A NEW APPROACH TO MOLECULAR WEIGHT CALCULATION FROM SEDIMENTATION EQUILIBRIUM EXPERIMENTS

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SUMMARY. The basic equation for the sedimentation equilibrium of homogeneous systems is solved directly in terms of the observable variable, viz. in ordinates of interference or scanner patterns. This allows one to calculate a molecular weight without an auxiliary run in the "low-speed" experiments with interference optics and without any artificial procedure for the proper base line determination on a scanner tracing. The suggested method provides a sufficient accuracy both with interference and absorption optics over a wide range of molecular weights.

The "low-speed" sedimentation equilibrium method requires a synthetic boundary run for labeling of the Rayleigh interference fringes (1). A number of procedures were proposed (2-4) to eliminate this additional experiment but none has as yet found a wide application. This paper deals with a method of calculation when the fringes are self-calibrated in terms of absolute concentrations whatever the mode of sedimentation equilibrium of homogeneous systems. In experiments with absorption optics this method allows one to establish the correct base line, thus markedly improving the accuracy of the scanning system.

Theoretical. The curve depicted in Fig. 1 schematically represents an interference fringe or an absorption curve at sedimentation equilibrium. On interferograms the ordinate y is counted off from the origin of the microcomparator scale,

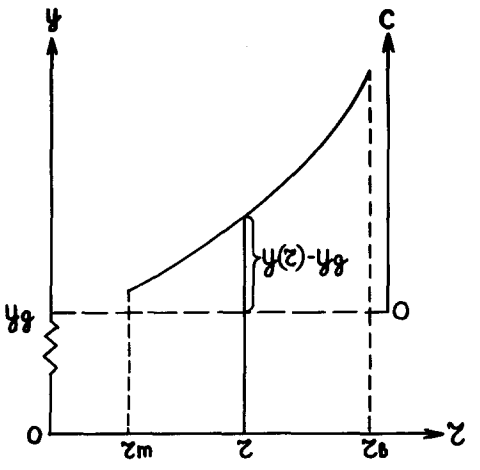

Fig. 1. Refractive index or absorbance distribution in the cell at sedimentation equilibrium. On the vertical axis are plotted ordinates of sedimentation pattern, y, and concentration of solute, C; r is the distance from the center of rotation.

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and on the scanner patterns - from an arbitrary level. We assume the base line ordinate y_g to be an unknown value constant along the solution column in the cell.

For the interference optics and also for a scanner, if its calibration is linear, we have

$$C(r) = \text{const} \times (y - y_g) \qquad /1/$$

The base equation for sedimentation equilibrium in ideal solutions (5) with this substitution becomes:

$$\frac{dy}{dr} = qr(y-y_g) \qquad /2/$$

where

$$q = \frac{M(1-\bar{V}\rho)\omega^2}{RT}$$

(all symbols have the usual meaning).

If both y_g and q are assumed to be constant Eq. /2/ gives

$$y = y_g + be^{\frac{1}{2}qr^2} \qquad /3/$$

with b being the integration constant.* In terms of a new variable $z = \exp(\frac{1}{2}qr^2)$ Eq. /3/ becomes linear;

$$y = y_g + z \qquad /4/$$

Now, if we vary q in such a manner that the sum of squared residuals S(q) (calculated by the least squares curve fitting) diminishes, the value $q=\hat{q}$ at which $S(\hat{q}) = \min S(q)$ will be the best estimate of q (6).

Experimental test of suggested procedure was made with proteins of known molecular weights: RNase and human serum albumin (HSA). RNase (Serva Feinbiochemica GMBH) was dissolved in 0.01 M Tris-saline, pH 8 and used without purification or dialysis. HSA (Reanal, Hungary) in the same buffer was chromatographed through a Sephadex G-200 column and the homogeneity was checked at 60,000 rpm. All equilibrium experiments were performed at 20°C for 20-24 h in a Beckman Model E ultracentrifuges. The interference optics was aligned according to Richards et al. (7,8), the camera lens focused two thirds - way through cells with sapphire windows. A six-channel cell was used, the channel nearest to the rotor axis was filled with solution of HSA, C_o=0.4 mg/ml, the next one - with HSA: RNase mixture (2:1 w/w), C_o=0.6 mg/ml, the third one - with RNase solution, C_o = 0.8 mg/ml. The speed 21,740 rpm was chosen to secure the "low-speed" equilibrium for RNase and to approximate "meniscus depletion" for HSA (see Table). The "blanks" were obtained with buffer in all channels. Interferograms were

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* If the calibration of the scanner is nonlinear, i.e. $A = a_o+a_1y+a_2y^2$ (A - absorbance, a_o, a_1, a_2 - constants), then the values y in Eq. /3/ must be replaced by $yx(1 + \frac{a_2}{a_1}y)$.

TABLE

Optics	Protein	$\hat{q}$	$\frac{C_b^*}{C_m}$	s**	y_g (mm)	Mx10^{-3}
				μm		
Interf.	RNase	0.95	5.2	3.3	72.384	14.8
	HSA	3.60	$2x10^3$	4.0	71.981	64.9
	Mixture HSA: RNase (2:1 w/w)	2.85		26	71.714	47.8
				mm		
	HSA	4.51	$8x10^3$	0.2	2.6	67.4
Scanner	"	4.40	$1.4x10^3$	0.2	1.7	65.7
	"	4.34	$1.3x10^3$	0.3	2.1	64.9
	tail sheaths T4***	3.4	$0.3x10^3$	0.17	3.4	9,455

* The ratio of concentration on meniscus to that on bottom is calculated by means of $\hat{q}$ and taking into account the position and thickness of the solution in the cell.

** Residual standard deviations $s = (S_{min}/(n-2)^{1/2}$, where n denotes number of points.

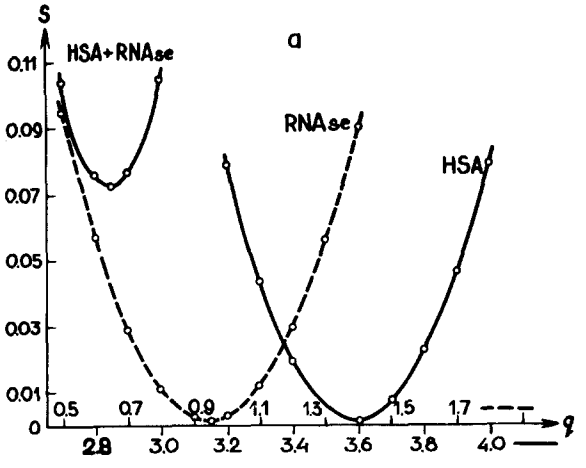
*** One of the experiments (11); 1.800 r.p.m., 22°C, 44h, $A_{280}^{12mm} = 0.24$.

measured at 50 μm radial increments using a two-coordinate microcomparator UIM-23 (USSR, scale division 1 μm) at 30^x magnification. The fringe ordinates were averaged from five fringes.

The experiments with absorption optics were performed with HSA in a six-channel cell at 23,840 rpm (the speed was

checked with a digital frequency meter). The cell was scanned at a lowest speed (1:1) at 280 nm, a 2 mm monochromator and 0.12 mm scanner slits, 5 mm/sec chart speed. The initial absorbance A^{12mm}_{280nm} varied from 0.2 to 0.5. The attainment of equilibrium was checked at 2–4 h intervals.

Calculations were carried out with an ALGOL program. The



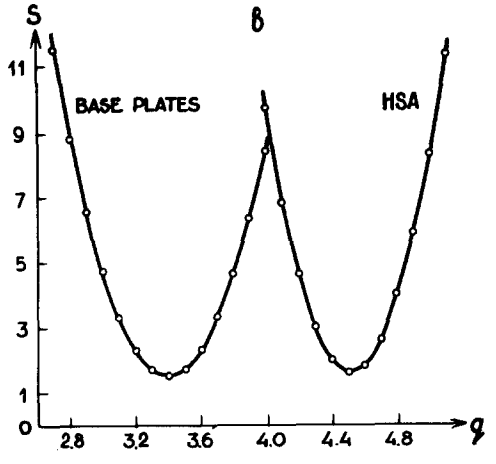


Fig. 2. Typical plots of sum of squared residuals S vs. q. a) Experiments with interference optics; b) with a UV scanner. S (q) in arbitrary units.

unknown paramter q was varied in the vicinity of $q=\hat{q}$ where S(q) was expected to have a minimum. Typical plots of S vs. q are given in Fig. 2. The Table lists some conditions and results of these experiments. One can see that the residual standard deviations s exceed the errors of measurements on plates or scanner charts only in the case of heterogeneous mixture. Molecular weights were calculated with $\bar{V}$ = 0.693 ml/g for RNase (9), 0.734 for HSA (10), 0.72 for the HSA: RNase mixture. The molecular weight of RNase is by approximately 8% higher than the formula weight, 13.683. A most likely reason for this is that we had not purified this solution from aggregates if there had been any. The molecular weight of HSA was determined with a good accuracy; the measurements with the scanner give $M = (66.0\pm0.9)\times10^3$ and with the interference optics $M=(64.9\pm0.6)\times10^3$. The high molecular weight of tail sheaths of T4 phage $M=(9.51\pm0.16)\times10^6$ was determined by means of this method also with an accuracy better than ±2% (11). $\hat{q}$ and M for HSA: RNase mixture are close to weight average values.

These results show that the proposed procedure of calculation may be applied to homogeneous systems over a sufficiently wide range of molecular weights; the equilibrium conditions may be both "low" and "high-speed". For polydisperse systems the method provides weight averages $\hat{q}_w$ and M_w.

REFERENCES

1. Richards E.G., Teller D.C., Schachman, H.K. (1968) Biochem. 7, 1054-1076.
2. La Bar, F.E. (1965) Proc.Nat.Acad.Sci. US, 54, 31-36.
3. Chervenka, C.H. (1966) Anal.Chem. 38, 356-358.
4. Nazarian, G.M. (1968) Anal.Chem. 40, 1766-1769.
5. Svedberg, T., Pedersen, K.O. (1940) "The Ultracentrifuge", p.8, Oxford, Clarendon Press.
6. Barham, R.H., Drane, W. (1972) Technometrics 14, 758-763.

7. Richards, E.G., Teller, D.C., Schachman, H.K. (1971) Anal. Biochem. 41, 189-214.
8. Richards, E.G., Teller, D.C., Hoagland, V.D., Haschemeyer, R.H., Schachman, H.K. (1971) Anal.Biochem. 41, 215-247.
9. McMeekin, F.A. (1962) Biochem.Biophys.Res.Commun. 7, 151-154.
10. Edsall, J.T. in "The Proteins" (ed. H.Neurat a. K.Bailey), v. 2, 263, Moscow, IL (1956).
11. Chernyak, V.Ya., Volkova, T.P., Magretova, N.N., Tluchorova, J., Poglazov, B.F. (1975) Dokl.Akad.Nauk SSSR 221, 235-239.