METHODS FOR THE EVALUATION OF IDEAL MOLECULAR WEIGHTS FROM SINGLE SEDIMENTATION EQUILIBRIUM EXPERIMENTS

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Molecular weights of biological (and other) macromolecules are routinely estimated from sedimentation equilibrium experiments, using the general equation (1)

$$\frac{M_o(1-\bar{\nu}\rho)\omega^2}{RT} \times cx = \frac{dc}{dx}\left(1 + \frac{\partial \ln\gamma}{\partial c} \times c\right) \qquad (1)$$

where M_o is the 'ideal' molecular weight (i.e. appertaining at zero solute concentration) of the macromolecule, $\bar{\nu}$ is its partial specific volume, ρ the solution density, ω the angular velocity, x the radial position, c the solute concentration and γ its activity coefficient. R and T are the gas constant and absolute temperature, respectively.

Since the activity coefficient is in general unknown, it is customary to measure 'apparent molecular weights' (M_{app}) ar real concentrations, defined as

$$\frac{1}{M_{app}} = \frac{1}{M_o}\left(1 + \frac{\partial \ln\gamma}{\partial c} \times c\right) \qquad (2)$$

and then to extrapolate a plot of $\frac{1}{M_{app}}$ against c to zero concentration. The intercept on the ordinate thus yields an estimate for $\frac{1}{M_o}$.

This procedure is laborious. For a system showing appreciable non-identity, some seven or eight experiments are required to obtain a single estimate for M_o. In addition to the obvious difficulties involved in standardising conditions between multiple experiments – the sample may change, even if all else is adequately controlled – certain theoretical difficulties arise: the 'molecular weight' calculated from the usual derived forms of eqn. 1 is *not* generally M_{app} as defined in eqn. 2 (Yphantis [2]). The form of the extrapolation can therefore be in doubt.

We have sought to obtain derived equations which would enable M_o to be calculated from the data obtained in a single experiment. On the assumption that the term $\partial \ln\gamma/\partial c$ in equation 1 can be replaced by a single constant α, we have found this to be possible.

For convenience, we re-define terms in eqn. 1 to give

$$k_o cx = \frac{dc}{dx}(1 + \alpha c) \qquad (3)$$

where

$$k_o \equiv \frac{M_o(1-\bar{\nu}\rho)\omega^2}{RT} \qquad (4)$$

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It can then be shown that for data tabulated as dc/dx against x (as from Schlieren optics), the following relation holds:

$$Z_d = \frac{k_0}{2} T_d - 2\alpha \qquad (5)$$

whilst for data tabulated as c against x (as from interference or absorption optics) a closely related equation can be employed:

$$Z_c = \frac{k_0}{2} T_c - \alpha \qquad (6)$$

Plots of the Z functions against the T functions (or linear regression analyses) will therefore yield an estimate for k_0, and hence M_0, from their gradient.

These functions are defined as follows:

$$Z_d = \frac{\int_{x_i,(\frac{dc}{dx})_i}^{x_j,(\frac{dc}{dx})_j} \left[\ln(\frac{1}{x} \times \frac{dc}{dx})\right]}{\int_{x_i,(\frac{dc}{dx})_i}^{x_j,(\frac{dc}{dx})_j} \frac{dc}{dx} \times dx} \qquad T_d = \frac{\int_{x_i}^{x_j} \left[x^2\right]}{\int_{x_i,(\frac{dc}{dx})_i}^{x_j,(\frac{dc}{dx})_j} \frac{dc}{dx} \times dx}$$

$$Z_c = \frac{\int_{c_i}^{c_j} \left[\ln c\right]}{\int_{c_i}^{c_j} \left[c\right]} \qquad T_c = \frac{\int_{x_i}^{x_j} \left[x^2\right]}{\int_{c_i}^{c_j} \left[c\right]}$$

They may be computed by the differencing of tables of values of $\ln(1/x \times dc/dx)$ and x^2 (eqn. 5), or $\ln c$, c and x^2 (eqn. 6). A simple numerical integration must also be performed for eqn. 5. The units of c are not important, absolute fringe numbers or optical density units may be substituted.

The full derivation of these equations is to be published *in extenso* elsewhere (Rowe and Rowe, currently submitted for publication). No assumptions, either mathematical or physical, are involved in the derivation of eqn. 6. In the case of eqn. 5, a minor term has been neglected in one of the intermediate equations. Detailed analysis shows that estimated M_0 values will not be in error to a significant extent from this cause. The same analysis shows, however, that the intercept on the ordinate of a $Z-T$ plot will not yield a reliable estimate for the concentration dependance term (α): the latter quantity is better obtained by substitution of M_0 and M_{app} values into eqn. 2.

A detailed analysis of the practical application of these new equations is being performed (Rowe, Rowe, and Khan, in preparation). They have been applied with success to a range of macromolecules, as shown in table 1. Fig. 1 shows the $Z-T$ plot for bovine serum albumin, at a concentration of 20 mg/ml. The gradient yields an estimated M_0 = 65,800. For comparison, the molecular weight estimated from a Lamm plot at this concentration is 52,200, showing strong non-ideality. Even at 100 mg/ml concentration of albumin, estimated M_0 values remain at about 65,000.

Thus for a single (solute) component, non-ideal system, our new equations provide a rapid and theoretically sound technique for the evaluation of M_0 (solute).

In addition, we have also extended the analysis for single (solute) component, ideal systems. Defining terms as before, it can readily be shown that k_x (k defined at radial distance x) can be obtained from

$$k_x \frac{\frac{d^2c}{dx^2} - \frac{1}{x} \times \frac{dc}{dx}}{\frac{dc}{dx} \times x} \qquad (7)$$

Table 1

Values for the ideal molecular weight, M_0, of four macromolecular solutes, estimated by the use of equation 5 (see text). In all cases data was obtained and processed as described in the legend to fig. 1.

Solute component	Solute concn. (mg/ml)	M_0 (estimated)	M_0 (theoretical)
Bovine serum	20	65,800	67,000 [3]
Albumin egg white	5	14,300	14,300 [4]
Lysozyme			
Pancreatic ribonuclease	5	13,900	13,700 [5]
Raffinose	10	501	504.5

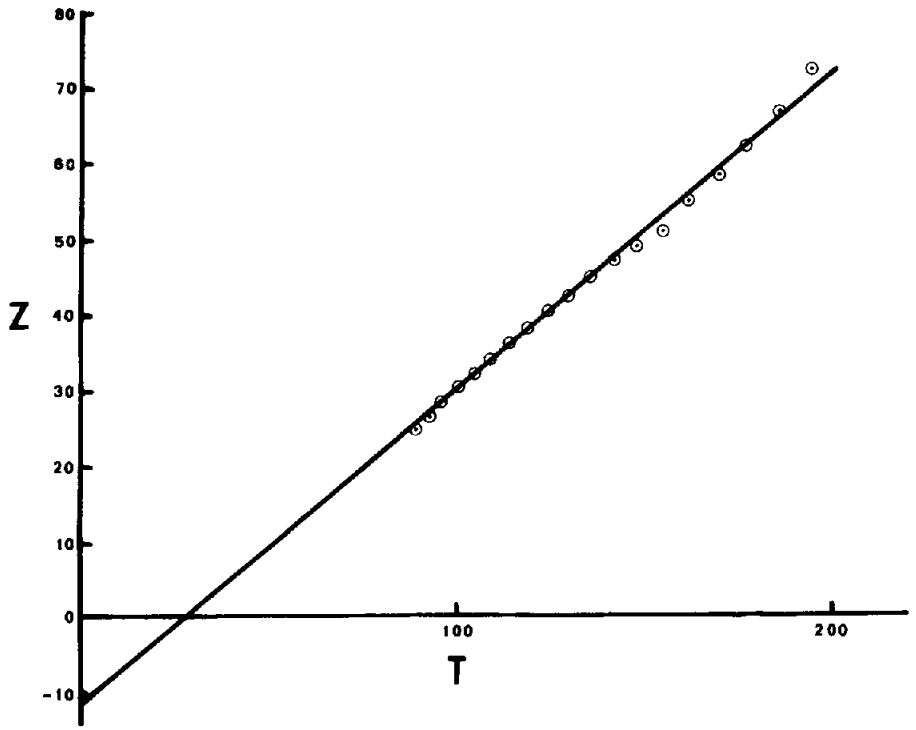

Fig. 1. A $Z-T$ plot for a sample of bovine serum albumin, concentration 20 mg/ml. Data taken from a short-column equilibrium experiment, performed at 6°, at a velocity of 10,220 rpm, using a double-sector cell of 10 mm optical pathlength in an MSE Analytical Ultracentrifuge. The equilibrium trace, photographed using Schlieren optics, was measured using a Joyce-Loebl scanning microdensitometer. A table of x and dc/dx values was derived from the scans. The values were smoothed by curve-fitting using a Fourier series, and processed to give a table of Z and T values, all by the use of an Algol program on an Elliott 4130 computer.

and this equation forms the basis for the very rapid evaluation of M_x values from single Schlieren diagrams taken at equilibrium, without the need for ancillary, synthetic boundary runs, or graphical plots of any kind.

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