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EVALUATION OF ULTRACENTRIFUGAL SCHLIEREN PATTERNS FROM EXPERIMENTS EMPLOYING THE ARCHIBALD PRINCIPLE

A DEVICE ENABLING THE USE OF A PHOTOGRAPHIC ENLARGER IN APPLYING TRAUTMAN'S RADIUS-CUBED SCALE

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SUMMARY

1. An apparatus has been devised and constructed for the use of TRAUTMAN's radius-cubed scale in combination with a photographic enlarger.

2. This allows a rapid numerical evaluation, using the Archibald principle, of the changes in concentration which take place at the meniscus, at any particular time in a centrifugation experiment.

THEORETICAL PART

Molecular weight determinations with the analytical ultracentrifuge, using TRAUTMAN's modification[1,2] of the Archibald procedure, involve the evaluation of two experimental variables:

(1) The concentration gradient at the meniscus $(\partial c/\partial r)_m$ which, at any time during the experiment, is obtained by extrapolation of the gradient curve to the exact geometrical position of the meniscus, followed by measurement of the intercept Δy_m and proper introduction of optical constants[3,4] (Δy is the difference in height of solute gradient curve and baseline in enlarged tracings).

(2) The change in concentration of the solute at the meniscus, Δc_m, at any time from the beginning of the experiment. Δc_m is given by the area integral of the enlarged schlieren pattern between the meniscus position x_m and a position x_p in the region of the plateau. The radial dilution is incorporated in the integral.

$$\Delta c_m = \frac{\text{tg}\,\theta}{a'b'F_xF_y\Delta n} \frac{1}{x_m^2} \int_{x_m}^{x_p} x^2 \Delta y \,\mathrm{d}x \tag{1}$$

($x = F_x \cdot r$ is magnified radius, F_x is total radial magnification, F_y is total vertical magnification, a' is optical path of cell used, b' is optical lever arm of centrifuge, θ is schlieren angle used, Δn is specific refractive increment of solute.)

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A number of different procedures have been suggested for the computation of the above integral[5,6,7].

TRAUTMAN[1] has proposed an elegant solution for the evaluation of Δc_m by introducing a radius-cubed scale, at the spacings of which a summation of ordinates under the gradient curve yields the numerical value of the above integral directly. In practice, TRAUTMAN defined a fixed scale:

$$z = \left(\frac{10\,x}{x_r}\right)^3 \tag{2}$$

which yields

$$x^2 dx = \frac{x^3{}_r}{3000}\, dz \tag{3}$$

after differentiation and rearrangement (x_r is a reference point on the scale). Substitution of the latter form into Eqn. 1 yields:

$$\Delta c_m = \frac{x_r^3 \operatorname{tg} \theta}{3000 a' b' F_x F_y \Delta n} \frac{1}{x_m^2} \int_{x_m}^{x_p} \Delta y \, dz \tag{4}$$

which can be written as a summation of ordinates Δy_j under the enlarged gradient curve at the centre of small equal intervals Δz, or as:

$$\Delta c_m = \frac{x_r^3 \operatorname{tg} \theta}{3000 a' b' F_x F_y \Delta n x_m^2}\, 2\left(\tfrac{1}{2}\Delta y_m + \Delta y_{m+2} + \ldots + \Delta y_{p-2}\right) \tag{5}$$

if Δz is taken as 2, and Δy_j measurements are made at the spacings themselves. To do this, a tabulation has to be made with the help of Eqn. 2 to relate z- and x-values. For practical reasons, the reference point x_r is chosen just beyond the bottom of the cell. The actual value of x_r depends on the total radial magnification of the patterns. For $x = x_r$, it follows that $z = 1000$. All other z-values are then related to values for x (in millimetres) until the top of the cell is reached.

When a microcomparator is used, the x-values corresponding to the Δz-spacings are taken from the table and the comparator is adjusted with its millimetre scale, after which the Δy_j readings can be made.

If the radius-cubed scale is used in combination with a photographic enlarger, the enlarged tracings have to be transferred to graph paper. This must also be done for the appropriate part of the z-scale by interpolation between the millimetre marks of the abcissa. It will be realized, however, that the extra transfer of the tracing, as well as of the z-scale to graph paper, is time consuming and a source of additional errors. In this laboratory, a device has therefore been constructed that allows measurements of Δy_j, at the correct Δz-spacings, directly from drawings of the enlarged schlieren patterns on blank paper*.

CONSTRUCTION OF THE DEVICE

A blank transparent plate of Perspex of 5 mm thickness from I.C.I. Ltd. (Great Britain), was fraised in square dimensions of 450 × 450 mm. On the lower surface of this plate, a line pattern was engraved (line thickness 0.1 mm) with the aid of a milling machine and a 60° burin, as shown in Fig. 1.

* ERLANDER AND FOSTER[8] constructed an adjustable triangle on which the z-scale and the corresponding millimetre scale were laid out.

ABCD is a square (400 × 400 mm) and is divided into two halves by a horizontal line EF. The abcissa, AB, of the lower rectangle contains the radius-cubed scale and covers the entire length of the solution-compartment of a standard analytical ultracentrifuge cell from Spinco. The length of the z-scale in millimetres is further determined by the total radial magnification in going from the dimensions of the cell

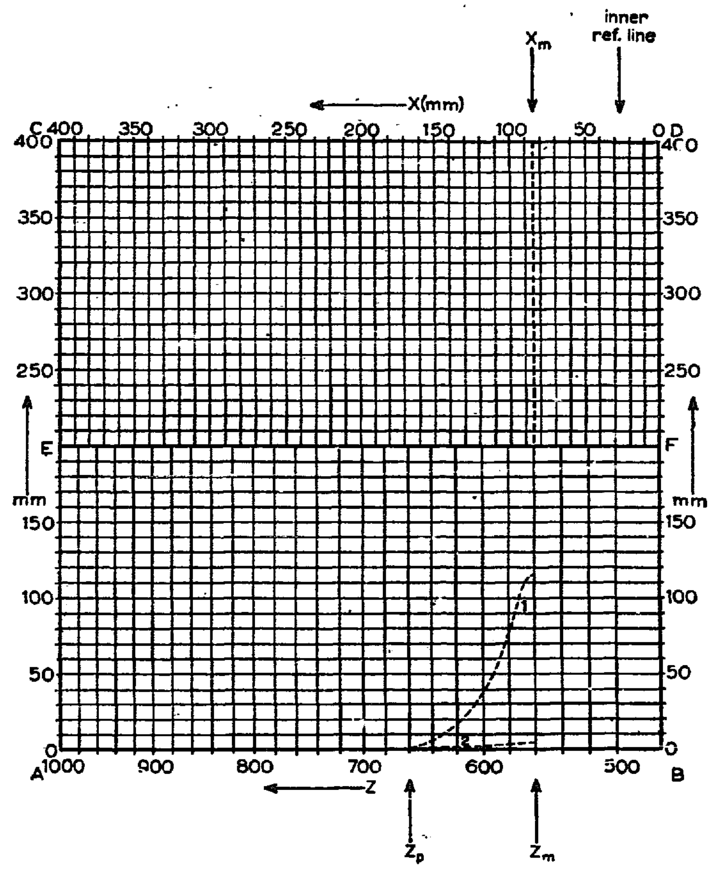

Fig. 1. Schematic drawing of z-scale device with engraved line pattern and enlarged tracing of ultracentrifuge gradient curve. 1, gradient curve; 2, base line.

to the enlarged tracing. Therefore, this magnification factor has to be fixed for every z-scale device and with the device described here was chosen to be 25. (This necessarily implies that the fixed magnification factor has to be applied whenever use is made of the z-scale device.) The reference point x_r was chosen to correspond with a distance of 1800 mm from the centre of rotation (in true dimensions 1800/25 = 72 mm), and thus represents a point between the bottom of the cell and the outer reference line. On the z-scale device, this point was fixed at position A. With position A at $x = x_r =$ 1800 mm, it follows from Eqn. 2 that $z = 10^3 = 1000$. At intervals of two units of z, all corresponding x-values were calculated and marked on the abcissa AB until a value of z of 500 was reached. $z = 500$ corresponds with a point between the top of the solution-compartment of the cell and the inner reference line. On the z-scale device this point lies in a position close to B. At each mark on the abcissa AB, a thin vertical line of 200 mm in length was engraved parallel to AE. In this way a pattern of vertical lines was obtained, in the lower half of the z-scale device, in which each line was separated from its neighbours by 2 units of z*. The ordinates of both halves

* Because of difficulties in reproduction, line patterns in Fig. 1 are drawn only at intervals of 10 mm for millimetre scales and at intervals of 20 units of z for the z-scale.

of rectangle ABCD, as well as the abcissa of the upper half EFCD, were marked at millimetre intervals and then lines of 400 mm and 200 mm were engraved parallel to AB and EC, respectively. All sides of the rectangle, ABCD, are properly marked with the corresponding z-values at AB and in millimetres at the three other sides. When the device is properly positioned (with engraved surface down), z-values at AB, and millimetre values at CD, should increase from right to left. This is caused by the fact that the ultracentrifuge plates in the photographic enlarger should be positioned with the emulsion side facing downwards. Consequently, in the enlarged pattern, sedimentation proceeds from right to left.

For an actual measurement of Δc_m in an enlarged tracing, the z-scale device is placed on top of it, with the engraved surface facing downwards. The position of the inner reference line can be calculated for the particular device; and, in the present case, corresponds with the 25-mm marking at CD. After determination of the exact position of the meniscus[3] on the enlarged tracing in terms of distance in millimetres from the inner reference line, the z-scale device can be properly adjusted with the aid of its upper half containing the millimetre scale on the abcissa. The meniscus has to be adjusted parallel to the engraved vertical lines; the intersection of the baseline and the gradient curve should be positioned on abcissa AB (see Fig. 1). In most cases it will be found that the position of the meniscus does not correspond to the position of a specific z-line in the lower half of rectangle ABCD. The exact z-value can be calculated, however, or obtained by interpolation. The proper correction can then be made for the first reading of Δy. Only a small error is made, however, if the z-scale device is readjusted in such a way that the meniscus corresponds to the nearest z-line. In this way some extra calculation can be avoided. According to Eqn. 5, the numerical value of the integral is now readily obtained by reading the ordinates at the intersections of the enlarged tracings and the vertical z-lines on the device, and adding these figures to half the value obtained at the meniscus. In certain instances a curved base-line is produced. In such a case Δy_j readings have to be made separately for the baseline pattern, and to be substracted from the initial readings.

In our hands the device has performed satisfactorily in combination with an automatic calculator. On the average it takes 10–20 min (depending on the size of the area to be integrated) to perform an integration in duplicate.

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REFERENCES

1 R. TRAUTMAN, *J. Phys. Chem.*, 60 (1956) 1211.
2 R. TRAUTMAN AND C. F. CRAMPTON, *J. Am. Chem. Soc.*, 81 (1959) 4036.
3 R. TRAUTMAN, *Biochim. Biophys. Acta*, 28 (1958) 417.
4 J. M. PETERSON AND R. M. MAZO, *J. Phys. Chem.*, 65 (1961) 566.
5 A. GINSBURG, P. APPEL AND H. K. SCHACHMAN, *Arch. Biochem. Biophys.*, 65 (1956) 543.
6 A. EHRENBERG, *Acta Chem. Scand.*, 11 (1957) 1257.
7 D. B. SMITH, G. C. WOOD AND P. A. CHARLWOOD, *Can. J. Chem.*, 34 (1956) 364.
8 S. R. ERLANDER AND J. F. FOSTER, *J. Polymer. Sci.*, 37 (1959) 103.