacting systems. A system for use as a standard for analysis of interacting systems has recently been suggested by Joss and Ralston [18•].

Whole boundary analysis

In the various methods of whole boundary analysis, the evolution of the position and shape of the boundary during sedimentation is analyzed. Whole boundary analysis falls into two main categories. In the first, various extrapolation methods are employed to remove the effects of diffusion to reveal the distribution of sedimentation coefficients. The most recent treatments of this type have been presented by Demeler *et al.* [19•] for integral distributions, and by me [20] for differential distributions.

In the second, both diffusion and sedimentation are included in the treatment to obtain estimates of both the sedimentation coefficient, s, and the diffusion coefficient, D. The differential equation (Lamm equation; [21]) describing the development of the concentration profile during sedimentation has been solved analytically to various degrees of approximation (cf. [22,23]). For a single homogeneous macromolecule, the analytical solutions describe the boundary very closely in the shape of the error function. There are several methods of analysis that are based on curve fitting the boundary to an appropriate form of these approximate solutions. There is also a method based on a rearrangement of the Lamm equation that employs the time dependence of the concentration profile to determine both sedimentation and diffusion coefficients, developed in the early 1970s in the laboratory of Len Bethune [24–26]. This method will not be reviewed here but is mentioned because of its appeal as the basis for a direct fitting approach.

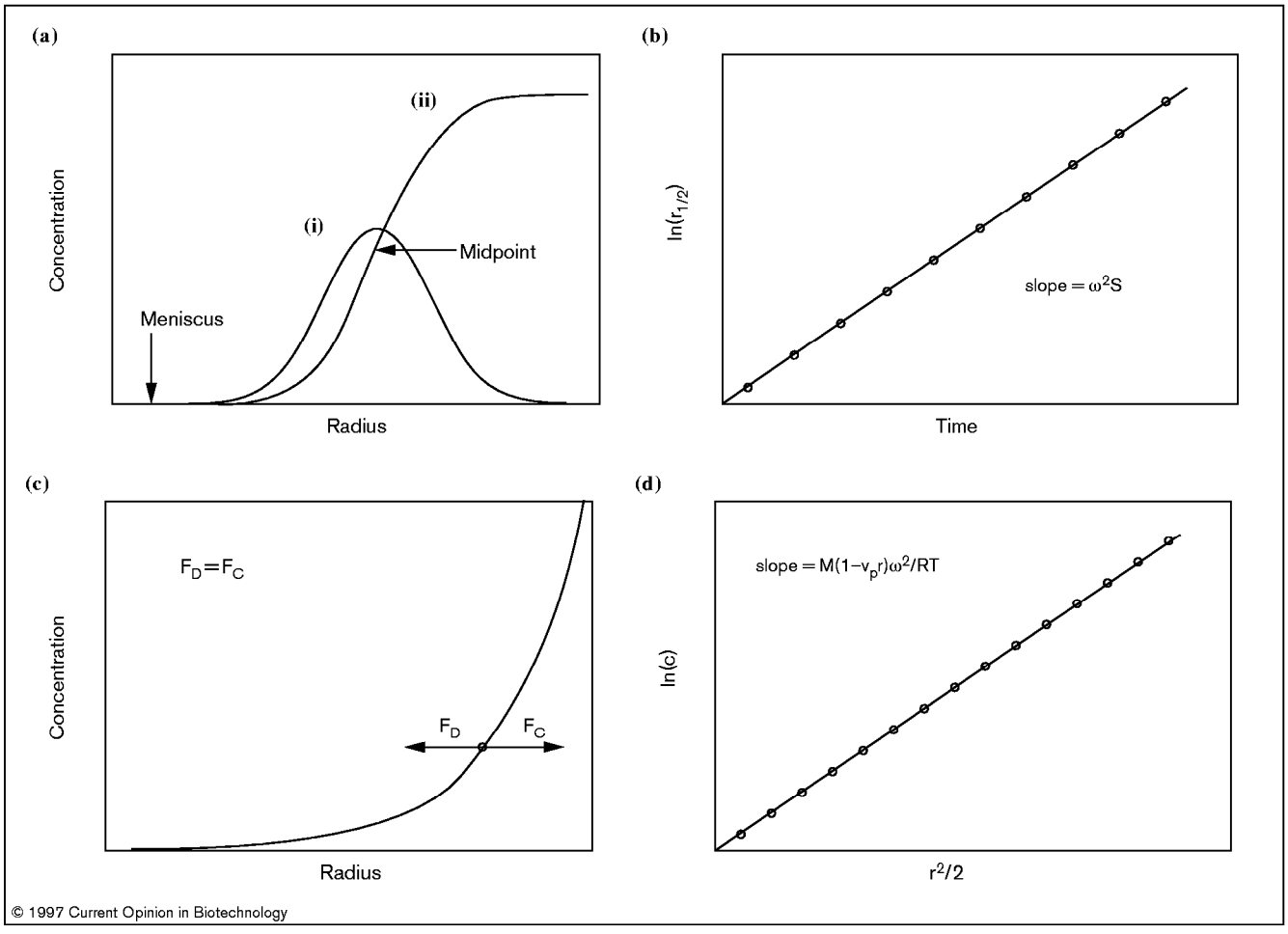
Extrapolation method of van Holde and Weischet

Although the van Holde and Weischet [27] method of whole boundary analysis is not new, it had received little attention until recently when it was implemented in an easily used computer program and was employed extensively by Hansen *et al.* [19•,28–31] to explore chromatin structure as well as other systems. It is an extrapolation method for the elimination of the contribution of diffusion to the shape of the sedimentation boundary. In this method, at a series of time points, one computes an apparent sedimentation coefficient s* (see Eqn 2) at each level in the boundary. The values of s* at each level are then plotted against $1/t^{0.5}$ and extrapolated to infinite time (i.e. to $1/t^{0.5} = 0$; see Fig. 2) . Because boundary spreading due to diffusion is proportional to the square root of time and the separation between components is proportional to the first power of time, the contribution due to diffusion becomes insignificant at infinite time and the true distribution of sedimentation coefficients is revealed. It is best applied to large noninteracting macromolecular assemblies for which diffusion coefficients are not desired but maximum resolution between components is sought.

The mathematical form used for the extrapolation is valid only for nonconcentration-dependent systems; nevertheless, extrapolations for both non-ideal and interacting systems yield characteristic plots that are quite useful for the identification of the type of concentration dependence. A tutorial for the use of this software can be found on the World Wide Web at http://bioc09.uthscsa.edu/.biochem/tutor.html. Quantitative analysis of concentration-dependent systems should be carried out using other methods. When using sedimentation velocity to study interacting systems, one must analyze them either by standard methods, as reviewed recently by Lee and Rajendran [32], or by the time-derivative methods developed by me [33,34].

In many cases, sedimentation equilibrium may be the preferred method because a broad range of high-quality data can be obtained from a single cell loading. Velocity and equilibrium sedimentation provide complementary and corroborative information. Both techniques should be used where possible. The shape of a sedimentation velocity boundary produced by an interacting system can be quite characteristic of the mode of interaction and this information can help resolve ambiguities inherent in resolving components in an equilibrium experiment.

Figure 1



Comparison of sedimentation velocity and sedimentation equilibrium. **(a)** Typical concentration distribution, c versus r, in a sedimentation velocity experiment showing the boundary formed between the solvent and the solution. **(i)** Concentration distribution, c versus r, after formation of the boundary and **(ii)** the corresponding concentration gradient curve, dc/dr versus r. The maximum in the gradient corresponds very closely to the midpoint position. **(b)** Plot of the logarithm of the boundary midpoint position as a function of time. The slope of this plot is proportional to the sedimentation coefficient, S, for an ideal monodisperse system. **(c)** Typical concentration distribution in a sedimentation equilibrium experiment showing the more gradual concentration gradient formed at sedimentation–diffusion equilibrium, at which the force due to the centrifugal field, F_C, is balanced by the contrary force due to diffusion from the region of higher to lower concentration, F_D. **(d)** Plot of the logarithm of the equilibrium concentration distribution as a function of $r^2/2$. The slope of this line is proportional to the molecular mass for an ideal, homogeneous system.

Moreover, time-derivative methods are especially useful at low concentration when either materials are scarce or complexes are too tight to be analyzed at the higher concentrations required by sedimentation equilibrium.

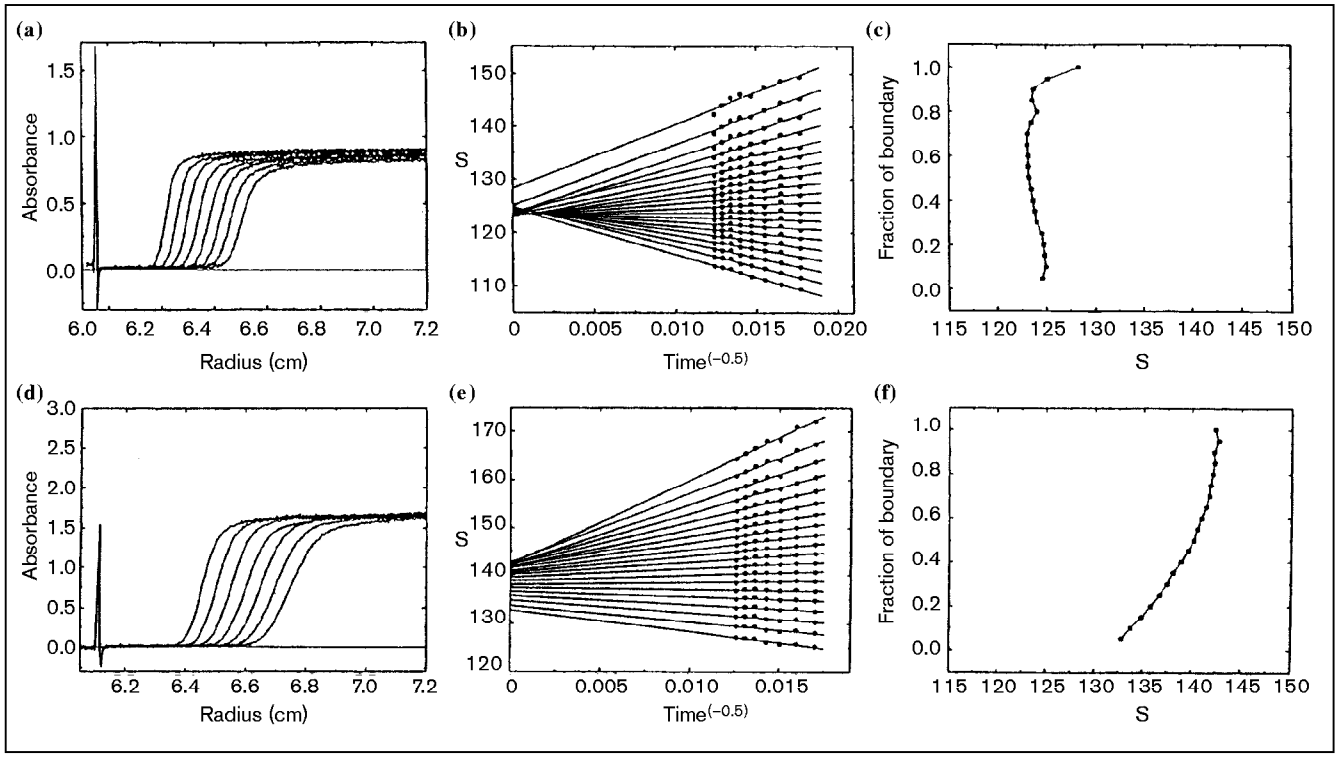
Whole boundary curve fitting

Most methods of whole boundary analysis involve some form of nonlinear parameter estimation to obtain values of s and D. Approximate closed forms of solutions to the Lamm equation exist only for the simplest cases and are limited to ideal solutions containing a few species exhibiting no concentration dependence. The methods to be described below involve fitting a series of concentration profiles to approximate analytical solutions to the Lamm equation.

One of the earliest applications of whole boundary fitting of approximate solutions of the Lamm equation was reported by Les Holladay, who derived an approximate solution and used it as a whole boundary fitting function [35,36]. It was most accurate at early times but gave less good fits as time went on. It was not widely used, mainly because the analytical ultracentrifuge was not widely used at that time. Recently, Holladay's type of approach has received more attention because of the need to analyze solutions of low molecular mass materials (10–20 kDa) or unstable samples that could not be analyzed sufficiently rapidly by sedimentation equilibrium.

John Philo [37] has developed a whole boundary fitting function that is implemented in a software program called

Figure 2



Integral sedimentation coefficient distributions: analysis of sedimentation velocity boundaries by the method of van Holde and Weischet [27]. The boundary is divided into levels relative to the plateau concentration. At each level, a value of s* is computed from Equation 2. The values of s* at corresponding levels are then plotted versus $1/t^{0.5}$ and extrapolated to $1/t^{0.5}=0$, corresponding to infinite time. The result is the diffusion-corrected integral distribution of s, G(s). Data were obtained in a Beckman XL-A analytical ultracentrifuge equipped with scanning absorption optics. **(a–c)** A preparation of bacteriophage T7MLD capsid II in which 0.01% of the mass of the capsids was subgenomic DNA [82]. **(d–f)** A preparation of bacteriophage T7 MLD capsid II in which the capsids contained measurable amounts of subgenomic packaged DNA [82]. The boundaries used for the analyses are shown in (a,d). Extrapolation plots are shown in (b,e). Integral distribution of s plots is shown in (c,f). The speed of the run was 10 000 rpm, and the temperature was 20 °C. Figure reproduced from [15].

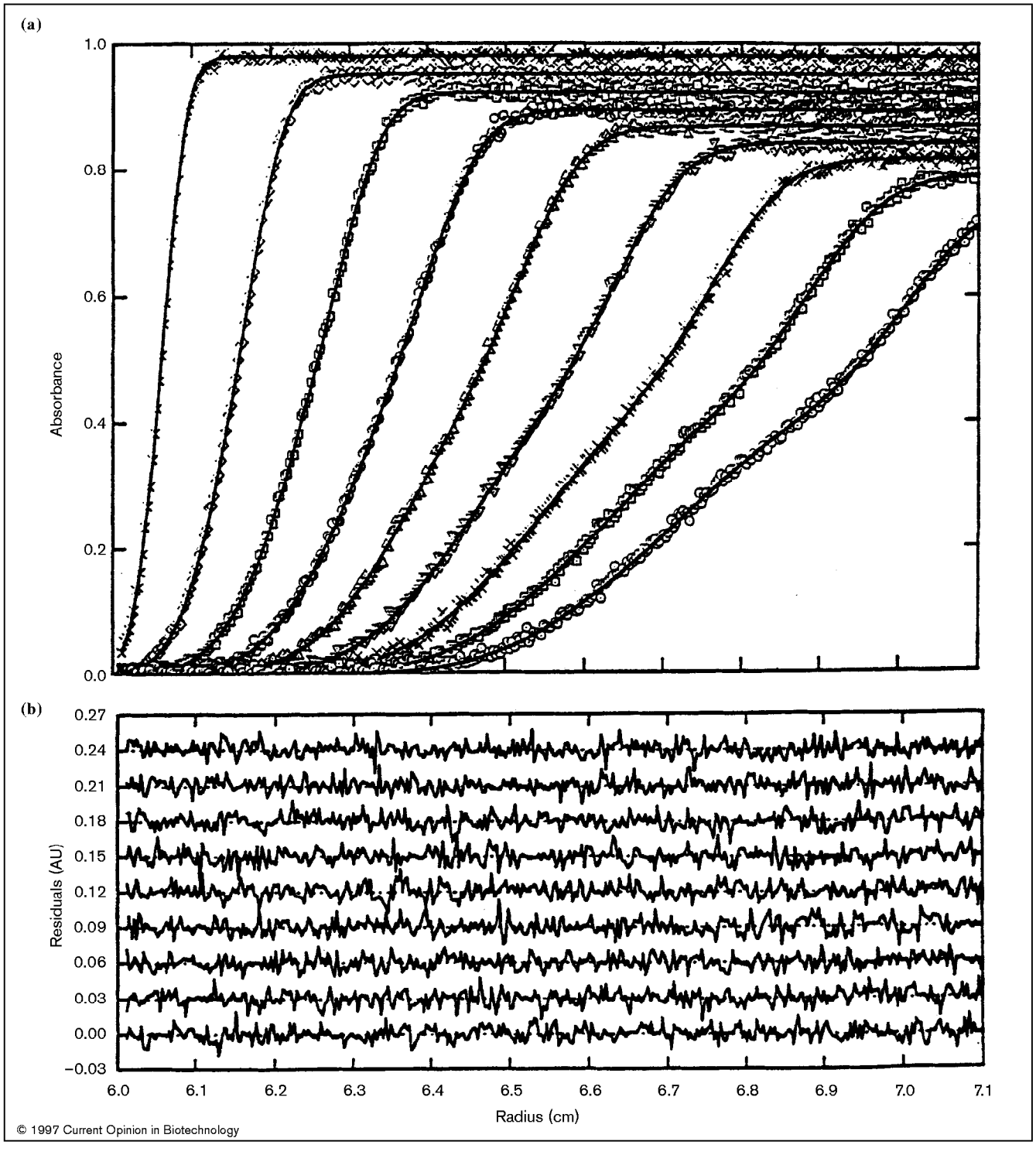
SVEDBERG. The first version of this method involved fitting sedimentation boundaries with an approximate Faxén-type solution to the Lamm equation. Faxén-type solutions assume an initially sharp free-standing boundary in a cell of infinite length [38] and, therefore, do not treat the effects of restricted diffusion and sedimentation at the meniscus and cell bottom [22]. Recently, Philo has improved this approach by using an expression (Fujita–MacCosham Equation) with two additional semi-empirical correction terms to give a better approximation that includes the effects of the boundary conditions at the meniscus and base of the cell [39]. The new method gives better fits for low molecular mass macromolecules and is better at resolving mixtures than the first method (Fig. 3).

The effects of moderate concentration dependence can be accommodated if runs are carried out at low concentration, although this results in reduced accuracy in both s and D. This method has received considerable attention since it was introduced in 1994 [40•,41,42,43•,44–46]. The software program SVEDBERG is available from the RASMB FTP software archive (see below).

Philo also gives a careful comparison of his method to the time-derivative method when applied to the analysis of low molecular mass macromolecules. As he points out, care must be exercised when using the time-derivative method for the analysis of proteins in the molecular mass range below 40 kDa. There is a small systematic shift from the correct value of s in the peak position of g(s*) to smaller values as the molecular mass decreases from 40 kDa (2% error) to 18 kDa (4% error) for a boundary that has travelled 0.6 cm from the meniscus. The error is larger when the boundary is nearer the meniscus and becomes negligible if the curves are extrapolated to infinite time (WF Stafford, unpublished data). The shift must be corrected if the values of s are to be used for shape analysis. However, the shift has very little effect on the determination of molecular mass derived from these curves because of compensating errors in the determination of the diffusion coefficient [47].

A whole boundary fitting approach also has been developed by Behlke and Ristau [48] using various solutions to the Lamm equation incorporating many high order terms

Figure 3



Whole boundary fitting. Simulated data for a mixture of three species in the ratio 2 to 1 to 1: 5.80S, 4.32F; 4.49S, 5.07F; and 3.54S, 6.59F. This system is meant to simulate the experimental system described by Hensley (adapted from Philo [39]). **(a)** Concentration distributions and fits for various times of sedimentation. **(b)** Residuals for a three-species fit returning the values of 5.769 (5.779–5.811)S, 4.42 (4.30–4.55)F; 4.473 (4.413–4.530)S, 4.91 (4.59–5.22)F; and 3.539 (3.515–3.563)S, 6.82 (6.55–7.08)F. The returned values are in very good agreement with the input parameters and demonstrate the validity of this curve-fitting method.

into the fitting function. Using a computer program called LAMM, they have compared five different functions found in Fujita's monographs [22,23]. The equations containing the most terms seem to work best, but none

of them can correctly handle the meniscus region at early times. However, they get good results if the points near the meniscus are deleted from the fits. They have used at least one equation that can account for concentration dependence in a synthetic boundary experiment. The software program LAMM is available from the RASMB FTP software archive (see below).

Direct curve fitting to approximate closed-form equations is restricted to ideal, noninteracting systems at the present time. A more versatile yet computationally more demanding approach is to generate numerical solutions for various guesses of parameters and compare these solutions to the experimental data. This approach has been incorporated into a nonlinear least squares fitting algorithm originally described by Todd and Haschemeyer [49]. It has been revived recently by Demeler (personal communication), who has exploited it for the analysis of complex boundaries. In this algorithm, the function generator is sedimentation simulation software that uses the rapid finite element method of Claverie [50]. Demeler has recently offered two commercial versions of the curve-fitting software, one for several non-interacting components and another one that can be used for fitting to a rapidly reversible monomer–dimer system to give the equilibrium constant.

A potential disadvantage of fitting procedures is that they are model dependent and, more importantly, require adequate baseline corrections to the patterns before reliable fits can be obtained. The are a number of other conditions that must be met before least squares analysis can be applied with confidence. For a detailed discussion of the use of least squares for parameter estimation, the reader is referred to the excellent reviews by Johnson and co-workers [51,52].

Time-derivative methods

The time-derivative is computed from a set of the concentration profiles and converted into an apparent sedimentation coefficient profile, g(s*) versus s* according to the following equation [33]

$$g(s^*) = \left(\frac{\partial c}{\partial s^*}\right)_t = \left[\left(\frac{\partial c}{\partial t}\right)_r + 2\omega^2 \int_{s^*=0}^{s^*=s^*} s^* \left(\frac{\partial c}{\partial s}\right)_t ds^*\right]\left[\left(\frac{\partial t}{\partial s^*}\right)_r\right] \quad (1)$$

where c is the concentration in arbitrary units, r is the radius, t is the time, and s* is defined by

$$s^* = \left[\frac{1}{\omega^2 t}\right] ln\left(\frac{r}{r_m}\right) \quad (2)$$

where ω is the angular velocity of the rotor and r_m is the radius of the meniscus.

The first term inside the first set of square brackets is the total time derivative computed point-by-point by subtracting pairs of concentration profiles. The second term involving the integral may be considered a correction term to account for dilution that occurs as the sample moves down the sectorially shaped cell. The second factor in square brackets is essentially a scale factor that changes the units of the time derivative so that the area under the curve is equal to the concentration in the plateau region beyond the boundary. Note that g(s*) is a function of itself through the term inside the integral and, therefore, this equation must be solved iteratively. The g(s*) versus s* curves are geometrically similar to the corresponding curves of dc/dr versus r that one would have obtained from the refractive index gradient patterns obtained with schlieren optics. Software that computes g(s*) from a set of concentration profiles, called DCDT, is available from the RASMB FTP software archive (see below). The essentials of the method are summarized in Figure 4.

Computation of the time derivative results in complete elimination of the time-independent baseline contributions to the sedimentation boundary patterns and, when combined with signal averaging, can result in 2–3 orders of magnitude increase in the signal-to-noise ratio compared to other methods [33,53,54]. Use of the time-derivative method has been reported by a number of investigators recently [43•,55–59,60•,61••,62–64,65•,66–69].

A curve-fitting method based on the time derivative has been devised to obtain the molecular mass directly from g(s*) versus s* curves (Fig. 4) [47]. In this method, fits to simple Gaussian functions are used to estimate values of D from the standard deviation of the apparent distribution function, g(s*) versus s*

$$D = \frac{(\sigma r_m \omega^2 t)^2}{2t} \quad (3)$$

where r_m is radius of the meniscus, σ is the standard deviation as defined by

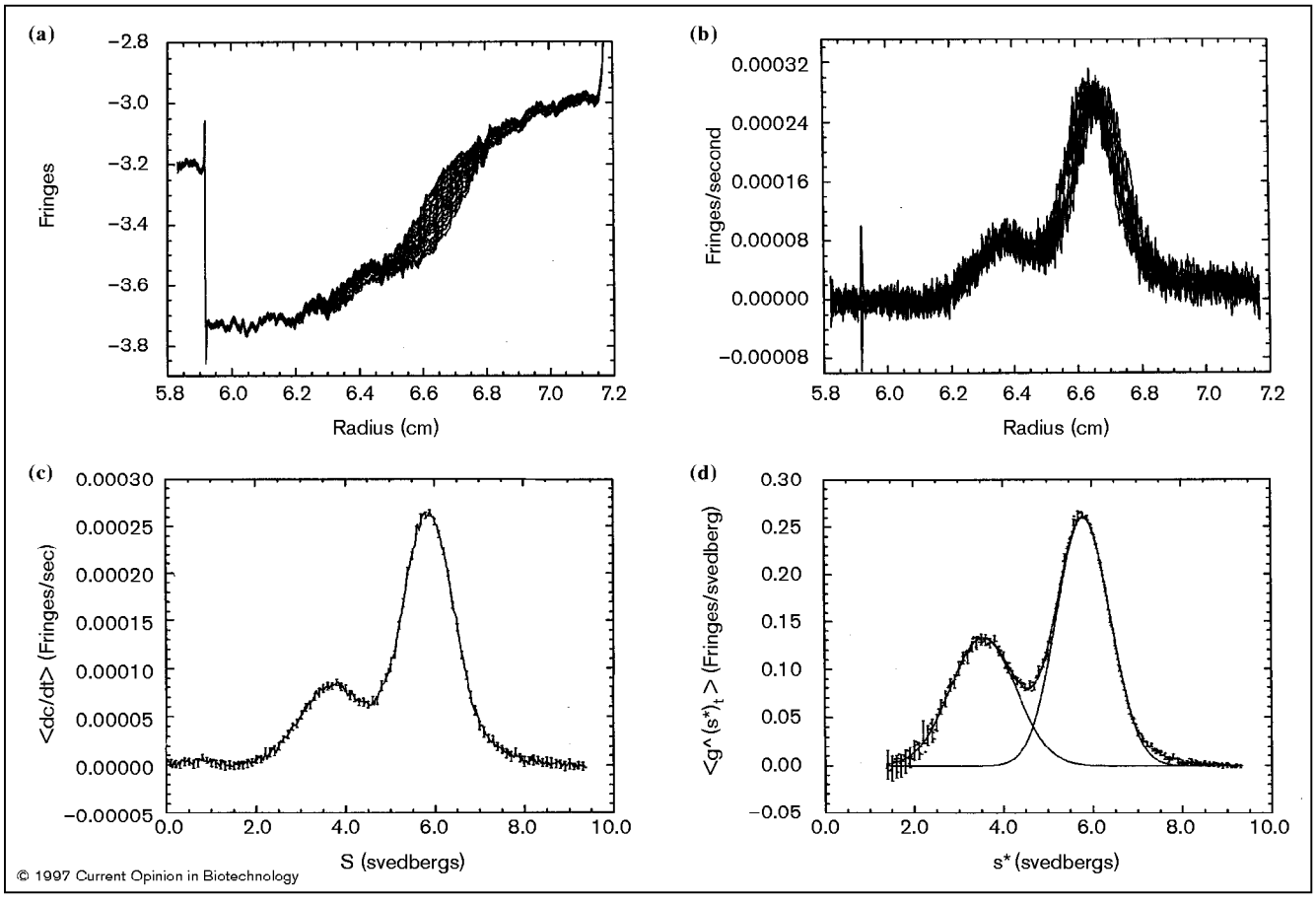
$$g(s*) = Aexp\left[-0.5\frac{s-s*)^2}{\sigma^2}\right] \quad (4)$$

where s is the sedimentation coefficient corresponding to the maximum in the g(s*) versus s* curve. This value of D is combined with the value of s using the Svedberg equation to get the molecular mass

$$M = \frac{s}{D}\frac{RT}{(1-\nu\rho).} \quad (5)$$

The method is based on the observation that the g(s*) curves are essentially Gaussian. A relationship between the standard deviation of the g(s*) curve and the diffusion

Figure 4



Time-derivative method of sedimentation velocity analysis. **(a)** Concentration profiles obtained from an online Rayleigh system on a Beckman Instruments Model-E analytical ultracentrifuge. Forty curves (only every second curve is shown) were obtained every 20 s over a period of approximately 13 min. **(b)** Time-derivative curves plotted as a function of radius obtained by subtracting pairs of curves shown in (a) (only 10 are shown). **(c)** Averaged time-derivative curves. Curves in (b) were plotted as a function of s* to align them by compensating for the time difference between them according to Equation 2 and then averaged to produce this plot. The error bars represent the standard error of the mean at each point. **(d)** Plot of g(s*) versus s* after transforming dc/dt into dc/ds* using Equation 1 so that now the area under the curve is equal to the concentration. The g(s*) versus s* curves are geometrically similar to the corresponding plots of dc/dr versus r that would have been obtained with schielren optics, which measure the refractive index gradient (Fig. 1). However, g(s*) versus s* curves generally have a signal-to-noise ratio that is 2–3 orders of magnitude higher. The solid curves are the result of fitting Gaussian curves according to Equations 3 and 4 to the bimodal g(s*) curve. The left-hand peak is the Fab. It has a molecular mass by mass spectrometry of 48.3 kDa; the curve fitting returned values of s=3.55S and D=6.48F, giving a molecular mass of 49.8 kDa (cf. Eqn 5). The right hand peak is the 2:1 complex comprising two moles of Fab and one mole of IL-5 dimer; it has a molecular mass from mass spectrometry of 125 kDa; the curve fitting returned values of s=5.80S and D=4.20F corresponding to a molecular mass of 126 kDa in very good agreement with the value obtained by mass spectrometry.

coefficient was derived from a Faxén-type solution to the Lamm equation. The method gives results for molecular mass that are accurate to within about 2–5%. This method has been used recently to analyze a strong, irreversible interaction in solution between an anti-IL-5 Fab and IL-5 dimer (IL, interleukin). A preliminary description of these experiments is available on the World Wide Web at http://www.beckman.com/biorsrch/sympo/dscvry/binding.htm. Another application of this method has also appeared recently [70].

Rapidly reversible interacting systems

A single homogeneous protein might undergo self-association to form higher oligomers, or two different proteins may interact to form a complex. These types of interaction can be either very strong, in which case the binding is complete and no re-equilibration can take place on the timescale of the experiment, or they can be rapidly reversible on the timescale of the experiment, such that re-equilibration takes place continuously during transport. Interacting systems of either type can be studied conveniently by sedimentation velocity as well as by sedimentation equilibrium. Time-derivative methods have been applied to the analysis of interacting systems [34,71].

The apparent differential sedimentation coefficient distribution function, g(s*) versus s*, calculated from the time derivative can be used for the analysis of interacting

systems especially at low concentrations because of its relatively high signal-to-noise level. It has been applied to the analysis of an antigen–antibody system at low protein concentration using online Rayleigh optics in the 5–10 $\mu g\,ml^{-1}$ range to estimate equilibrium constants [54,60•,71].

The time-derivative method has been used extensively by Jack Correia and co-workers [59,63,69] to investigate the thermodynamics of tubulin self-association under various conditions. They have been able to separate the effects of drug binding to monomers from self-association. By taking into account the linkage of drug binding to polymers as a function of nucleotides, drugs, salt and divalent cations, they are dissecting the energetics of this system. Because the binding events are all linked to self-association, a description of simple structure–function relationships must include binding interactions and conformational changes induced by self-association. Occasionally, the free energy linkages appear to be simply additive [63], but cooperative free energies also appear to enter the picture. They have concluded that the $\Delta\Delta G$ enhancement for GTP to GDP is 0.9 kcal mol^{-1} in low salt, but only 0.4 kcal mol^{-1} in 150 mM NaCl. NaCl effects the GTP state more than the GDP state. Eventually, they should be able to assign energetic roles to drug modifications, different protein domains, post-translational modifications and isotypes in addition to other effects.

Hydrodynamic modeling from X-ray crystallographic data

Most hydrodynamic modeling of complex structures has been based on the work of Kirkwood [72] and has been extended to arbitrary shapes by several researchers [73,74], most notably by Garcia de la Torre and Bloomfield [75]. A computer program, HYDRO, for computing various hydrodynamic parameters from a model that consists of an arbitrary assemblage of spherical beads has been recently updated and made available by Garcia de la Torre and co-workers [76,77].

Recently, with the ever increasing availability of high-resolution structures from X-ray crystallography, a need has developed to represent these structures in hydrodynamically equivalent forms to make comparisons with solution measurements [78,79]. Because of the large number of atoms in any given structure, the direct introduction of atomic coordinates and radii into HYDRO would create a computational burden that is beyond the capacity of most readily accessible computers. Recently, an algorithm for deriving a bead model from a set of crystal coordinates was reported by Olwyn Byron [80••]. This algorithm was designed to create models that are composed of far fewer elements than the original crystal structure but that still faithfully represent its most important structural features [80••]. This algorithm promises to be of great utility. It is available from the RASMB FTP software archive (see below).

Analytical ultracentrifugation e-mail server and software FTP archive

An e-mail list server has been established for the exchange of information related to the study of reversible associations in structural and molecular biology (RASMB). Analytical ultracentrifugation has been a popular topic among the participants. Many of the exchanges deal with the problems and ideas of researchers who use these techniques on a day-to-day basis. One may subscribe to it by sending a request to rasmb-manager@bbri.harvard.edu. In addition to the e-mail server, a software archive has been established for the exchange of software for the analysis of ultracentrifuge data. The archive can be reached by anonymous FTP to bbri.harvard.edu/rasmb/spin. A current list of literature references dealing with the analytical ultracentrifuge can be obtained on the World Wide Web at http://www.bbri.harvard.edu/rasmb/xla_refs.html.

Other internet sites of interest are the National Analytical Ultracentrifuge Facility at the University of Connecticut at http://www.ucc.uconn.edu/~wwwbiotc/uaf.html and the Beckman Instruments World Wide Web site at http://www.beckman.com. The RASMB FTP site is mirrored at the University of Texas at http://bioc02.uthscsa.edu/. The XL-A literature reference database is also mirrored at http://bioc02.uthscsa.edu/.biochem/xla1.html and at http://www.beckman.com/biorsrch/xlaref/xlaref.htm.

The future

The new instruments would not have been possible without important contributions from those who have dedicated themselves to the development of new theoretical and practical approaches to computerized data acquisition and analysis. Both sedimentation equilibrium and sedimentation velocity have taken major strides toward the goal of accessibility to all laboratory scientists. Because of these recent developments, analytical ultracentrifugation will again take its proper place in the laboratory as a routinely applied research tool for the analysis of interacting systems in solution. We can expect continued progress toward more advanced instrumentation that is both more sensitive and easier to use.

An exciting prospect is the commercial development of fluorescence optics for the Beckman Optima XL series analytical ultracentrifuges. The feasibility of fluorescence optics for the Beckman Instruments Model-E analytical ultracentrifuge was demonstrated by Schmidt and Riesner [81] who described the analysis of fluorescein isothiocyanate-labeled bovine serum albumin (BSA) at 10^{-10} M (6.5 ng ml^{-1}) and of RNA and DNA labeled with ethidium bromide at concentrations of 6×10^{-11} M (530 pg ml^{-1}).

A prototypical fluorescence optical system for the XL-I has been developed by Tom Laue and will be presented at the SPIE (International Society for Optical Engineering) Bios97 meeting at the Symposium on Ultrasensitive

Clinical Diagnostics: Advances in Ultracentrifugation in San Jose 1997 and at the Workshop on Analytical Ultracentrifugation at the 1997 annual meeting of the Biophysical Society in New Orleans.

We will be looking forward to the commercial development of fluorescence optics for new centrifuges with the extended sensitivity and selectivity that fluorescence techniques can provide. We can probably expect 2–3 orders of magnitude extension of the range of concentrations allowing analysis of extremely dilute solutions and interacting systems of high affinity.

Conclusions

Sedimentation velocity analysis is a powerful tool for the investigation of the overall size and shape of proteins and nucleic acids under ordinary, as well as a wide range of not so ordinary, solution conditions. It can also provide information about the purity and state of aggregation of a protein agent under a particular set of formulation conditions. As we have seen, if carefully applied, it can be an effective tool for the characterization of interacting systems providing important thermodynamic information.

Of special interest to the biotechnology community, it can be a potent method for the characterization of protein formulations to be used as therapeutic agents. Because it is one of the few methods capable of giving information about protein–protein interactions under a specified set of conditions in solution, it is rapidly becoming a method of choice to provide this essential information.

Acknowledgements

I would like to thank John Philo, Olwyn Byron, Joachim Behlke, Jeff Hansen, Borries Demeler, Preston Hensley, Karen Fleming, and Todd Schuster for supplying preprints of their manuscripts prior to publication and Preston Hensley, Tom Laue, and John Philo for helpful discussions during the preparation of this manuscript.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Laue TM: **Rapid precision interferometry for the analytical ultracentrifuge [PhD thesis].** Storrs, Connecticut: University of Connecticut; 1981.

2. Runge MS, Laue TM, Yphantis DA, Lifsics MR, Saito A, Altin M, Reinke K, Williams RC Jr: **ATP-induced formation of an associated complex between microtubules and neurofilaments.** *Proc Natl Acad Sci USA* 1981, **78**:1431–1435.

3. Laue TM, Johnson AE, Esmon CT, Yphantis DA: **Structure of bovine blood coagulation factor Va. Determination of the subunit associations, molecular weights, and asymmetries by analytical ultracentrifugation.** *Biochemistry* 1984, **23**:1339–1348.

4. Yphantis DA, Laue TM, Anderson I: **Rapid precision interferometry for the analytical ultracentrifuge. II. A laser controller based on a rate-multiplying circuit.** *Anal Biochem* 1984, **143**:95–102.

5. Laue TM, Yphantis DA, Rhodes DG: **Rapid precision interferometry for the analytical ultracentrifuge. III. Determination of period of rotation, frequency of rotation, and elapsed time.** *Anal Biochem* 1984, **143**:103–112.

6. Laue TM: **On-line data acquisition and analysis from the Rayleigh interferometer.** In *Analytical Ultracentrifugation in Biochemistry and Polymer Science.* Edited by Harding SE. Cambridge: Royal Society of Chemistry; 1992:63–89.

7. Yphantis DA, Lary JW, Stafford WF, Liu S, Olsen PH, Hayes DB, Moody TP, Ridgeway TM, Lyons DA, Laue TM: **On line data acquisition for the Rayleigh interference optical system of the analytical ultracentrifuge.** In *Modern Analytical Ultracentrifugation: Acquisition and Interpretation of Data for Biological and Synthetic Polymer Systems.* Edited by Schuster TM, Laue TM. Boston: Birkhäuser–Boston; 1994:209–226.

8. Laue TM: **An on-line interferometer for the XL-A ultracentrifuge.** *Prog Coll Polym Sci* 1994, **94**:74–81.

9. Svedberg T, Pederson KO: *The Ultracentrifuge.* New York: Oxford University Press; 1940.

10. Williams JW, Van Holde KE, Baldwin RL, Fujita H: **The theory of sedimentation analysis.** *Chem Rev* 1958, **58**:715–806.

11. Schachman HK: *Ultracentrifugation in Biochemistry.* New York: Academic Press; 1959.

12. Cann JR: *Interacting Macromolecules.* New York: Academic Press; 1970.

13. Werner WE, Cann JR, Schachman HK: **Boundary spreading in sedimentation velocity experiments on partially liganded aspartate transcarbamoylase. A ligand-mediated isomerization.** *J Mol Biol* 1989, **206**:231–237.

14. Werner WE, Schachman HK: **Analysis of the ligand-promoted global conformational change in aspartate transcarbamoylase. Evidence for a two-state transition from boundary spreading in sedimentation velocity experiments.** *J Mol Biol* 1989, **206**:221–230.

15. Hansen JC, Lebowitz J, Demeler B: **Analytical ultracentrifugation of complex macromolecular systems.** *Biochemistry* 1994, **33**:13155–13163.

16. •• Hensley P: **Defining the structure and stability of macromolecular assemblies in solution – the re-emergence of analytical ultracentrifugation as a practical tool.** *Structure* 1996, **4**:367–373.
This is a good review of recent advances. It not only brings the reader up to date in this field, but discusses analytical ultracentrifugation in relation to other techniques used for solution interaction analysis.

17. •• Schuster TM, Toedt JM: **New revolutions in the evolution of analytical ultracentrifugation.** *Curr Opin Struct Biol* 1996, **6**:650–658.
A thorough review of the most recent literature.

18. • Joss LA, Ralston GB: **Beta-lactoglobulin B – a proposed standard for the study of reversible self-association reactions in the analytical ultracentrifuge.** *Anal Biochem* 1996, **236**:20–26.
A long-needed attempt to establish a standard system that can be used to test instrumental alignment and methods of data analysis for interacting systems.

19. • Demeler B, Saber H, Hansen JC: **Identification and interpretation of complexity in sedimentation velocity boundaries.** *Biophys J* 1997, **72**:in press.
This paper discusses in tutorial format how to use the extrapolation procedures developed by van Holde and Weischet [27]. A clear presentation is given of the types of curves one might expect to see for various types of concentration dependence.

20. Stafford WF: **Methods for obtaining sedimentation coefficient distributions.** In *Analytical Ultracentrifugation in Biochemistry and Polymer Science.* Edited by Harding SE, Rowe AJ, Horton JC. Cambridge: Royal Society of Chemistry; 1992:359–393.

21. Lamm O: **Die differentialgleichung der ultrazentrifugierung.** *Arkiv Math Astron Fysik* 1929, **21B**:1–4

22. Fujita H: *Foundations of Ultracentrifugal Analysis.* New York: John Wiley & Sons; 1976.

23. Fujita H: *Mathematical Theory of Sedimentation Analysis.* New York: Academic Press; 1962.

24. Bethune JL: **Time dependence of concentration distributions in sedimentation analysis.** *Biochemistry* 1970, **9**:2737–2744.

25. McNeil BJ, Bethune JL: **Experimental determination of sedimentation and diffusion coefficients employing**

concentration changes as a function of time. *Biochemistry* 1973, **12**:3254–3259.

26. Simpson RT, Bethune JL: **Ultracentrifuge time-lapse photography. Determination of molecular weights.** *Biochemistry* 1973, **9**:2745–2750.

27. Van Holde KE, Weischet WO: **Boundary analysis of sedimentation-velocity experiments with monodisperse and paucidisperse solutes.** *Biopolymers* 1978, **17**:1387–1403.

28. Hansen JC, Ausio J, Stanik VH, Van Holde KE: **Homogeneous reconstituted oligonucleosomes, evidence for salt-dependent folding in the absence of histone H1.** *Biochemistry* 1989, **28**:9129–9136.

29. Schwarz PM, Hansen JC: **Formation and stability of higher order chromatin structures. Contributions of the histone octamer.** *J Biol Chem* 1994, **269**:16284–16289.

30. Fletcher TM, Serwer P, Hansen JC: **Quantitative analysis of macromolecular conformational changes using agarose gel electrophoresis: application to chromatin folding.** *Biochemistry* 1994, **33**:10859–10863.

31. Schwarz PM, Felthauser A, Fletcher TM, Hansen JC: **Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains.** *Biochemistry* 1996, **35**:4009–4015.

32. Lee JC, Rajendran S: **Studies of macromolecular interaction by sedimentation velocity.** In *Modern Analytical Ultracentrifugation: Acquisition and Interpretation of Data for Biological and Synthetic Polymer Systems.* Edited by Schuster TM, Laue TM. Boston: Birkhäuser–Boston; 1994:138–155.

33. Stafford WF: **Boundary analysis in sedimentation transport experiments: a procedure for obtaining sedimentation coefficient distributions using the time derivative of the concentration profile.** *Anal Biochem* 1992, **203**:295–301.

34. Stafford WF: **Sedimentation boundary analysis of interacting systems: use of the apparent sedimentation coefficient distribution function.** In *Modern Analytical Ultracentrifugation: Acquisition and Interpretation of Data for Biological and Synthetic Polymer Systems.* Edited by Schuster TM, Laue TM. Boston: Birkhäuser–Boston; 1994:119–137.

35. Holladay L: **An approximate solution to the Lamm equation.** *Biophys Chem* 1979, **10**:187–190.

36. Holladay L: **Simultaneous rapid estimation of sedimentation coefficient and molecular weight.** *Biophys Chem* 1980, **11**:303–308.

37. Philo JS: **Measuring sedimentation, diffusion, and molecular weights of small molecules by direct fitting of sedimentation velocity concentration profiles.** In *Modern Analytical Ultracentrifugation.* Edited by Schuster TM, Laue TM. Boston: Birkhäuser; 1994:156–170.

38. Faxén H: **ber eine differentialgleichung aus der physikalischen chemie.** *Arkiv Math Astron Fysik* 1929, **21B**:5–9.

39. Philo JS: **An improved function for fitting sedimentation velocity data for low molecular weight solutes.** *Biophys J* 1997, **72**:in press.

40. • Benaroudj N, Batelier G, Triniolles F, Ladjimi MM: **Self-association of the molecular chaperone HSC70.** *Biochemistry* 1995, **34**:15282–15290.
Combines sedimentation velocity with sedimentation equilibrium to characterize this self-associating system.

41. Borza DB, Tatum FM, Morgan WT: **Domain-structure and conformation of histidine proline-rich glycoprotein.** *Biochemistry* 1996, **35**:1925–1934.

42. Benaroudj N, Triniolles F, Ladjimi MM: **Effect of nucleotides, peptides, and unfolded proteins on the self-association of the molecular chaperone HSC70.** *J Biol Chem* 1996, **271**:18471–18476.

43. • Herrera JE, Correia JJ, Jones AE, Olson MOJ: **Sedimentation analyses of the salt- and divalent metal ion-induced oligomerization of nucleolar protein B23.** *Biochemistry* 1996, **35**:2668–2673.
Application of time-derivative methods to the thermodynamic characterization of this self-associating system.

44. Narhi LO, Philo JS, Li TS, Zhang M, Samal B, Arakawa T: **Induction of alpha-helix in the beta-sheet protein tumor-necrosis factor-α: acid induced denaturation.** *Biochemistry* 1996, **35**:11454–11460.

45. Ralston G, Cronin TJ, Branton D: **Self-association of spectrins repeating segments.** *Biochemistry* 1996, **35**:5257–5263.

46. Serina L, Bucurenci N, Gilles AM, Surewicz WK, Fabian H, Mantsch HH: **Structural-properties of Ump-kinase from *Escherichia coli*.** *Biochemistry* 1996, **35**:7003–7011.

47. Stafford WF: **Rapid molecular weight determination by sedimentation velocity analysis [abstract].** *Biophys J* 1996, **70**:A231.

48. Behlke J, Ristau O: **Molecular mass determination by sedimentation velocity experiments and direct fitting of the concentration profiles.** *Biophys J* 1997, **72**:in press.

49. Todd GP, Haschemeyer RH: **General solution to the inverse problem of the differential equation of the ultracentrifuge.** *Proc Natl Acad Sci USA* 1981, **78**:6739–6743.

50. Claverie JM, Dreux H, Cohen R: **Sedimentation of generalized systems of interacting particles. I. Solution of systems of complete Lamm equations.** *Biopolymers* 1975, **14**:1685–1700.

51. Johnson ML: **Why, when, and how biochemists should use least squares.** *Anal Biochem* 1992, **206**:215–225.

52. Johnson ML, Faunt LM: **Parameter estimation by least-squares methods.** *Methods Enzymol* 1992, **210**:1–37.

53. Stafford WF: **Boundary analysis in sedimentation velocity experiments.** In *Methods in Enzymology Numerical Computer Methods,* part B. Edited by Johnson ML, Brand L. Orlando: Academic Press; 1994:478–501.

54. Stafford WF, Liu S: **Methods for increasing the sensitivity of sedimentation velocity analysis: a signal averaging Rayleigh optical system for the Beckman Instruments Optima XL-A analytical ultracentrifuge.** *Prog Biomed Optics* 1995, **2386**:130–135.

55. Correia JJ, Gilbert SP, Moyer ML, Johnson KA: **Sedimentation studies on the kinesin motor domain constructs K401, K366, and K341.** *Biochemistry* 1995, **34**:4898–907.

56. Dallmann HG, McHenry CS: **Dnax complex of *Escherichia coli* DNA-polymerase-Iii holoenzyme – physical characterization of the Dnax subunits and complexes.** *J Biol Chem* 1995, **270**:29563–29569.

57. Hester K, Luo J, Burns G, Braswell EH, Sokatch JR: **Purification of active E1-Alpha(2)Beta(2) of *Pseudomonas putida* branched-chain-oxoacid dehydrogenase.** *Eur J Biochem* 1995, **233**:828–836.

58. Laue TM, Starovasnik MA, Weintraub H, Sun XH, Snider L, Klevit RE: **MyoD forms micelles which can dissociate to form heterodimers with E47 – implications of micellization on function.** *Proc Natl Acad Sci USA* 1995, **92**:11824–11828.

59. Lobert S, Frankfurter A, Correia JJ: **Binding of vinblastine to phosphocellulose-purified and alpha beta-class III tubulin: the role of nucleotides and beta-tubulin isotypes.** *Biochemistry* 1995, **34**:8050–8060.

60. • McCartney JE, Tai MS, Hudziak RM, Adams GP, Weiner LM, Jin D, Stafford WF, Liu S, Bookman MA, Laminet AA *et al.*: **Engineering disulfide-linked single-chain Fv dimers [(sFv′)$_2$] with improved solution and targeting properties – anti-digoxin 26-10 (sFv′)$_2$ and anti-c-ErbB-2 741F8 (sFv′)$_2$ made by protein folding and bonded through C-terminal cysteinyl peptides.** *Protein Eng* 1995, **8**:301–314.
An example of the use of the time derivative to characterize reversible complex formation between an antigen and antibody.

61. •• Wingfield PT, Stahl SJ, Williams RW, Steven AC: **Hepatitis core antigen produced in *Escherichia coli* – subunit composition, conformational-analysis, and *in vitro* capsid assembly.** *Biochemistry* 1995, **34**:4919–4932.
A good example of the combined use of velocity and equilibrium sedimentation to characterize a complex system.

62. Stafford WF, Liu S, Prevelige PE: **New high sensitivity sedimentation methods: application to the analysis of the assembly of bateriophage P22.** In *Techniques in Protein Chemistry VI.* Edited by Crabb JW. San Diego: Academic Press; 1995:427–432.

63. Lobert S, Vulevic B, Correia JJ: **Interaction of vinca alkaloids with tubulin – a comparison of vinblastine, vincristine, and vinorelbine.** *Biochemistry* 1996, **35**:6806–6814.

64. Colfen H, Harding SE, Wilson EK, Packman LC, Scrutton NS: **Homodimeric and expanded behavior of trimethylamine dehydrogenase in solution at different temperatures.** *Eur Biophys J* 1996, **24**:159–164.

65. • Yang YR, Schachman HK: **A bifunctional fusion protein containing the maltose-binding polypeptide and the catalytic chain of aspartate transcarbamoylase: assembly, oligomers, and domains.** *Biophys Chem* 1996, **59**:289–297.
A good example of the combined use of velocity and equilibrium sedimentation to characterize this self-associating system.

66. Rodgers KK, Bu Z, Fleming KG, Schatz D, Engleman DM, Coleman JE: **A zinc-binding domain involved in the dimerization of RAG1.** *J Mol Biol* 1996, **260**:70–84.

67. MacPhee CE, Chan RYS, Sawyer WH, Stafford WF, Howlett GJ: **The hydrodynamic properties of homogeneous lipid emulsions and the complexes formed with lipoprotein lipase [abstract].** *Biophys J* 1996, **70**:A231.

68. MacPhee CE, Chan RYS, Sawyer WH, Stafford WF, Howlett GJ: **The hydrodynamic properties of homogeneous lipid emulsions and the complexes formed with lipoprotein lipase.** *J Lipid Res* 1997, in press.

69. Lobert S, Boyd CA, Correia J: **Divalent cation and ionic strength effects on vinca alkaloid-induced tubulin self-association.** *Biophys J* 1997, **72**:in press.

70. Rodgers KK, Fleming KG: **Metal-dependent structure and self association of the RAG1 zinc-binding domain.** In *Techniques in Protein Chemistry,* volume VIII. Edited by Marshak DR. San Diego: Academic Press; 1997:in press.

71. Liu S, Tai M-S, McCartney J, Oppermann H, Hudziak RM, Houston LL, Weiner LM, Huston JS, Stafford WF: **Physical characterization of recombinant anti-c-erbB-2 and 741F8 single chain antibody dimer $(sFv')_2$ and analysis of its interaction with recombinant extracellular domain of c-erbB-2 oncogene product by analytical ultracentrifugation [abstract].** *Biophys J* 1995, **68**:A407.

72. Kirkwood JG: **The statistical mechanical theory of irreversible processes in solutions of flexible macromolecules.** *Rec Trav Chim - JR Neth Chem Soc* 1949, **68**:649–660.

73. Teller DC, Swanson E, De Haen C: **The translational frictional coefficients of proteins.** *Methods Enzymol* 1979, **61**:103–124.

74. De Haen C, Easterly RA, Teller DC: **Translational friction of rigid assemblies of spheres: derivation and application of new hydrodynamic interaction tensors.** *Biopolymers* 1983, **22**:1133–1143.

75. Garcia de la Torre J, Bloomfield V: **Hydrodynamic properties of complex, rigid, biological macromolecules: theory and applications.** *Q Rev Biophys* 1981, **14**:81–139.

76. Garcia de la Torre J: **Sedimentation coefficients of complex biological particles.** In *Analytical Ultracentrifugation in Biochemistry and Polymer Science.* Edited by Harding SE, Rowe AJ, Horton JC. Cambridge: Royal Society of Chemistry; 1992:333–345.

77. Garcia de la Torre J, Navarro S, Lopez Martinez MC, Diaz FG, Lopez Cascales JJ: **HYDRO: a computer program for the prediction of hydrodynamic properties of macromolecules.** *Biophys J* 1994, **67**:530–531.

78. Perkins SJ, Smith KF, Sim RB: **Molecular modelling of the domain structure of factor I of human complement by X-ray and neutron solution scattering.** *Biochem J* 1993, **295**:101–108.

79. Morgan PJ, Hyman SC, Byron O, Andrew PW, Mitchell TJ, Rowe AJ: **Modeling the bacterial protein toxin, pneumolysin, in its monomeric and oligomeric form.** *J Biol Chem* 1994, **269**:25315–25320.

80. •• Byron O: **Construction of hydrodynamic bead models from high-resolution X-ray crystallography or nuclear magnetic resonance data.** *Biophys J* 1997, **72**:in press.
This paper describes a useful algorithm for deriving hydrodynamically equivalent bead models from X-ray crystallographic data.

81. Schmidt B, Riesner D: **A fluorescence detection system for the analytical ultracentrifuge and its application to proteins, nucleic acids, viroids and viruses.** In *Analytical Ultracentrifugation in Biochemistry and Polymer Science.* Edited by Harding SE, Rowe AJ, Horton JC. Cambridge: Royal Society of Chemistry; 1992:176–207.

82. Khan SA, Griess GA, Serwer P: **Assembly-associated structural changes of bacteriophage T7 capsids. Detection by use of a protein-specific probe.** *Biophys J* 1992, **63**:1286–1292.