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Advances in Sedimentation Velocity Analysis

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ABSTRACT On February 20, 1996, a workshop titled "Advances in Sedimentation Velocity Analysis" was held at the Biophysical Society meeting in Baltimore, Maryland, in honor of Professor David Yphantis's 65th birthday. Although he is known more for his work with sedimentation equilibrium, David's work on instrumentation and data analysis is the foundation for many of the recent advances in both equilibrium and velocity sedimentation. Over the years he has trained numerous graduate students, most of whom have gone on to emphasize the use of analytical ultracentrifugation to answer biochemical questions involving macromolecular assembly. His laboratory was one of very few that continued to use and develop analytical ultracentrifugation during its nadir in the 1970s and early 1980s. The rebirth and resurgence of analytical ultracentrifugation owe a great deal to his persistence and enthusiasm. These efforts have borne fruit. In the last five years, through his work at the National Analytical Ultracentrifugation Facility, he has helped train nearly 100 individuals in the delicate art of nonlinear least-squares analysis of equilibrium sedimentation data. Furthermore, the number of researchers using the ultracentrifuge and the number of papers published has skyrocketed in the last few years. This workshop, then, was a way to thank David for his years of devotion to analytical ultracentrifugation.

INTRODUCTION

There are two distinct methods associated with analytical ultracentrifugation, sedimentation equilibrium and sedimentation velocity. Although sedimentation velocity is the older of the two methods, there have been several recent advances in instrumentation and data analysis. Collected here are some of the papers demonstrating this new-found vibrancy.

The fundamental descriptor determined from a sedimentation velocity measurement is the sedimentation coefficient, s. This coefficient may be expressed either as the ratio of the particle velocity to the gravitational field, $s = v/a$, which describes the experimental measurement, or as the ratio of the particle's buoyant mass to its frictional coefficient, $s = M_b/f$, which relates the measurable parameter to molecular mass and molecular size and shape (through their effects on f). If a discrete number of boundaries are observed during a sedimentation velocity experiment, it is also possible to determine the diffusion coefficient from the spreading of the boundary during the course of the experiment. This is useful because measurement of D provides an independent means of determining the frictional coefficient.

There are, of course, several issues that must be dealt with when determining and interpreting the sedimentation coefficient. In this collection of papers, two (Philo, 1997; Behlke and Ristau, 1997) describe refinements to earlier methods (Holladay, 1979, 1980) for fitting concentration profiles directly to transport equations. These methods permit direct estimates of s and D as long as the correct form of the transport equation is used. However, an improper choice of the fitting function will lead to erroneous values of s and D and an improper interpretation of the experiment. Some guidance as to which transport equation might be most appropriate is available in the paper by Demeler et al. (1997). This work builds on their extensive experience with the analysis method first described by Van Holde and Weischet (1978). The method described is robust, although it requires some (fully computer-automated) data manipulation.

Once a reliable estimate of the sedimentation or diffusion coefficient is obtained, it can be used to learn more about the shape and size of the sedimenting particle. Traditionally, this has been done by computing the ratio of the frictional coefficient calculated from $s = M_b/f$ with the frictional coefficient calculated for a sphere of equal mass and density (f_o). This value of f/f_o is then used with Perrin's equations to determine the axial ratio of oblate or prolate ellipsoids (e.g., Laue et al., 1992). Although it is a useful exercise for estimating the asymmetry of a molecule, this procedure is inappropriate in cases in which independently determined structural information is available. In such cases, the bead-model methods (Bloomfield et al., 1967; de la Torre, 1992) are most appropriate. However, these methods are computer intensive and, in principle, require detailed structures. The paper by Byron (1997) addresses the issue of just how detailed a model is required to account for an observed s and, conversely, explores the level of structural detail that can be gleaned from sedimentation data.

Advances in data acquisition have an impact on the extent and quality of analysis available from sedimentation velocity. The paper by Lobert et al. (1997) provides an excellent example of how sedimentation velocity can be used to answer important questions concerning the assembly of tubulin. Full advantage is taken of the recent advances in instrumentation to gather the copious numbers of data points needed for this project. In addition to being a *tour de*

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force, this project shows the use of the time derivative analysis described by David Yphantis (Runge et al., 1981; Yphantis, 1984) and advanced by one of his students, Walter Stafford (Stafford, 1992).

Finally, it should be noted that these papers describe only part of the ongoing modernization of sedimentation velocity analysis. In the future, we can expect to learn of advances in differential sedimentation, an underexploited technique first described by Richards and Schachman (1957) that can provide exquisite sensitivity to changes in molecular hydrodynamics. Likewise, the elegant method of gravitational sweep analysis described by Mächtle (1984, 1988) for the analysis of polymer dispersions should find wide use in the biological sciences. Sedimentation velocity analysis also will be advanced by changes in the instrumentation. Work that takes advantage of the inherent accuracy and speed of the automated interference optics, long championed by David Yphantis (Yphantis, 1964, 1994), is appearing. It should not be too long before fluorescence optics like those described for Model E (Schmidt and Riesner, 1992) are included in the choices for optical detectors on the new ultracentrifuge. With their great sensitivity and selectivity, fluorescence detection capabilities will expand sedimentation velocity analysis into entirely new areas.

There can be no doubt that analytical ultracentrifugation is undergoing a renaissance. While the advances in sedimentation velocity analysis build on the solid foundation of earlier work, the new capabilities afforded by rapid data acquisition and computer-based analysis are permitting ultracentrifugation to be used in new ways. David Yphantis recognized long ago just how important these advances would be, and endured the lean decades of the 1970s and 1980s while working on these improvements. Happy birthday, David, and thank you for your perseverance!

REFERENCES

Behlke, J., and O. Ristau. 1997. Molecular mass determination by sedimentation velocity experiments and direct fitting of the concentration profiles. *Biophys. J.* 72:000–000.

Bloomfield, V., W. O. Dalton, and K. E. Van Holde. 1967. Frictional coefficients of multisubunit structures. I. Theory. *Biopolymers.* 5:135–148.

Byron, O. 1997. The construction of hydrodynamic bead models from high resolution x-ray crystallographic or NMR data. *Biophys. J.* 72:000–000.

de la Torre, J. G. 1992. Sedimentation coefficients of complex biological particles. *In* Analytical Ultracentrifugation in Biochemistry and Polymer Science. S. E. Harding, A. J. Rowe and J. C. Horton, editors. Royal Society of Chemistry, Cambridge, England. 333–358.

Demeler, B., H. Saber, and J. C. Hansen. 1997. Identification and interpretation of complexity in sedimentation velocity boundaries. *Biophys. J.* 72:000–000.

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

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

Laue, T. M., B. Shah, T. M. Ridgeway, and S. L. Pelletier. 1992. Computer-aided interpretation of sedimentation data for proteins. *In* Analytical Ultracentrifugation in Biochemistry and Polymer Science. S. E. Harding, J. C. Horton and A. J. Rowe, editors. Royal Society of Chemistry, Cambridge, England. 90–125.

Lobert, S., C. A. Boyd, and J. J. Correia. 1997. Divalent cation and ionic strength effects on *Vinca* alkaloid-induced tubulin self-association. *Biophys. J.* 72:000–000.

Mächtle, W. 1984. Characterisierun von Dispersionen durch gekoppelte H_2O/D_2O Ultrazentrifugenmessuungen. *Makromol. Chem.*, 185: 1025–1039.

Mächtle, W. 1988. Coupling particle size distribution technique. *Angew. Makromol. Chem.* 162:35–52.

Philo, J. S. 1997. An improved function for fitting sedimentation velocity data for low molecular weight solutes. *Biophys. J.* 72:000–000.

Richards, E. G., and H. K. Schachman. 1957. A differential ultracentrifuge technique for measuring small changes in sedimentation coefficients. *J. Am. Chem. Soc.* 79:5324–5325.

Runge, M. S., T. M. Laue, D. A. Yphantis, M. R. Lifsics, A. Saito, M. Altin, L. Reinke, and R. C. Williams. 1981. ATP-induced formation of an associated complex between microtubules and neurofilaments. *Proc. Natl. Acad. Sci. USA.* 78:1431–1435.

Schmidt, B., and D. Riesner. 1992. 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. S. E. Harding, A. J. Rowe and J. C. Horton, editors. Royal Society of Chemistry, Cambridge, England. 176–207.

Stafford, W. F., III. 1992. Boundary analysis in sedimentation transport experiments: a procedure for obtaining sedimentation coefficient distributions using the time derivative of the concentration profile. *Anal. Biochem.* 203:1–7.

Van Holde, K. E., and W. O. Weischet. 1978. Boundary analysis of sedimentation-velocity experiments with monodisperse and paucidisperse solutes. *Biopolymers.* 17:1387–1403.

Yphantis, D. A. 1964. Equilibrium ultracentrifugation of dilute solutions. *Biochemistry.* 3:297–317.

Yphantis, D. A. 1984. Sedimentation coefficient distributions from ultracentrifuge interferograms. *Biophys. J.* 45:324a.

Yphantis, D. A., J. W. Lary, W. F. Stafford, S. Liu, P. H. Olsen, D. B. Hayes, T. P. Moody, T. M. Ridgeway, D. A. Lyons, and T. M. Laue. 1994. On line data acquisition and analysis 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. T. M. Schuster and T. M. Laue, editors. Birkhäuser, Boston. 209–226.