Editor's Note

After we published a review on modern analytical ultracentrifugation by Jacob Lebowitz, Marc S. Lewis, and Peter Schuck (*Protein Science* **11**: 2067–2079 [2002]), we received a commentary from Henryk Eisenberg, who raised several points of disagreement with the approaches advocated by Dr. Lebowitz and colleagues. Because *Protein Science* does not have a standing provision for commentary on articles published in the journal, I consulted several colleagues and then decided to invite a “point/counterpoint” pair of articles by Drs. Lebowitz and Eisenberg to focus on the principal points of disagreement as a way of exposing our readers to differing views within the field. The articles follow.

—Mark Hermodson
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Modern analytical ultracentrifugation in protein science: Look forward, not back

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Summary

The reasons for replacing the classical strictly two-component velocity and equilibrium analytical ultracentrifugation Svedberg equations by multicomponent equations, applicable in the study of biological macromolecular systems, are given.

Keywords: Analytical ultracentrifugation; Svedberg equations; multicomponent systems; thermodynamic equations; protein and nucleic acid solutions; detergents

A recent “tutorial” review (Lebowitz et al. 2002) emphasizes the new role of analytical ultracentrifugation, reborn by the development of novel instrumental design and the advanced application of computer analysis. A weak point, not affecting the renewed use of analytical ultracentrifugation, is the fact that current authors in this topical field of study essentially refer to the classical Svedberg analysis, which was the basis for the development of the technology, and has been known for a long time to apply strictly to

two-component systems only. This is correct for synthetic no-charge-carrying organic polymers in single organic solvents; however, it is often not applicable to charge-carrying proteins or nucleic acids in aqueous salt, sugar, detergent, or denaturant solutions. For the development and use of the thermodynamic theory applicable to multicomponent systems, the reader is referred to the following papers: Casassa and Eisenberg (1964), Fujita (1975, 1994), Eisenberg (1976, 1994, 1999, 2000, 2003), and Ebel et al. (2000), where references to work by additional workers will be found. Consideration of the multicomponent nature of biological macromolecular solutions is essential in approaching this problem and evaluating errors and loss of information connected with its neglect.

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To keep matters simple in this presentation, a three-component system containing a cosolvent component 3 only in addition to the solvent component 1 and the macromolecular component 2 will be considered. This does not limit extension to any number of low-molecular or macromolecular components. For finite macromolecular concentrations, a powerful connection exists between the equilibrium sedimentation differential equation and the osmotic pressure concentration derivative ($d\Pi/dc_2$)

$$(d\ln c_2/dr^2) = (\omega^2/2)(\partial\rho/\partial c_2)_\mu / d\Pi/dc_2 \quad (1)$$

where r is the distance from the center of rotation in, ω is the angular velocity of the ultracentrifuge, c_2 is in grams per milliliter, and the density increment $(\partial\rho/\partial c_2)_\mu$ is at constant chemical potentials μ of solutes diffusible through a semipermeable membrane and measurable at low concentrations by the Kratky (Kratky et al. 1973) Paar DMA 5000 densitometer accurate to six significant figures. A 1-mL dialyzed reusable solution at $c_2 \sim 1$ mg/mL is required.

The osmotic pressure concentration derivative ($d\Pi/dc_2$) can be expanded in a virial series, yielding the molar mass in grams per mole at vanishing macromolecular concentrations,

$$(d\Pi/dc_2)/RT = M_2^{-1} + 2A_2 c_2 + \dots \quad (2)$$

In the limit of vanishing concentration c_2 equation 1 reduces to

$$d\ln c_2/dr^2 = (\omega^2/2RT)(\partial\rho/\partial c_2)_\mu M_2 \quad (3)$$

In examining the dimensionality of equation 3, the ratio M_2/c_2 is in units of milliliters per mole and M_2 will be given in whatever units are used for the concentration (Eisenberg 1994, 2000, 2003).

The ratio of the velocity sedimentation and diffusion coefficients s and D in the limit of vanishing component 2 concentration is given in similar fashion by

$$s/D = (\partial\rho/\partial c_2)_\mu M_2/RT, \quad (4)$$

The density increment $(\partial\rho/\partial c_2)_\mu$ for the three-component system is given by

$$(\partial\rho/\partial c_2)_\mu = (1 - \rho \bar{v}_2) + \xi_3 (1 - \rho \bar{v}_3) \quad (5)$$

where $\bar{v}_2$ and $\bar{v}_3$ are the partial specific volumes of components 2 and 3, $\xi_3 = (\partial w_3/\partial w_2)_\mu$ is an interaction parameter indicating the change in gram molality w_3 with the change in gram molality w_2 at constant chemical potentials of components 1 and 3 diffusible through a semipermeable membrane, and ρ is the density of the solvent in the absence of component 2. Protein volumes and hydration are well defined (Ebel et al. 2000; Eisenberg 2000).

For symmetry reasons equation 5 can also be written as

$$(\partial\rho/\partial c_2)_\mu = (1 - \rho \bar{v}_2) + \xi_1 (1 - \rho \bar{v}_1) \quad (6)$$

where $\xi_1 = (\partial w_1/\partial w_2)_\mu$ and ξ_1 and ξ_3 are related by

$$\xi_1 = \xi_3/w_3. \quad (7)$$

The interaction coefficients ξ_1 and ξ_3 cannot be associated with specific interaction with only either component 1 or 3, but should each be considered as relating to both components 1 and 3. A zero value of either ξ_1 or ξ_3 does not indicate lack of interaction with component 1 or 3. Furthermore a positive value of ξ_1 yields a negative value of ξ_3 or vice versa by equation 7.

The density increment $(\partial\rho/\partial c_2)_\mu$ is substituted sometimes by $(1 - \rho\phi')$, mimicking the two-component buoyancy equation (Eisenberg 1976). This should be avoided as ϕ' has no defined meaning and should therefore not be used (Eisenberg 2003).

It is now seen that in the absence of component 3 $(\partial\rho/\partial c_2)_\mu$ in equations 5 and 6 reduces to $(1 - \rho\bar{v}_2)$, and equations 3 and 4 reduce to the classical two-component Svedberg equations at vanishing macromolecular concentrations. Neglect of the consideration of the multicomponent nature of protein and nucleic acids solutions leads to errors of 9% for DNA/NaCl (1 mole/L) solutions, 0.4% for BSA/NaCl (1 mole/L) solutions, 16.7% for BSA/GdnCl (4 moles/L) solutions, and 4.9% for aldolase/sucrose (0.399 moles/L) solutions in molecular weight determinations (Table 1 in Eisenberg 2000). Furthermore, additionally important information relating to solvent interactions, hydration, cosolvent interactions, and fractal probing of the macromolecular particles are not considered in this neglect (Eisenberg 1994, 2003). Analytical ultracentrifugation powerfully connects in a broad complementary sense to light-, X-ray-, and neutron-scattering experiments (Eisenberg 1999), providing additional structure, shape, and interaction information.

There are no unusual difficulties or material requirements in the determination of the density increments $(\partial\rho/\partial c_2)_\mu$ in most systems.

In a newly proposed, simplified experimental approach requiring less material (Courtenay et al. 2000; Anderson et al. 2002), water vapor pressure osmometry in an attractive procedure replaces equilibrium dialysis density increment measurements, comparing with electromotive-force determination and isopiestic distillation (Eisenberg 1976). Interaction parameters are obtained that, combined with partial specific volumes and density data, enable the calculation of the density increments required in analytical ultracentrifugation. Additional work is required to establish the significance of this new procedure.

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