*ACHIEVEMENT OF SEDIMENTATION EQUILIBRIUM**

BY P. E. HEXNER, L. E. RADFORD,† AND J. W. BEAMS

UNIVERSITY OF VIRGINIA

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The equilibrium method is generally considered to be the most reliable of the different ways that have been used for determining molecular weights with the ultracentrifuge.[1, 2] This results from the fact that the method is based upon equilibrium thermodynamics theory and yields absolute molecular weight values. On the other hand, the method requires centrifuging for a comparatively long time at a constant temperature and with a constant or slowly decreasing rotor speed which is free of hunting. The magnetically suspended equilibrium ultracentrifuge[3] solves the temperature and rotor speed problems and, by providing a uniform slowly decreasing speed, also significantly reduces the centrifuging time without lowering the precision. However, it is often important to reduce the centrifuging time still further in order to prevent deactivation of the substances or to increase the number of measurements in a given time, etc. Van Holde and Baldwin[4] have proposed the use of very short columns of solution in the centrifuge cell. This reduces the centrifuging time required to reach equilibrium, but also reduces the accuracy.[5, 6] Pasternak *et al.*[7] have considered starting the centrifuging with an accurately calculated step distribution of concentration using a synthetic boundary cell which also reduces the equilibrium time. The purpose of this paper is to describe a method of introducing a predetermined step-function reduction in angular speed of the rotor which greatly reduces the centrifuging time without sacrifice of accuracy. The procedure consists of centrifuging the substance under test at an angular velocity ω' until the concentration distribution in the ultracentrifuge cell most closely approximates the concentration distribution at equilibrium if the rotor were spun at a lower angular velocity ω. When this distribution is reached, the angular velocity is reduced from ω' to ω and the centrifuging continued until equilibrium is obtained.

In order to estimate ω' and the length of time necessary to centrifuge at ω' such that the proper concentration distribution is achieved, the solution of the following well-known differential equation[8] for sedimentation of an ideal solute in a uniform gravitational field is employed. This, of course, is not strictly applicable to the centrifuge where the centrifugal field is not constant, but it is sufficiently accurate for the purpose.[9]

$$\frac{\partial c}{\partial t} = A\frac{\partial^2 c}{\partial r^2} - B\frac{\partial c}{\partial r} \qquad (1)$$

with the boundary conditions

$$A\frac{\partial c}{\partial r} = BC \quad \text{for } r = 0 \text{ and } r = L \qquad (2)$$

and the initial condition

$$c(r,0) = f(r). \qquad (3)$$

The equation is reduced to dependence on a single parameter by the substitutions:

$$y = \frac{r}{L}, \quad \alpha = \frac{A}{BL}, \quad \beta = \frac{L}{B}.$$

The solution to the above problem for the concentration as a function of position and time is:

$$c(y,t) = \frac{e^{y/\alpha}}{\alpha(e^{1/\alpha} - 1)} \int_0^1 f(y)dy + e^{y/2\alpha} \sum_{m=1}^{\infty} S_m(t)[\sin m\pi y + 2\pi m\alpha \cos m\pi y], \quad (4)$$

where

$$S_m(t) = \frac{2e^{-P_m t/\beta} \int_0^1 f(y) e^{-y/2\alpha}[\sin m\pi y + 2\pi m\alpha \cos m\pi y]dy}{(1 + 4\alpha^2 m^2 \pi^2)}$$

and $P_m = \alpha m^2\pi^2 + \frac{1}{4\alpha}\cdot$

When the initial distribution is uniform, say $f(y) = c_0 =$ constant, then,

$$\frac{c(y,t)}{c_0} = \frac{e^{y/\alpha}}{\alpha(e^{1/\alpha} - 1)} + e^{y/2\alpha} \sum_{m=1}^{\infty} T_m(t)[\sin m\pi y + 2\pi m\alpha \cos m\pi y], \quad (5)$$

where

$$T_m(t) = \frac{16\alpha^2\pi e^{-P_m t/\beta} m[1 - (-1)^m e^{-1/2\alpha}]}{[1 + 4\pi^2 m^2 \alpha^2]^2},$$

which agrees with the solution obtained by Mason and Weaver.[8]

Now suppose that the initial distribution is the solution (5) just obtained. That is,

$$f(y) = \frac{e^{y/\alpha'}}{\alpha'(e^{1/\alpha'} - 1)} + e^{y/2\alpha'} \sum_{n=1}^{\infty} T_n(t')[\sin n\pi y + 2\pi n\alpha' \cos n\pi y]. \quad (6)$$

Then the concentration as a function of position and time is:

$$\frac{c(y,t)}{c_0} = \frac{e^{y/\alpha}}{\alpha(e^{1/\alpha} - 1)} + e^{y/2\alpha} \sum_{m=1}^{\infty} \frac{A_m e^{-P_m t/\beta}[\sin m\pi y + 2\pi m\alpha \cos m\pi y]}{[1 + 4\alpha^2 m^2 \pi^2]}, \quad (7)$$

where

$$A_m = \frac{[2m\pi - 4\alpha m\pi Q][1 - (-1)^m e^Q]}{[\alpha'(e^{1/\alpha'} - 1)][Q^2 + m^2\pi^2]} + 16\alpha'^2\pi \sum_{n=1}^{\infty} n e^{-P_n' t'/\beta'}[1 - (-1)^n e^{-1/2\alpha}][1 - (-1)^{m+n} e^R] F_{mn}$$

and

$$F_{mn} = \frac{2\pi^2(m + n)(\alpha' n + \alpha m) + R(1 - 4\pi^2\alpha\alpha' mn)}{R^2 + (m + n)^2\pi^2} + \frac{2\pi^2(m - n)(\alpha' n - \alpha m) - R(1 + 4\alpha\alpha' mn\pi^2)}{R^2 + (m - n)^2\pi^2}$$

$$Q = \left(\frac{1}{\alpha'} - \frac{1}{2\alpha}\right), \quad R = \left(\frac{1}{2\alpha'} - \frac{1}{2\alpha}\right), \quad P_n' = \left(\alpha' n^2\pi^2 + \frac{1}{4\alpha'}\right).$$

When Equation (1) is applied to sedimentation in the ultracentrifuge,[4] the parameters are:

b = radius of bottom of cell,
a = radius of meniscus,
$L = b - a$,
$y = \frac{r - a}{L}$,

$$\alpha = \frac{RT}{M(1 - \bar{V}\rho)\omega^2\bar{r}(b - a)}, \quad \beta = \frac{(b - a)RT}{M(1 - \bar{V}\rho)\omega^2\bar{r}D}, \quad \bar{r} = \frac{b + a}{2},$$

D = diffusion constant,
R = gas constant,
M = molecular weight,
T = absolute temperature,
$(1 - \bar{V}\rho)$ = buoyancy factor,
ω = angular velocity.

It is now assumed that the experiment has proceeded for a period of time t', at an angular velocity ω', such that the concentration distribution in the cell will be as in (6). Since the exponential containing the time in (7) involves m^2, it is clear that equilibrium will be most closely approximated if the first term in the series is made to vanish. This is assured if equality (8) holds.

$$\frac{[2\pi - 4\alpha\pi Q][1 + e^Q]}{[\alpha' e^{1/\alpha'} - 1][Q^2 + \pi^2]} = -16\alpha'^2\pi \sum_{n=1}^{\infty} \frac{n e^{-P_n' t''}[1 - (-1)^n e^{-1/2\alpha'}][1 - (-1)^{n+1} e^R]}{[1 + 4\alpha'^2 n^2\pi^2]^2} F_n, \quad (8)$$

where

$$t'' = \frac{t'}{\beta'}.$$

There exists a wide range of choices of ω' and t'' for the above equality to hold. Equality (8) is solved for t'', and Figure 1, obtained with the aid of a Burroughs 205 digital computer, shows a plot of t'' against α' for values of α shown. The curves were calculated using only the first term in the series in (7), which accounts for the fact that they approach large values of t'' at small values of α'. This is insignificant for experimental purposes due to the angular velocities generally used in equilibrium ultracentrifugation and provided the duration of an experiment is of the order of 30 min or more.

The procedure employed in this laboratory is to choose an α for the substance under test, preferably from experience, which maximizes the capabilities of the apparatus. If no information is available concerning the magnitude of α, an initial qualitative experiment usually gives the necessary data with which a good selection can be made. Figure 1 is then used to determine the smallest experimentally convenient α' and consequently the proper t'. Although with an actual system the value of α may be incorrectly chosen or there may be a series of α values due to polydispersity, the shape of the curves in Figure 1 is such that a small error will not materially alter the time for the experiment.

The theoretical results have been confirmed experimentally using the magnetically suspended ultracentrifuge.[3] Due to the stability of this system, the reduction in angular velocity produced no apparent stirring or other disturbances such as convection currents in the system.

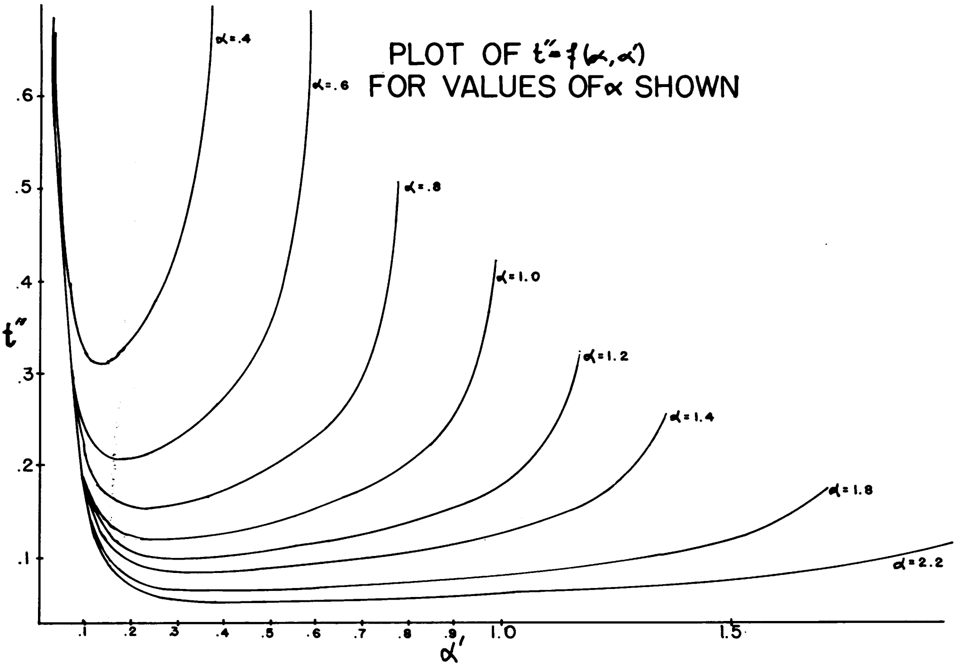

FIG. 1.

Typical experimental results with ribonuclease, insulin in 30 per cent acetic acid, and six times recrystallized lysozyme kindly furnished by Dr. K. Hayashi of Kyushu University, Kyushu, Japan, are shown in Table 1, where L is the length of the ultracentrifuge cell in *mm*, N' is the initial rotor speed in r.p.s., t' the time in hours of centrifugation at N', N the final rotor speed, τ the total time in hours for the experiment, and τ_e the time normally required for an experiment at a constant rotor speed N. M_w is the weight average molecular weight obtained.

TABLE 1

Substance	L	N'	t'	N	τ	τ_e	M_w
Ribonuclease	3	350	1.1	220	2.2	14	13,690 ± 60
Insulin	8	320	15.2	240	16	76	5,800 ± 40
Lysozyme	3	250	4.1	216	4.8	17	14,750 ± 60

In addition to the fact that this method markedly reduces the time required to reach sedimentation equilibrium, it is interesting to note from Table 1 that with lysozyme, for example, there is a better than threefold reduction in time with the speeds shown, although the time required to reach equilibrium at a constant velocity of 250 r.p.s. is approximately the same as that required for 220 r.p.s.

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† Present address: U.S. Military Academy, West Point, New York.

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*RELATIVISTIC UNIVERSES WITH SHEAR**

BY BRUCE B. ROBINSON

YERKES OBSERVATORY, UNIVERSITY OF CHICAGO, WILLIAMS BAY, WISCONSIN†

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1. *Introduction.*—In the classic cosmological solutions of Einstein's field equations, one assumes that the rotation tensor,

$$\omega_{\mu\nu} \equiv {}^1/_2(u_{\mu;\nu} - u_{\nu;\mu}), \tag{1}$$

and the shear tensor,

$$q_{\mu\nu} \equiv {}^1/_2(u_{\mu;\nu} + u_{\nu;\mu}) - {}^1/_3(g_{\mu\nu} - u_\mu u_\nu)u^\lambda{}_{;\lambda}, \tag{2}$$

vanish.[1] The quantities on the right hand side of (1) and (2) have their usual meanings, and the semicolon signifies covariant differentiation. It is well known that these solutions are characterized by a point singularity in the distant past at which time the universe was effectively created and that one of the characteristic features of relativistic cosmology (associated with the names Robertson and Lemaître) is the fact that solutions which adequately describe the present state of the universe assign an age to the universe (time since the point singularity) which is exceeded by the ages assigned, by present theories, to some astronomical bodies.

Raychaudhuri[2] investigated the possibility of increasing the age of the universe by removing the condition that $q_{\mu\nu}$ must vanish and discovered that the time since the singularity is maximum when $q_{\mu\nu} = 0$.

Heckmann and Schücking[3] briefly discussed universes with shear and pointed out the importance of investigating the nature of the singularity when shear is present. They pointed out the existence of two line elements which show that the "big bang" theory must be substantially revised if any shear is present.

It appears that one of the Heckmann and Schücking solutions is the only permissible solution with purely time-dependent shear in a homogeneous universe, also that the family of the solutions with constant curvature all possess the Heckmann and Schücking singularity.