THE ULTRACENTRIFUGAL "STEADY STATE"

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Steady state conditions are of interest in all transport processes (cf. Hoch, 1950) because of their simple mathematical description; in the ultracentrifuge the condition is important because it provides a new approach to the analysis of heterogeneity. Owing to radial dilution and the varying field, a true steady state cannot be attained; nevertheless, a particular time-invariant condition exists, which can be approached in practice sufficiently closely for useful results to be obtained. The basis of the method is that, for all non-aggregating systems, the sedimentation coefficient decreases with increasing concentration; thus a sedimenting boundary tends to be sharpened by concentration-dependence, but spread by diffusion and heterogeneity. In the steady state, the sharpening and spreading effects are balanced.

Theory - For a two-component (single pure solute) system, where the sedimentation (s) and diffusion(D) coefficients vary with concentration (c), s is usually measured by reference to a cylindrical surface moving in the radial direction with velocity v:

$$v = \omega^2 r s_x \qquad (1)$$

Here ω is the angular velocity, r the distance from the center of rotation, and $s_x = s(c_x)$, where c_x is the concentration in the plateau region. The velocity v is equal to that of a point r_*, which is the square root of the second moment of the boundary distribution, and in general close to r_p, the peak position. The steady state condition is that all points of fixed fractional concentration (c/c_x) are required to move with this same velocity v.

This restriction may be applied to the Lamm (1929) flow equation, when after some manipulation the fundamental equation of the steady state is obtained:

$$\frac{(dc/dr)}{rc} = \frac{\omega^2[s(c) - s(c_x)]}{D(c)} \tag{2}$$

Here c and dc/dr are the concentration and concentration gradient at the point r: the significance of the equation is that although c_x, etc., vary with time, the particular distribution defined by the equation applies at all times, for arbitrary dependence of s and D on c (provided that $s(c) > s(c_x)$). When s varies linearly with c:

$$s = s^o(1 - kc) \tag{3}$$

substitution in (2) gives

$$\frac{(dc/dr)}{rc(c_x - c)} = \frac{\omega^2 s^o k}{D(c)} \tag{4}$$

whereas if (1/s) is linear in c:

$$(1/s) = (1/s^o)(1 + Kc) \tag{5}$$

we have

$$\frac{(1 + Kc)(1 + Kc_x)(dc/dr)}{rc(c_x - c)} = \frac{\omega^2 s^o K}{D(c)} \tag{6}$$

Equations (4) and (6) define steady state distributions which are strictly time-invariant; a simpler method than the implied analysis is desirable, however, to test the attainment of a steady state. Such a method follows from (4). Assuming that the maximum gradient occurs at $c_p = c_x/2$, and applying the radial dilution law

$$c_x = c_o(r_a/r_p)^2 \tag{7}$$

where r_a refers to the meniscus, we have

$$r_p^3(dc/dr)_p = r_a^4 c_o^2 \omega^2 s^o k/4D(c_p) \tag{8}$$

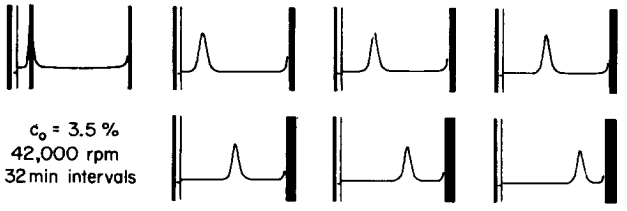
which shows that the product $r_p^3 H$ (where H is the height of the peak) is constant in the steady state within the constancy of $D(c_p)$, for systems where s is linear in c. Since c_p may vary by about 10% during the steady state experiment, the approximation is useful only where dD/dc is small.

In general, this is not a serious restriction. For cases where 1/s is linear in c, the approximation is less accurate.

Equation (8) provides a ready means of recognition of attainment of the steady state, and incidentally emphasizes that a peak does change height considerably while remaining in the steady state. From the stable value of $r_p{}^3H$ attained, a value of $D(c_p)$ is easily found; if this should agree with the ordinary diffusion coefficient as measured in static experiments, one must conclude that the sedimenting substance is indeed one component - i.e., it is homogeneous in sedimentation. If the value obtained from $r_p{}^3H$ exceeds the true diffusion coefficient, then heterogeneity is present, and the quantity is designated as $D^\dagger$, the 'spreading coefficient'. The difference between D and $D^\dagger$ as c varies from zero to c_x carries all the information on heterogeneity; however, only a qualitative interpretation is possible at present.

Experimental - Fig. 1 illustrates the sedimentation patterns obtained in a typical steady state experiment on a nearly monodisperse system. They refer to a preparation of horse γ-globulin which gave almost symmetrical peaks in conventional sedimentation velocity experiments. The experiment is conducted as follows. The peak is first brought well into view by a period at maximum speed (first frame), the rotor is then braked to low speed and held there until the peak height has decreased to a convenient value. At the end of this diffusion period, the rotor is re-accelerated to a predetermined speed, at which it is anticipated the peak height will be close to the steady state value (second frame). A first approximation to this speed may generally be calculated

Fig. 1 Steady-state experiment on horse γ-globulin



from equation (8), using the known value of D, and allowing for the slight decrease in peak height during the re-acceleration period. When the variables of height and speed are thus correctly controlled, the boundary will attain a steady state fairly quickly. It is apparent from the patterns that little change with time occurs over the 3 hour duration of the experiment. It is very desirable to vary the diffusion time so that in a pair of experiments at the same speed, the stable value of $r_p{}^3H$ is attained both from above and below (Fig. 2). It must be emphasised that the peak height must be close to its steady state value at the start of the main part of the experiment; the ultracentrifuge cell is too short to allow for the prolonged sedimentation necessary if the steady state is to be attained from a markedly different initial state.

Fig.2 Attainment of steady-state

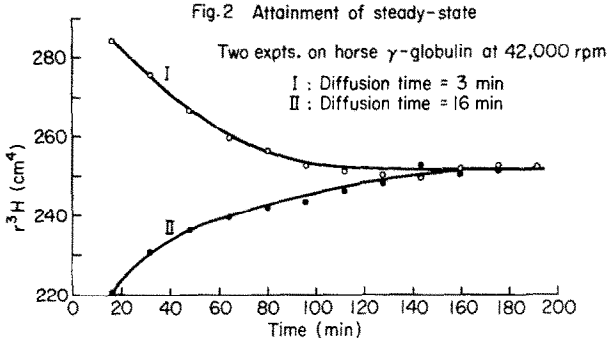


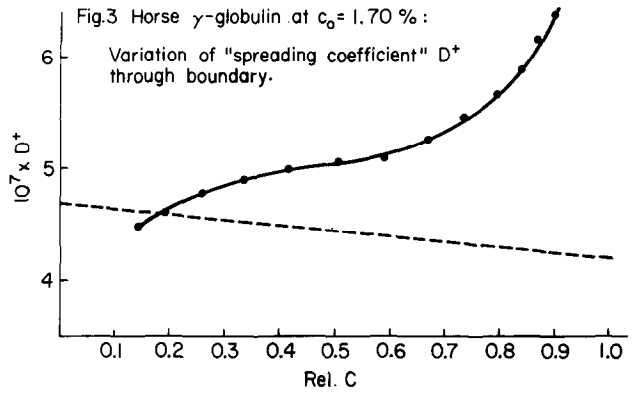
Fig. 3 shows the results obtained by applying the detailed analysis to a typical steady state boundary. Experience has shown that use of the individual gradient values leads to excessive scatter for $c/c_x < 0.25$ and > 0.75, so equation (4) was applied in its logarithmic form. For measurements of c at equal intervals in r, this takes the form

$$D^{\dagger}(c) = -\frac{c_x \omega^2 s^o k \Delta r}{2.303} \cdot \frac{\bar{r}}{\Delta \log_{10}[(c_x - c)/c]} \qquad (9)$$

(The equivalent form for calculating D when 1/s is linear in c is

$$D^{\dagger}(c) = -\frac{c_x}{1 + Kc_x} \cdot \frac{\omega^2 s^o K \Delta r}{2.303} \cdot \frac{r}{\Delta[(1 + Kc_x)\log_{10}(c_x - c) - \log_{10} c]} \qquad (10))$$

For γ-globulin under the conditions used, $s^o k = -0.60$ sec. dl. g.$^{-1}$



The dashed line in Fig. 3 represents the concentration dependence of the true diffusion coefficient (data of Pain, 1963). As can be seen, the values of $D^\dagger$ and D coincide at low values of c, but $D^\dagger$ is appreciably greater than D for the higher values, particularly as $c \rightarrow c_x$ ("Rel c" = c/c_x). This is clear evidence for the presence of a faster 'impurity' in the preparation, and it is an indication of the sensitivity of the method that the difference between $D^\dagger$ and D is so well-defined in a case where the schlieren peaks themselves appear almost symmetrical.

The main experimental difficulty with the method is that relatively high concentrations must be used to avoid excessive spreading; for the γ-globulin the minimum concentration was about 1.2%. With very polydisperse materials, the gradients may pass through a maximum before the steady state is attained, and allowance must be made for this in selecting the conditions.

The method provides a convenient and rapid test of homogeneity, and a means of comparing degrees of heterogeneity among similar materials. It should be particularly useful for those highly concentration-dependent systems where existing methods (see review by Baldwin and van Holde, 1960) are inapplicable. Full details of the method, and the results obtained with a polydisperse system following equation (5), will be published elsewhere.

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