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This Week's Citation Classic

van Holde K E & Baldwin R L. Rapid attainment of sedimentation equilibrium.
J. Phys. Chem. 62:734-43, 1958.
[Depts. Biochemistry, Chemistry, and Dairy and Food Industries, Univ. Wisconsin, Milwaukee, WI]

Sedimentation of macromolecules can be attained rapidly by the use of short solution columns. The approach to equilibrium can be quantitatively predicted. Experiments with sucrose and ribonuclease demonstrate that very accurate molecular weight values can be obtained by this method. [The *SCI*® indicates that this paper has been cited in over 590 publications since 1961.]

K.E. van Holde
Department of Biochemistry and Biophysics
Oregon State University
Corvallis, OR 97331

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"My graduate studies at the University of Wisconsin were with J.W. Williams, who had, in turn, worked with T. Svedberg. In using the sedimentation equilibrium method to study synthetic polymers, I was impressed with its potential accuracy, but dismayed by the time required—often a week or more—for each experiment. Aside from the inconvenience, this made the method impractical for sensitive biological materials.

"Upon joining the research laboratories of E.I. du Pont de Nemours, I began some studies to seek ways to shorten this time. In 1955, I left industrial research, and returned to Wisconsin as a postdoctoral fellow. Shortly thereafter, I began a theoretical analysis of the problem, which showed that the time to reach equilibrium depended on the *square* of the solution column length. Clearly, using very short columns was the answer, but an experimental demonstration was essential. However, I had no idea of how to do an experiment that would convince biochemists of the method's utility.

"At this point, I had the good luck to renew an acquaintance with R.L. Baldwin, who had just joined the faculty at Wisconsin. We found that we had been working along very similar lines, so we decided to join forces. Baldwin had a new Spinco ultracentrifuge and, more importantly, a thorough understanding of contemporary problems in biochemistry. He suggested that we employ ribonuclease as a test substance, for sequencing of that protein had proceeded to the point where the molecular weight was exactly known. My own knowledge of biochemistry was so meager that I was quite unclear as to the difference between ribonuclease and ribonucleic acid! Baldwin was, in addition, an expert on ultracentrifuge theory, and together we were able to work out new methods for data analysis.

"With Baldwin's expertise, and his superbly tuned centrifuge, the experiments went splendidly. Equilibrium could be attained in hours, rather than days, and the theory for the approach to equilibrium was quantitatively confirmed. The known molecular weights of sucrose and ribonuclease were reproduced almost exactly.

"In a sense, the paper made practicable the application of sedimentation equilibrium to biochemical problems. Extensions of the method soon followed. Especially notable were the use of the interferometric optical system[1,2] and the development by D.A. Yphantis of the technically simpler, yet accurate, 'meniscus depletion method.'[3] Later, the extension of the technique to reversibly associating macromolecules[4,5] opened a whole new area of research. The extensive use of sedimentation equilibrium during the following years doubtless explains the numerous citations. For a recent review, see 'Sedimentation analysis of proteins.'[6]

"In retrospect, it seems to me that the work was possible only because of the standards of excellence that had been set by Williams and his protégés. Perhaps the most important of these to the younger scientists was Louis Gosting, who inspired each of us to do every experiment with uncompromising attention to detail."

1. **Richards E G & Schachman H K.** Ultracentrifuge studies with Rayleigh interference optics. I. General applications. *J. Phys. Chem.* 63:1578-91, 1959.
2. **LaBar F E & Baldwin R L.** A study by interference optics of sedimentation in short columns. *J. Phys. Chem.* 66:1952-9, 1962.
3. **Yphantis D A.** Equilibrium ultracentrifugation of dilute solutions. *Biochemistry* 3:297-317, 1964.
4. **Adams E T, Jr. & Fujita H.** Sedimentation equilibrium in reacting systems. (Williams J W, ed.) *Ultracentrifuge analysis in theory and experiment.* New York: Academic Press, 1963. p. 119-29.
5. **Adams E T, Jr. & Williams J W.** Sedimentation equilibrium in reacting systems. II. Extension of the theory to several types of association phenomena. *J. Amer. Chem. Soc.* 86:3454-61, 1964.
6. **van Holde K E.** Sedimentation analysis of proteins. (Neurath H & Hill R L, eds.) *The proteins.* New York: Academic Press, 1975. Vol. I. p. 225-91.