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A Stability Criterion for the Measurement of Diffusion Coefficients in the Zonal Ultracentrifuge

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SUMMARY:

Experiments have been conducted to determine the onset of droplet sedimentation in diffusion experiments in the zonal ultracentrifuge. The data obtained have been used to provide a guide for the choice of appropriate macromolecular and supporting solute concentrations in such experiments. Comments are made on the effect of introducing perturbed zones into the rotor.

INTRODUCTION:

In a previous publication[1] we described a method for measuring translational diffusion coefficients in the zonal ultracentrifuge. It was pointed out that droplet sedimentation[2,3] may invalidate the results. This is probably caused by a density inversion at the interface between the macromolecu-

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lar zone and the supporting plateau of salt, resulting in extensive band spreading by convection. The phenomenon may be avoided by the choice of appropriate conditions, and theoretical approaches[4,5] have yielded differing stability criteria. We have found that the zonal rotor can be used to give a tremendous magnification of interface instabilities, and we have used this property to obtain experimental values for the stability criterion to be used when diffusion coefficients are to be measured.

MATERIALS:

Sodium bromide was Mallinkrodt Analytical Reagent lot #VKH B and required filtering to remove suspended matter before use. Bovine serum albumin (BSA) was Pentex crystallized.

PROCEDURE:

The zonal rotor (B-XV, titanium, Oak Ridge type) was loaded with a sodium bromide solution of uniform density (determined refractometrically), the rotor speed being maintained at 3000 rpm. When full, 10 mℓ of a C% solution of BSA in sodium bromide at a lower known density (2 w/w% decrement) than the underlay was injected slowly via a syringe into the center of the rotor and the time noted (t_1). This was followed slowly by sodium bromide at a lower density (2 w/w% decrement) than that in the macromolecular zone until a specified radius was reached. The system was then left for a known period of time (between 5 and 20 min), after which the rotor contents were displaced by pumping dense sodium bromide to the edge of the rotor. Pumping was initially fairly rapid (~100 mℓ/min) until the position of the layered zone was approached. The displaced solution was then pumped slowly by a Buchler polystaltic pump through a flowthrough cell installed in a Gilford recording spectrophotometer monitoring at 280 mμ. The chart record was calibrated against the polystaltic pump during every run. Effluent solution temperatures were monitored with a Tele-thermometer (Model 42SC, Yellow Springs Instrument Co., Ohio). The time t_2 was noted when the leading edge of the zone (nearest the center of rotation) reached the flowthrough cell. All line volumes were kept as small as was practically possible within the limitations imposed by our equipment.

Longer (~15 hr) runs were performed to determine the diffusion coefficient of BSA as per reference 1. In these experiments the BSA concentration was kept constant, and the concentration of the density making solute varied.

CALCULATIONS:

The general form of the stability criteria is taken to be

$$\frac{\Delta\rho_m}{\Delta\rho_s} = \frac{C_m(1 - \bar{v}_m\rho''_{salt})}{\rho'_{salt} - \rho''_{salt}} < \left(\frac{D_m}{D_s}\right)^n \qquad \text{(Eq. 1)}$$

for stability, where

C_m = macromolecular concentration in the zone at time t = 0 in gm/mℓ,

$\bar{v}_m$ = partial specific volume of macromolecule,

ρ''_{salt} = initial density of salt in the zone,

ρ'_{salt} = initial density of salt in the underlay,

D_m = diffusion coefficient of macromolecule in the experimental medium in Ficks,

D_s = diffusion coefficient of the salt in the medium in Ficks,

n is given as 1/2 by reference 4, and as 3/2 by reference 5.

The measurements taken in the experiments together with tabulated data[7,8] give all the unknowns in this equality except n, which is then calculated. This value of n was plotted against the width of the peak at half height measured from the Gilford trace and converted to milliliters.

RESULTS:

In Fig. 1 can be seen a plot obtained by the method described in "Calculations." The break in the line depicts the onset of droplet sedimentation.

Figure 2 compares "stable" and "unstable" band profiles which have stood for equal periods of time in the zonal rotor.

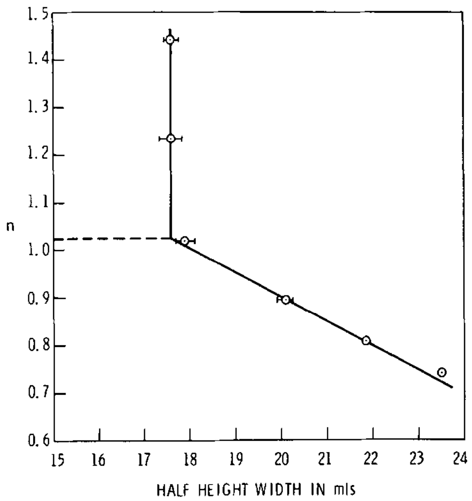

Figure 1. A plot of n calculated from Eq. (1) versus the zone half height width in milliliters. The break in the line at n = 1 is caused by the onset of droplet sedimentation.

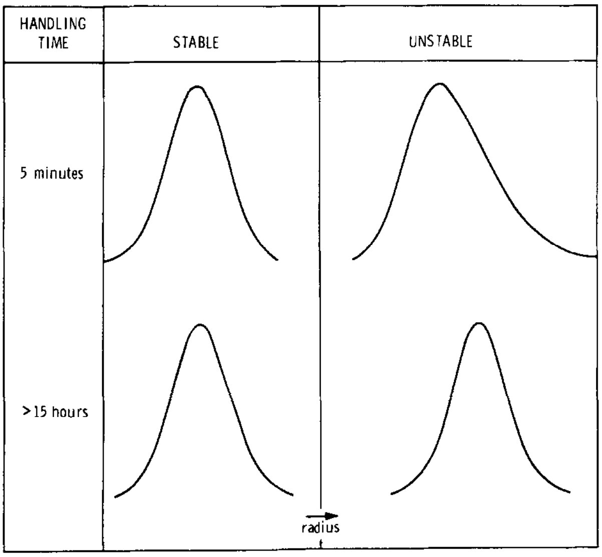

Figure 2. A comparison of the profiles of stable and unstable bands which have stood for similar lengths of time in the rotor. The profiles (5 min) are plotted on the same abscissal scale. The 15-hr scale has been much contracted. Ordinate scales have been normalized to facilitate comparison.

Table 1 gives the results of calculation of the diffusion coefficient[1] for BSA from ~15-hr runs with both stable and unstable initial boundary conditions.

$D_{20,W}$(Ficks)	n	Stability
6.05	1.010	+
6.73	0.925	–
7.11	0.889	–
7.34	0.853	–

Table 1. Diffusion coefficient of bovine serum albumin at 0.5 wt % determined by the method of ref. 1. The "stability" column refers to the initial interface condition between the macromolecular zone and the underlay, using n = 1 as the lower limit for stability.

DISCUSSION:

It has been assumed in this work that certain characteristics of the system we used produce an increase in zone width, apart from droplet sedimentation itself, and that this spreading should be small and reproducible. This is borne out by the small spread in data at the same concentration and the constancy of the stable zone half height widths at a variety of concentrations. (By far the greatest zone broadening effect was that of molecular diffusion of the BSA, even at these short time intervals) it follows then that any gross deviations in half height width at constant handling time are attributable only to droplet sedimentation (see Fig. 2). The onset of such convection is some progressive function of the macromolecular concentration, and therefore requires magnification to see small amounts of convection. The property of the zonal rotor that volume is approximately proportional to the radius squared was found to provide this magnification adequately by pumping the zone to a radius of approximately 5 cm. Using this system, and the calculations described earlier, we find (Fig. 1) the exponent, n, in Eq. (1) to be equal to 1 experimentally for the step concentration conditions used in diffusion experiments in the zonal rotor.*

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*A more practical value to use routinely, however, would be somewhat higher, e.g., 1.10, since this reduces the difficulties encountered when layering solutions extremely close in density.

We may speculate with some confidence on the fate of a zone broadened by droplet sedimentation. Unstable zones which have been left in the rotor for a considerable period of time (~15 hr), show a remarkably Gaussian profile (Fig. 2). The degree of convection is reflected in the magnitude of the diffusion coefficients calculable from the profile compared with the value obtained from a stable zone (Table 1). However, it seems apparent that the initially unstable zone convects and "expands" until a point of stability is reached. Molecular diffusion then takes place normally. Cox's prediction,[6] using computer simulation, that initially skew boundaries become Gaussian by diffusion, fits in well with this. This ability of a skew zone to correct itself is very important to remember when interpreting the results of a diffusion experiment. If the stability criterion reported here is abided by, and density steps between zones are kept small (to eliminate cross-diffusion effects), then any skew or non-Gaussian property of the diffused peak is due not to hydrodynamic disturbances during or immediately after layering the zone, but probably inhomogeneity or association-dissociation of the sample under study.

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