APPENDIX

Analysis of Data from Boundary Sedimentation Experiments in the Preparative Ultracentrifuge

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The boundary sedimentation experiment described by Schumaker & Rees (1972) provides a simple method of making approximate estimates of sedimentation coefficients. In many instances it may be the only practicable method, as for example in the present study where infective aggregates can only be obtained in mixtures that may also contain many non-infective aggregates. One of the limitations of the method as described is that interpretation of the results depends on extrapolation of graphs which have a complex shape and are not therefore amenable to statistical analysis. However, the equations can be transformed to yield a straightforward linear plot.

In the experiment, a centrifuge tube with radius of cross-section R is filled with a mixture containing the particles to be studied at concentration $C_0$. The radial distance from the axis of rotation of the rotor to the meniscus ($r_0$) is measured. (For swinging bucket rotors, of course, radial distances refer to the position assumed by the tube during centrifugation). The tube is then centrifuged at a given speed, measuring the time taken for acceleration, the time at full speed and the time taken for deceleration. The quantity $\int \omega^2 dt$ for the centrifugation is calculated as described by Funding & Steensgaard (1973). The top $V$ ml is then removed from the tube, and the radial distance to the new meniscus so formed ($r_e$) is measured. The concentration, $C_e$, of particles in the sample removed is measured.

Now consider a particle, initially at a radial distance $r_p$ from the axis of rotation ($r_0 < r_p < r_i$), which after centrifugation has just reached the position of the new meniscus $r_e$. The sedimentation coefficient, $s$, of this particle is given by

$$s = \frac{1}{\int \omega^2 dt} \ln \frac{r_i}{r_p}$$

Of an initial population of such particles at uniform concentration $C_0$ throughout the tube, only particles which started from radial positions closer to the axis of rotation than $r_p$ will remain in the sample of $V$ ml; that is, particles which started in a region of volume $\pi R^2 (r_p - r_0)$. The total concentration of particles in the sample, $C_t$, is therefore given by

$$C_t = \frac{C_0 \pi R^2 (r_p - r_0)}{V} = \frac{C_0 \pi R^2 (r_p - r_0)}{\pi R^2 (r_i - r_0)}$$

Therefore

$$\frac{C_t}{C_0} = \frac{r_i - r_0}{r_i - r_0}$$

But from equation (1), $r_p = r_i e^{-\int \omega^2 dt}$

Therefore

$$\frac{C_t}{C_0} = \frac{r_p e^{-\int \omega^2 dt} - r_0}{r_i - r_0} = \frac{r_0 e^{-\int \omega^2 dt}}{r_i - r_0}$$

Let

$$\frac{r_0}{r_i - r_0} = K$$
Then
\[ \frac{C_t}{C_0} + K = \frac{r_\ell e^{-\int \omega^2 dt}}{r_t - r_0} \]

Therefore
\[ \ln \left[ \frac{C_t}{C_0} + K \right] = \ln \left[ \frac{r_t}{r_t - r_0} \right] - s \int \omega^2 dt \]
\[ = \ln \left[ \frac{r_t}{r_0} \cdot K \right] - s \int \omega^2 dt. \]

For a series of such experiments with varying values of \( \int \omega^2 dt \), a plot of \( \ln \left[ \frac{C_t}{C_0} + K \right] \) against \( \int \omega^2 dt \) yields a straight line of slope \(-s\). The intercept on the y-axis where \( \int \omega^2 dt = 0 \) is \( \ln \left[ \frac{r_t}{r_0} \cdot K \right] \), which can be calculated. The straight line which best fits the experimental data, with the constraint that it must pass through this point, is found by regression, using standard statistical methods (see, for example, Bliss, 1967), and the slope of this line gives the best estimate of \( s \). The rectilinear form of the relationship makes it relatively simple to calculate the error in the estimate.

This type of experiment suffers from a number of obvious defects, many of which are discussed by Schumaker & Rees (1972). In addition, when the particles studied are heterogeneous with respect to \( s \), as is probably true of the aggregates considered here, it must be borne in mind that the calculated value of \( s \) will only represent an average. Nevertheless such a crude estimate may for some purposes be sufficient to draw useful conclusions. The method of analysis presented here should enable the optimum use to be made of the data.

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REFERENCES

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