PART II. VELOCITY SEDIMENTATION

I. Introduction

The number of investigators using the ultracentrifuge as a research tool has increased greatly in the past two decades; this has been made possible by the development of new commercial instruments. As the instruments have become more widely available, interest has grown in using the ultracentrifuge for as many purposes as possible. For example, two recent applications of sedimentation velocity experiments are the measurement of molecular weights by use of the boundary condition (the Archibald method) and the binding of small molecules by proteins. The experiments have been made more accurate, chiefly by careful control of rotor speed and temperature, and refinements have been made of the equations used in measurement. Also, there have been striking developments in the basic theory; together with the observation by Archibald that one can measure such purely thermodynamic quantities as molecular weights and activity coefficients from sedimentation velocity experiments has come the realization that gradients of thermodynamic potentials are the driving forces which cause sedimentation. Today there is a smooth transition from the theory of the sedimentation velocity experiment to that of the equilibrium experiment.

A. SCOPE OF PART II

Part II contains results obtained from two fundamental equations for the ultracentrifuge: the conservation of mass and the expression for the flow of a solute. Starting with these two equations one can develop relations for measuring sedimentation coefficients and heterogeneity in sedimentation behavior, for finding molecular weights, and for studying interacting systems. Neither methods of computation nor experimental results have been included, and mathematical solutions to the continuity equation are considered in Part III. This review includes the period of roughly two decades since the publication of *The Ultracentrifuge* by Svedberg and Pedersen (89).

An attempt has been made to derive many of the results given here in a unified manner. Consequently the method of derivation often differs somewhat from the one used in the original article, and also the form of the result may differ. This does not mean that a particular derivation or the form of a final equation is an improvement over that given by the original authors; rather, it is the natural result of trying to derive diverse equations by a common route.

B. QUANTITIES MEASURED IN SEDIMENTATION EXPERIMENTS

In a sedimentation experiment one takes photographs which yield the curve of concentration (c) or concentration gradient versus distance. Also one measures the temperature (T) and angular velocity (ω) of the rotor, and the times (t) at which photographs are taken. Different optical systems are in use. Most common is the schlieren optical system described by Philpot (73) and by Svensson (91). In a schlieren photograph the abscissa is a linear function of the radial distance

r, and the ordinate is related in the same manner to the gradient of refractive index ($\partial n/\partial r$), from which the concentration gradient ($\partial c/\partial r$) can be obtained. Philpot and Cook (74) have described an optical system based on the Rayleigh interferometer, which yields a curve of n vs. r, and this is beginning to be used. The original light-absorption system (89, 90) is finding favor again, for studying substances at low concentrations which absorb light strongly and which show a marked dependence of sedimentation behavior on concentration.

There are certain theoretical problems in obtaining a curve of $\partial c/\partial r$ vs. r from a schlieren photograph (1, 16, 50, 95) or in obtaining a curve of c vs. r from a photograph of Rayleigh fringes (27), but this subject will not be reviewed here. It will be assumed that n and $\partial n/\partial r$ (or c and $\partial c/\partial r$ when there is a single solute) are directly measurable quantities.

II. Fundamental Equations

A. Coördinate System

The equations for the ultracentrifuge take a simple form when cylindrical coördinates are used. The ultracentrifuge cell itself is a truncated sector of a cylinder (see figure 1). It is necessary to use a cell of this shape because sedimentation occurs radially and use of a rectangular cell would cause convection, by reason of accumulation of solute at the cell walls. In this coördinate system h is the distance parallel to the axis of rotation and θ is the sector angle. Since the concentration of a solute is usually a function only of r, at a given time, the other two coördinates rarely appear in the final equations.

The area (A) of a cylindrical surface parallel to the h axis and cut by the sector angle θ is

$$A = \theta h r \tag{1}$$

and the volume contained in the sector is

$$V = \tfrac{1}{2}\theta h r^2 \tag{2}$$

so that

$$\frac{dV}{dr} = \theta h r \tag{2a}$$

B. Conservation of Mass

Consider a section of volume such as that shown in figure 1, with cylindrical surfaces parallel to the h axis at r_1 and r_2. Provided that the solute does not take part in chemical reactions, the amount of solute flowing in through the surface at r_1 minus the amount flowing out at r_2 must equal the rate of change with time of the amount (g_i) remaining in this volume.

$$(AJ_i)_{r_1} - (AJ_i)_{r_2} = \frac{\partial g_i}{\partial t} \tag{3}$$

where J_i is the *flow* (or flux) of i, the amount crossing unit area in unit time.

$$J_i = c_i v_i \tag{4}$$

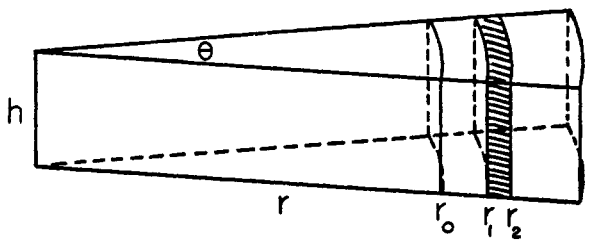

FIG. 1. Diagram of the solution contained in an ultracentrifuge cell (not to scale), showing the cylindrical system of coördinates used to describe sedimentation processes. The darkened section represents an illustrative element of volume. Light from the optical system passes through the solution parallel to the h axis.

In equation 4 v_i is the velocity of i (centimeters per second) and c_i is the concentration of i on a *weight per volume* scale (here grams per cubic centimeter). The amount of i in the section of volume is given by

$$g_i = \int_{r_1}^{r_2} c_i \, dV = \theta h \int_{r_1}^{r_2} r c_i \, dr \tag{5}$$

where θ and h have been taken outside the integral sign because c_i is in general a function only of r. Combination of equations 1, 3, and 5 gives the important result:

$$(rJ_i)_{r_1} - (rJ_i)_{r_2} = \frac{\partial}{\partial t} \int_{r_1}^{r_2} r c_i \, dr \tag{6}$$

1. The continuity equation

Differentiation of equation 6 with respect to r at constant t gives the continuity equation.

$$\left(\frac{\partial c_i}{\partial t}\right)_r = -\frac{1}{r}\left[\frac{\partial (rJ_i)}{\partial r}\right]_t \tag{7}$$

If chemical reactions occur, then terms must be added to describe the reaction. Thus for the reaction

$$x\mathrm{A} + y\mathrm{B} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} u\mathrm{C} + v\mathrm{D} \tag{8}$$

one has continuity equations of the form

$$\left(\frac{\partial c_A}{\partial t}\right)_r = -\frac{1}{r}\left[\frac{\partial (rJ_A)}{\partial r}\right]_t + xM_A\{-k_1 c_A^x c_B^y + k_2 c_C^u c_D^y\} \tag{9}$$

where the molecular weights M_A, M_B, etc. are required because the concentrations are expressed as weight per volume rather than moles per volume. It will be noted that when equation 9 is summed over products and reactants one has

$$\sum_i \left(\frac{\partial c_i}{\partial t}\right)_r = -\frac{1}{r}\left[\sum_i \frac{\partial (rJ_i)}{\partial r}\right]_t \tag{10}$$

since

$$xM_A + yM_B = uM_C + vM_D \tag{11}$$

Many experimental procedures in sedimentation analysis are based on solutions to equation 7, which is a partial differential equation. These solutions give c or $\partial c/\partial r$ as a function of r and t. They are usually based on a simple flow equation (55)

$$J_i = c_i s_i \omega^2 r - D_i \left(\frac{\partial c_i}{\partial r}\right)_t \tag{12}$$

in which s_i and D_i are sedimentation and diffusion coefficients and ω is the angular velocity in radians per second. This equation is applicable only in certain conditions; a more general flow equation is given later (equation 29). The different solutions to equation 7 vary chiefly in the functional dependence assigned to s_i and D_i (whether they are treated as constants or as functions of concentration or pressure) and in the choice of initial and boundary conditions. This subject is considered in detail in Part III of the review.

C. FLOW EQUATIONS

Perhaps the most important development of recent years in the theory of sedimentation is the derivation of general flow equations from thermodynamics of irreversible processes (37, 70, 71, 76) by de Groot, Mazur, and Overbeek (38), by Hooýman (44, 45, 46), and by Haase (40). All of the equations used in sedimentation analysis depend upon a proper formulation of the flows, and one might say that the fundamental problem in the descriptive or phenomenological theory of sedimentation is the derivation of accurate flow equations. The ones derived by thermodynamics of irreversible processes are believed to be far more accurate than present experiments. They are limited to systems close to equilibrium; however, this does not seem to be a serious limitation in studying sedimentation and diffusion in liquids.

1. Origin and properties of the equations

The following procedure was used (38, 45, 46) to derive flow equations for the ultracentrifuge. By combining four basic physical principles (conservation of mass, conservation of momentum, conservation of energy, and the second law of thermodynamics) an expression was obtained for the dissipation function of Onsager (70, 71) denoted (46) by $T\sigma$, where σ is the rate of production of entropy by the irreversible processes and T is the absolute temperature. This expression contains the flows and can be written in a compact form

$$T\sigma = \sum_{i=1}^{q} (J_i) X_i \tag{13}$$

in which the X_i are identified as the "forces" causing the flows, and the flows have been enclosed in parentheses to indicate that the frame of reference has not been specified. (The equations given here apply to a transport process oc-

curring in one dimension and are not given in vector notation.) In an isothermal system containing $q + 1$ components it is necessary to describe the flows of only q components; here the remaining component is specified to be the solvent and is labelled component 0. Next the flows were assumed to be linear functions of the forces appearing in equation 13.

$$(J_i) = \sum_{k=1}^{q} (L_{ik})X_k \tag{14}$$

The equations represented by equation 14 are called *phenomenological* equations and the coefficients (L_{ik}) are termed phenomenological coefficients. They are functions of temperature, pressure, and composition and of the frame of reference. Equation 14 is limited to small values of X_k or, in other words, to small departures from equilibrium. Combination of equation 13 and 14 yields

$$T\sigma = \sum_{i=1}^{q} \sum_{k=1}^{q} (L_{ik})X_i X_k \tag{15}$$

In the procedure used by Hooýman (45) the reciprocal relations of Onsager (70, 71) take the form

$$(L_{ik}) = (L_{ki}) \tag{16}$$

when equation 14 is written in terms of q flows and forces, and the forces have been chosen to give the entropy production (equation 13).

A primary result of applying this procedure to the problem of sedimentation and diffusion in a centrifugal field is the equation for $T\sigma$ (equation 31 of Hooýman, Holtan, Mazur, and de Groot). It is given here for an isothermal system, with the omission of a minor term involving the Coriolis force and with the assumption of electroneutrality; also the system is treated as being at mechanical equilibrium.

$$T\sigma = \sum_{i=0}^{q} (J_i)_a \left\{ (1 - \bar{v}_i \rho)\omega^2 r - \frac{z_i}{M_i} \epsilon \frac{\partial \varphi}{\partial r} - \left(\frac{\partial \mu_i}{\partial r} \right)_{P,T} \right\} \tag{17}$$

(Note that this summation includes all $q + 1$ components.) In equation 17 $(J_i)_a$ denotes flow relative to an arbitrary frame of reference (a), $\bar{v}$ is the partial specific volume (cubic centimeters per gram), ρ is the density of the solution (grams per cubic centimeter), z is the valence of an ion (+1 for Na^+, −1 for Cl^-, etc.), M is the molecular weight, ϵ is the charge on one mole of protons, $\partial\varphi/\partial r$ is the electrostatic potential gradient, and μ is the chemical potential *per gram*.

$$\mu_i = \mu_i^\circ + \frac{RT}{M_i} \ln y_i c_i \tag{18}$$

In equation 18 μ° is the reference potential (a function of temperature and pressure) and y is the activity coefficient on the c scale. A more compact form of equation 17 is

$$T\sigma = -\sum_{i=0}^{q} (J_i)_a \left(\frac{\partial \bar{\mu}_i}{\partial r} \right)_t \tag{19}$$

in which $\bar{\mu}$ is the total potential per gram (the sum of the chemical, centrifugal, and electrostatic potentials)

$$\bar{\mu}_i = \mu_i - \frac{\omega^2 r^2}{2} + \frac{z_i}{M_i}\epsilon\varphi \tag{20}$$

and in which the subscript t denotes a partial derivative taken with only time held constant. From this point on, discussion of the flow equations will be limited to nonionizing solutes ($z_i = 0$ for $i = 1, \cdots, q$).

When the flows relative to an arbitrary frame of reference are specified to be those relative to the solvent (component 0), equation 19 reduces to an equation in q flows and q gradients

$$T\sigma = -\sum_{i=1}^{q} (J_i)_0 \left(\frac{\partial \bar{\mu}_i}{\partial r}\right)_t \tag{21}$$

since the flow of solvent relative to solvent is zero.

$$(J_0)_0 = 0 \tag{22}$$

Thus, following the procedure discussed above, Hooýman (45) obtained the flow equations

$$(J_i)_0 = -\sum_{k=1}^{q} (L_{ik})_0 \left(\frac{\partial \bar{\mu}_k}{\partial r}\right)_t \qquad (i = 1, \cdots, q) \tag{23}$$

with

$$(L_{ik})_0 = (L_{ki})_0 \tag{23a}$$

The experimentalist requires an equation for flows relative to the cell. A convenient method of obtaining such an equation (45) is to convert equation 23 into one in which the flows are expressed relative to the mean volume velocity v'.

$$v' = \sum_{j=0}^{q} \bar{v}_j J_j \tag{24}$$

The flows relative to the cell are then identical with those relative to v' when the partial specific volumes are constant, independent of pressure and composition.

$$v' = 0 \qquad (\bar{v}_j = \text{constant}) \tag{24a}$$

It will be assumed here that the partial volumes can be treated as constants and therefore flows relative to the cell (denoted simply by J) will not be distinguished from those relative to v'.

To obtain an expression for flow relative to the cell in terms of the flows relative to solvent one may begin with the equation

$$J_i = (J_i)_0 + c_i v_0 \tag{25}$$

in which v_0 is the velocity of solvent relative to the cell. Then an expression for v_0 is obtained by multiplying equation 25 by $\bar{v}_i$, summing over all components, and making use of equations 24, 24a, and $\sum_i \bar{v}_i c_i = 1$.

$$v_0 = -\sum_{j=1}^{q} \bar{v}_j (J_j)_0 \tag{26}$$

The following expression for flow relative to the cell is obtained by substituting equations 23 and 26 into equation 25; this is equation 22 of Hooýman (45), limited to the case of constant partial volumes.

$$J_i = -\sum_{k=1}^{q} L_{ik} \left(\frac{\partial \bar{\mu}_k}{\partial r}\right)_t \qquad (i = 1, \cdots, q) \tag{27}$$

$$L_{ik} = (L_{ik})_0 - c_i \sum_{j=1}^{q} (L_{jk})_0 \bar{v}_j \tag{27a}$$

$$-\left(\frac{\partial \bar{\mu}_k}{\partial r}\right)_t = (1 - \bar{v}_k \rho)\omega^2 r - \left(\frac{\partial \mu_k}{\partial r}\right)_{P,T,t} \tag{27b}$$

It will be observed that a simple and compact set of flow equations has been obtained, but that this last set of equations does not satisfy equation 13 and the reciprocal relations do not apply in the form of equations 16 and 23a.

$$L_{ik} \neq L_{ki} \tag{27c}$$

However, given the L_{ik} one could make use of the reciprocal relations in other ways: for example, by finding the coefficients $(L_{ik})_0$ in terms of the coefficients L_{ik} and then using equation 23a. Since the aim was to obtain a correct and useful set of equations for the flows relative to the cell and to determine the status of the reciprocal relations for this set of equations, it is unimportant that they no longer fit into the scheme of equations 13 and 14.

2. A practical flow equation for multicomponent systems

For the purposes of the experimentalist it is convenient to express equation 27 in a form which contains directly measurable quantities and the least number necessary of unknown coefficients. First examine the variables and the coefficients in this equation.

$$J_i = \sum_{k=1}^{q} L_{ik} \left\{ (1 - \bar{v}_k \rho)\omega^2 r - \sum_{j=1}^{q} \mu_{kj} \left(\frac{\partial c_j}{\partial r}\right)_t \right\} \tag{28}$$

In Hooýman's notation (45)

$$\mu_{kj} = \left(\frac{\partial \mu_k}{\partial c_j}\right)_{\substack{T,P,c_m \\ (m \neq 0)}} \tag{28a}$$

One may regard $\partial c_j/\partial r$ and $\omega^2 r$ as variables and the other terms as coefficients. Then a practical form of equation 28 is (*cf.* Hooýman (45))

$$J_i = c_i s_i \omega^2 r - \sum_{j=1}^{q} D_{ij} \left(\frac{\partial c_j}{\partial r}\right)_t \qquad (i = 1, \cdots, q) \tag{29}$$

in which

$$s_i = \frac{1}{c_i} \sum_{k=1}^{q} L_{ik}(1 - \bar{v}_k \rho) \qquad (i = 1, \cdots, q) \tag{30}$$

$$D_{ij} = \sum_{k=1}^{q} L_{ik} \mu_{kj} \qquad (i, j = 1, \cdots, q) \tag{31}$$

It will be observed that s_i and D_{ij}, like the coefficients L_{ik}, are functions of the frame of reference and of temperature, pressure, and composition. In Lamm's flow equation (55) (equation 12), which is strictly applicable only to two-component systems, the total flow is a simple summation of the flows from sedimentation and from diffusion. This is also true of equation 29. Measurement of the diffusion coefficients D_{ij} has been reviewed by Gosting (36).

Equation 30 allows one to see what use can be made of the reciprocal relations in interpreting sedimentation measurements. For a system containing q solutes there are q sedimentation coefficients (the sedimentation coefficient of the solvent is given implicitly by equations 24 and 24a) and q^2 coefficients L_{ik}. Since there are only $(\frac{1}{2})(q)(q - 1)$ restrictions on the L_{ik} from the reciprocal relations (see equation 23a), the number of coefficients L_{ik} exceeds the number of sedimentation coefficients plus the number of restrictions from the reciprocal relations by $(\frac{1}{2})(q)(q - 1)$. Thus, except for the unique case $q = 1$, one can obtain from sedimentation coefficients alone only combinations of the coefficients L_{ik}.

On the other hand one can use studies of diffusion to find all q^2 coefficients L_{ik}, as Hooýman (44) has pointed out; it is necessary to determine the various μ_{kj} from equilibrium measurements. Theory and methods have been worked out for using the Gouy diffusiometer to study interacting flows in diffusion (23, 30, 36, 68) and the four diffusion coefficients of several three-component systems have been determined (21, 22, 23, 68).

3. Correlation of sedimentation and diffusion measurements; equations for molecular weights

Since the same coefficients L_{ik} enter into the expressions for the sedimentation and diffusion coefficients (equations 30 and 31), one can correlate measurements of sedimentation and diffusion not only for two-component but also for multicomponent systems. Consider first the standard case of a two-component system ($q = 1$). Equations 29, 30, and 31 become

$$J_1 = -D_{11} \left(\frac{\partial c_1}{\partial r}\right)_t + c_1 s_1 \omega^2 r \tag{32}$$

$$s_1 = L_{11}(1 - \bar{v}_1 \rho)/c_1 \tag{33}$$

$$D_{11} = L_{11} \frac{\partial \mu_1}{\partial c_1} \tag{34}$$

These equations assume a more familiar form when L_{11}/c_1 is written as M_1/f_1, f_1 being a translational frictional coefficient per mole, and $\partial\mu_1/\partial c_1$ is evaluated by differentiating equation 18.

$$\frac{\partial\mu_1}{\partial c_1} = \frac{RT}{c_1 M_1}\left(1 + c_1\frac{\partial \ln y_1}{\partial c_1}\right) \tag{35}$$

Then

$$s_1 = M_1(1 - \bar{v}_1\rho)/f_1 \tag{33a}$$

$$D_{11} = D = \frac{RT}{f_1}\left(1 + c_1\frac{\partial \ln y_1}{\partial c_1}\right) \tag{34a}$$

In equation 34a D_{11} is written simply as D, because it is the same for solute and solvent; the sedimentation coefficients of solute and solvent are different, but one can be found from the other by use of equations 24 and 24a.

Derivation of these equations by the methods of thermodynamics of irreversible processes answers certain questions which were left unanswered by derivation from kinetic theory. (*1*) Does the expression for the sedimentation coefficient contain $M\bar{v}$ or some other measure of the volume of the solute? (*2*) Should ρ, in equation 33a, be taken as the density of the solvent or as that of the solution? (*3*) Are the values of f obtained from s and D the same at all concentrations, or only at infinite dilution? These questions were asked, and left unanswered, at the conference on the ultracentrifuge in 1949, according to the report by Longsworth (62) on the proceedings of the conference. Indeed, it was thought probable that the answer to question 3 is negative (*cf.* Kegeles, Klainer, and Salem (51)).

An expression for M_1 is obtained by eliminating L_{11} between equations 33 and 34.

$$M_1 = \frac{RTs_1\left(1 + c_1\frac{\partial \ln y_1}{\partial c_1}\right)}{D(1 - \bar{v}_1\rho)} \tag{36}$$

In the limit of zero c_1 this confirms Svedberg's equation (88, 89), as noted by Hooýman, Holtan, Mazur, and de Groot (46) (see their equation 80).

$$\lim_{c_1\to 0} s_1 = M_1(1 - \bar{v}_1\rho)D/RT \tag{36a}$$

The same procedure can be used to find an expression for M_1 when three-component systems are studied. Studies of the sedimentation and diffusion of proteins must be made in systems of at least three components (protein, salt, water) in order to avoid charge effects. Since the expression for s_1 contains the two coefficients L_{11} and L_{12}

$$s_1 = [L_{11}(1 - \bar{v}_1\rho) + L_{12}(1 - \bar{v}_2\rho)]/c_1 \tag{37}$$

it is necessary to write expressions for only two of the four D_{ij}.

$$D_{11} = L_{11}\mu_{11} + L_{12}\mu_{21} \tag{38a}$$

$$D_{12} = L_{11}\mu_{12} + L_{12}\mu_{22} \tag{38b}$$

Then equations 38a and 38b can be solved to give L_{11} and L_{12} in terms of D_{11}, D_{12} and the four μ_{ij}. Substitution of the resulting expressions into equation 37 and evaluation of the μ_{ij} by differentiating equation 18 give an equation for M_1 (12) analogous to equation 36. In the limit of zero c_1 this becomes

$$\lim_{c_1 \to 0} s_1 = \frac{M_1(1 - \bar{v}_1\rho)D_{11}}{RT}\left\{1 + c_2\left[\frac{M_2(1 - \bar{v}_2\rho)}{M_1(1 - \bar{v}_1\rho)}\right] \times \left[\frac{\frac{1}{D_{11}}\left(\frac{\partial D_{12}}{\partial c_1}\right)_{T,P,c_2} - \left(\frac{\partial \ln y_1}{\partial c_2}\right)_{T,P,c_1}}{1 + c_2\left(\frac{\partial \ln y_2}{\partial c_2}\right)_{T,P,c_1}}\right]\right\} \tag{39}$$

The result bears a strong resemblance to Svedberg's equation (see equation 36a) and also to the equation for finding M_1 from sedimentation equilibrium studies of three-component systems (*cf.* Part I).

Since charge effects are responsible for studying proteins in systems of three components, one wants an expression like equation 39 which will apply to systems containing ionized solutes. For simplicity, consider a system containing a macromolecular ion P of fixed charge $+z$, a counter-ion X of charge -1, and another cation B of charge $+1$. Details of the derivation (to be published) cannot be given here. The neutral components are chosen as follows: 1 = PX_z, 2 = BX. In the limit of zero c_1 the result is

$$\lim_{c_1 \to 0} s_1 = \frac{M_1(1 - \bar{v}_1\rho)D_{11}}{RT}\left\{1 + \frac{c_2}{2}\left[\frac{M_2(1 - v_2\rho)}{M_1(1 - \bar{v}_1\rho)}\right] \times \left[\frac{\frac{1}{D_{11}}\left(\frac{\partial D_{12}}{\partial c_1}\right)_{T,P,c_2} - (z/c_2) - (z+1)\left(\frac{\partial \ln y_1}{\partial c_2}\right)_{T,P,c_1}}{1 + c_2\left(\frac{\partial \ln y_2}{\partial c_2}\right)_{T,P,c_1}}\right]\right\} \tag{40}$$

Thus the valence of the macromolecular ion enters as an unknown into the equation for the molecular weight in very much the same manner as it does in the expression for sedimentation equilibrium. This conclusion is in agreement with the earlier treatment of Tiselius (89, 93).

4. *Translational frictional coefficients*

The sedimentation and diffusion coefficients of a two-component system yield, when extrapolated to zero c_1, not only the molecular weight but also the translational frictional coefficient f. Several theories have been developed for randomly coiled molecules which relate f to an average configuration (20, 64, 79). Similar theories have been derived for macromolecules which can be represented by rigid ellipsoids of revolution; these relate f to the dimensions of the ellipsoid or to an "equivalent ellipsoid" (66, 69, 80, 82). The latter theories have been intended for use with proteins. However, proteins must in general be studied in three-component systems and the expressions for s_1, D_{11}, and D_{12} do not yield a single translational frictional coefficient. Instead the interactions of one solute with another solute and with the solvent must be expressed separately. Thus Lamm (57) has used four frictional coefficients to describe the flows of the solutes in diffusion of a three-component system.

This problem has been known to exist for several years but only recently have measurements been made which begin to show the magnitude of the effect. Recent studies by Dunlop (21, 22) on the diffusion of three-component systems show that although the cross-term diffusion coefficients D_{12} and D_{21} are small, they are not in general negligible in comparison with the main coefficients D_{11} and D_{22}. Electrophoretic studies by Longsworth (60) show that neutral molecules such as sucrose and urea have appreciable electrical mobilities in the presence of salts, again demonstrating a significant interaction of flows. Very accurate values of f are required in a theory such as that of Scheraga and Mandelkern (82) in order to obtain only a fair accuracy in the dimensions of the ellipsoid. It seems, therefore, that the theory for measuring and interpreting translational frictional coefficients must be extended to three-component systems in order to study proteins.

III. Measurement of Sedimentation Coefficients

In the last two decades there have been significant advances in the measurement of sedimentation coefficients, both in experiment and in the theory. Experiments of the 1930's and early 1940's often show a variability of 5 per cent in s. In 1948 Cecil and Ogston (19) showed that they could reproduce the sedimentation coefficient of β lactoglobulin within 0.5 per cent, and recently measurements on bovine albumin (11) and thyroglobulin (67) have been reported which show a precision of 0.2 per cent. Improvements in control and measurement of rotor speed and temperature account for this in part; better methods of computing sedimentation coefficients are in part responsible. An important factor in the precision achieved by Cecil and Ogston (19) was the use of an integrated equation relating boundary position to time, rather than the differential form used in the past. Later Alberty (4) showed the value of taking the dependence of s on c into account when integrating the equation for the boundary position.

Sedimenting boundaries are in general diffuse, and the position in the boundary

which is to be used for measuring s requires careful definition. By starting from the conservation of mass, Gutfreund and Ogston (39) showed how the sedimentation coefficients of small molecules could be computed from the changes with time of boundary curves which never form "peaks" in the schlieren photographs, and Goldberg (34) showed that the correct boundary position for freely sedimenting boundaries is given by the square root of a second moment of the boundary gradient curve.

Cells have been devised by Kegeles (49) and by Pickels, Harrington, and Schachman (75) which will form a boundary between two different solutions while the rotor is revolving, and these have made possible new types of measurements.

A. DEFINITION OF SEDIMENTATION COEFFICIENT AND OF BOUNDARY POSITION

In order to measure a sedimentation coefficient it is first necessary to define it in such a way that it can be determined from experimental quantities. A frequent definition of s is velocity per unit field strength, and sometimes s is defined as $M(1 - \bar{v}\rho)/f$. However, molecular velocities are not in general directly measurable, and the second definition suffers both from a lack of generality (see equation 30) and from being a theoretical rather than an experimental definition. With two provisions, one can give an experimental definition of s_i in terms of the *flow* J_i. The first provision is that there be a region of the cell in which all concentration gradients are zero and solute i is present. The second provision is that the curve of c_i vs. r be accepted as a measurable quantity. Then one can use equation 29 to define s_i by

$$s_i \equiv (J_i/c_i\,\omega^2\,r) \qquad \left(\frac{\partial c_j}{\partial r} = 0,\ j = 1, \cdots, q\right) \tag{41}$$

To show that J_i is a measurable quantity, write equation 6 with $r_1 = r_0$ (the position of the meniscus) and $r_2 = r_w$, the position at which one wishes to measure J_i.

$$(rJ_i)_{r_0} - (rJ_i)_{r_w} = \frac{\partial}{\partial t}\left(\int_{r_0}^{r_w} rc_i\,\mathrm{d}r\right) \tag{42}$$

There is no flow of any solute through either end of the cell, and consequently

$$(J_i)_{r_0} = 0 \qquad (i = 1, \cdots, q) \tag{43}$$

Therefore

$$(rJ_i)_{r_w} = -\frac{\partial}{\partial t}\left(\int_{r_0}^{r_w} rc_i\,\mathrm{d}r\right) \tag{42a}$$

Provided that one allows the use of numerical integration and differentiation to find the right-hand side, equation 42a shows that J_i is expressed in terms of measurable quantities.

In practice, the sedimentation coefficient of a solute usually is measured from the velocity of the boundary formed by this solute. Equations 41 and 42a

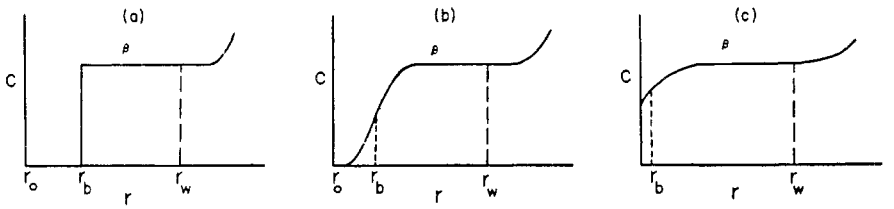

FIG. 2. Diagrams of concentration versus distance for certain types of boundaries: r_0 is the position of the meniscus, r_b is the boundary position, and r_w is in a region (β) where $\partial c/\partial r = 0$. (a) A hypothetical, perfectly sharp boundary (no diffusion). (b) A typical, freely sedimenting boundary ($c = 0$ at r_0). (c) The type of boundary formed by small molecules; c may remain non-zero at r_0 throughout the experiment.

provide the basis for this. Consider first the hypothetical case in which the boundary is perfectly sharp at all times. The boundary curve is sketched in figure 2a: r_b denotes the position of the boundary and c^β is the concentration of the solute beyond the boundary, between r_b and r_w. The integral in equation 42a is readily evaluated.

$$\int_{r_0}^{r_w} rc\, dr = (r_w^2 - r_b^2)c^\beta/2 \tag{44}$$

Before differentiating with respect to time, it is helpful to note that in a zero-gradient region equations 7 and 29 give

$$\frac{\partial c_i}{\partial t} = -2\omega^2 c_i s_i \qquad \left(\frac{\partial c_j}{\partial r} = 0,\ j = 1, \cdots, q\right) \tag{45}$$

Then substitution of equation 44 into equation 42a, followed by differentiation and use of equations 45 and 41, shows that the rate of movement of the boundary yields s^β.

$$s^\beta = \frac{1}{\omega^2 r_b}\frac{dr_b}{dt} \tag{46}$$

Equation 45 shows that c changes with time in the region ahead of the boundary. Combination of equations 45 and 46, followed by integration, yields a simple expression for c^β in which c° is the initial concentration of the solute.

$$c^\beta r_b^2 = c^\circ r_0^2 \tag{47}$$

This is the radial dilution rule of Trautman and Schumaker (96), which applies whether or not s varies with c.

In general the boundaries are not perfectly sharp. However, one can define the boundary position by equation 44, as Goldberg (34) pointed out, and use the resulting equation to measure r_b. A convenient expression for r_b in terms of c is obtained by subtracting equation 44 from $2c^\beta\int_{r_0}^{r_w} r\, dr$.

$$r_b^2 = r_0^2 + 2\int_r^{r_w} r(c^\beta - c)\, dr/c^\beta \tag{48}$$

With a schlieren optical system one wants an expression for r_b in terms of $\partial c/\partial r$. When the left-hand side of equation 44 is integrated by parts the result is

$$r_b^2 = \left\{(r^2c)_{r_0} + \int_{r_0}^{r_w} r^2 \frac{\partial c}{\partial r}\, dr\right\} \Big/ c^\beta \tag{49}$$

Equations 48 and 49 can be used to measure the sedimentation coefficients of small molecules (*cf.* figure 2c) as well as those which form freely sedimenting boundaries. When $c = 0$ at r_0 equation 49 gives the boundary position in terms of the square root of a second moment; this is the well-known result of Goldberg (34). When r_b is found by numerical integration other forms of equation 49 may be more convenient for computation; often it is helpful to replace r by $r_0 + (r - r_0)$. Then

$$r_b^2 = r_0^2 + 2r_0 \int_{r_0}^{r_w} (r - r_0) \frac{\partial c}{\partial r}\, dr/c^\beta + \int_{r_0}^{r_w} (r - r_0)^2 \frac{\partial c}{\partial r}\, dr/c^\beta \tag{50}$$

Once the boundary leaves the meniscus it is customary to equate r_b with r_H, the position of the maximum gradient, provided the boundary appears symmetrical. Equation 49 gives a basis for estimating the error in this procedure. In a symmetrical boundary r_H coincides with $\bar{r}$, the first moment.

$$\bar{r} = \int_{r_0}^{r_w} r \frac{\partial c}{\partial r}\, dr/c^\beta \tag{51}$$

The boundary position is related to $\bar{r}$ by equation 49, with $c = 0$ at r_0.

$$r_b^2 = \bar{r}^2 + \sigma^2 \tag{52}$$

$$\sigma^2 = \int_{r_0}^{r_w} (r - \bar{r})^2 \frac{\partial c}{\partial r}\, dr/c^\beta \tag{52a}$$

When equation 52 is differentiated with respect to time and divided by $2\omega^2 r_b^2$, the result is:

$$\frac{1}{\omega^2 r_b} \frac{dr_b}{dt} = \left(\frac{\bar{r}}{r_b}\right)^2 \left(\frac{1}{\omega^2\, \bar{r}} \frac{d\bar{r}}{dt}\right) + \left(\frac{1}{2\omega^2\, r_b^2} \frac{d\sigma^2}{dt}\right) \tag{53}$$

If one denotes by s' the apparent sedimentation coefficient which would be measured from $(d\bar{r}/dt)/\omega^2\bar{r}$, then equation 53 becomes

$$\frac{s'}{s} = 1 + \frac{\sigma^2}{r_b^2} - \left(\frac{1}{2\omega^2\, sr_b^2}\right) \frac{d\sigma^2}{dt} + \cdots \tag{54}$$

For the case of a single solute with constant s and D, σ^2 is given (9, 25) by

$$\sigma^2 = \frac{D}{s\omega^2} (e^{2s\omega^2 t} - 1) + \cdots \tag{55}$$

and equation 54 becomes

$$\frac{s'}{s} = 1 - (D/s\omega^2\, r_b^2) - \cdots \tag{56a}$$

or

$$\frac{s'}{s} = 1 - [RT/M(1 - \bar{v}\rho)\omega^2 r_b^2] - \cdots \tag{56b}$$

Equation 56a is the same expression found by Lamm (56, 89), who studied the position of the maximum gradient given by Faxén's equation for a single solute with constant s and D. Equation 54 is limited to symmetrical boundaries (if $\bar{r}$ is to equal r_H) but it is applicable to concentration-dependent and polydisperse systems, as well as to a single solute with constant s and D. The error term in equation 56b is roughly 0.1 per cent for a solute of molecular weight 60,000 and 1 per cent for one of molecular weight 6000 (for $\omega^2 = 4 \times 10^7$ sec.$^{-2}$, $(1 - \bar{v}\rho) = 0.25$).

B. RELATION OF BOUNDARY POSITION TO TIME

When the sedimentation coefficient can be treated as a constant, independent of r and t, and ω does not vary with t, then integration of equation 46 yields the simple result (89):

$$\ln (r_b/r_0) = s\omega^2 t \tag{57}$$

If ω and the rotor temperature vary with time, the result is

$$\ln (r_b/r_0) = s_{20} \int_0^t \omega^2(\eta_{20}/\eta)\, dt \tag{57a}$$

when the product $s\eta$ can be treated as a constant (*cf.* Cecil and Ogston (19)); η is the viscosity of the solvent. Sufficient control of ω and T has been achieved for many instruments so that one need take account only of the variation of ω during the period of acceleration. If ω is proportional to t during acceleration, then (94)

$$\int_0^t \omega^2\, dt = \omega_f^2 [(t - t_f) + t_f/3] \tag{57b}$$

where ω_f is the value at full speed and t_f is the time required for acceleration. In the following equations ω and T will be treated as constants, with the understanding that variations can be treated in the manner just discussed.

In general, sedimentation coefficients depend on concentration and c^β varies with time (see equations 45 and 47). Alberty (4) showed that one can express s^β as a power series in t and then find the coefficients of this series by integrating equation 45. For example, if s is related to c by

$$s = s_0(1 - k_1 c - k_2 c^2) \tag{58}$$

then the dependence of s^β on t is given by

$$s^\beta = s^\dagger\{1 + 2(s_0\omega^2 t)[k_1 c^\circ + 2k_2(c^\circ)^2] + \cdots\} \tag{59}$$

$$s^\dagger = s_0[1 - k_1 c^\circ - k_2(c^\circ)^2] \tag{59a}$$

where c° and $s^\dagger$ are the initial concentration and the sedimentation coefficient. The boundary position is found by substituting equation 59 into 46 and integrating.

$$\ln (r_b/r_0) = s^\dagger\omega^2 t + s^\dagger s_0(\omega^2 t)^2[k_1 c^\circ + 2k_2(c^\circ)^2] + \cdots \tag{60}$$

Several methods of computing sedimentation coefficients by this or an equivalent equation have been discussed (4, 11, 97). Equation 46 has been integrated in closed form for the two cases $s = s_0/(1 + kc)$ and $s = s_0(1 - kc)$ (97) and the relation between $\ln (r_H/r_0)$ and t has been given (28) for the case in which $s = s_0(1 - kc)$.

The problems introduced by a dependence of s on pressure do not fit readily into this scheme of analysis. In the first place the basic equation for flow relative to the cell (equation 29) does not apply if the partial volumes vary significantly with pressure. In the second place Eriksson (24) and Fujita (29) have shown that a consequence of the dependence of s on P is a continuous concentration gradient from the boundary to the bottom of the cell. In these circumstances equation 41 no longer provides an experimental definition of s and equation 44 no longer defines the position of the boundary. A useful approach to the problem is solution of the continuity equation for the case in which s is an empirical function of pressure (see Fujita (29) and Oth and Desreux (72)).

C. POLYDISPERSE SYSTEMS

When the system contains several solutes one can obtain a weight-average sedimentation coefficient, or more generally a "weight-refractive" average. It will be assumed throughout this part of the review that the refractive index n can be represented by

$$n = n_0 + \sum_{i=1}^{q} R_i c_i \tag{61}$$

for a system containing q solutes. In equation 61 n_0 is the refractive index of the solvent, at the corresponding temperature and pressure, and the differential refractive increments R_i are taken to be constants, independent of concentration and pressure.

$$R_i = \left(\frac{\partial n}{\partial c_i}\right)_{\substack{T,P,c_j \\ (j\neq 0)}} \tag{62}$$

It is convenient to introduce the symbol n_c for the refractive increment, the difference in refractive index between solution and solvent.

$$n_c = n - n_0 \tag{63}$$

$$n_c = \sum_{i=1}^{q} R_i c_i \tag{63a}$$

One can proceed in the same manner as for a single solute. Definition of the boundary position by

$$\int_{r_0}^{r_w} r n_c \, dr = (r_w^2 - r_b^2) n_c^{\bar{a}}/2 \tag{64}$$

leads to an expression for r_b analogous to equation 49.

$$r_b^2 = \left[(r^2 n_c)_{r_0} + \int_{r_0}^{r_b} r^2 \frac{\partial n_c}{\partial r} \, dr \right] \Big/ n_c^\beta \tag{65}$$

Differentiation of equation 64 with respect to time, followed by use of equations 41, 42a, and 45, gives the rate of movement of the boundary position.

$$\frac{1}{\omega^2 r_b} \frac{dr_b}{dt} = \sum_i R_i c_i^\beta s_i^\beta / n_c^\beta \tag{66}$$

In deriving equation 66 one obtains an expression for dn_c^β/dt from equation 45:

$$\frac{dn_c^\beta}{dt} = -2\omega^2 \sum_i R_i c_i^\beta s_i^\beta \tag{66a}$$

Combination of equations 66 and 66a, followed by integration, shows that the radial dilution rule (96) applies also to mixtures.

$$r_b^2 n_c^\beta = r_0^2 n_c^0 \tag{67}$$

The refractive increment of the initial solution is represented by n_c^0.

Before integrating equation 66 to give r_b as a function of t one must express the dependence of s_i on concentration. In general s_i is a function of all the solute concentrations and this function can be represented by a Taylor's series taken about $s_{i,0}$, the sedimentation coefficient of solute i at zero concentration of all solutes.

$$s_i = s_{i,0} - \sum_j a_j R_j c_j - \sum_j \sum_k a_{jk} R_j c_j R_k c_k - \cdots \tag{68}$$

The coefficients a_j and a_{jk} are related to partial derivatives taken at zero concentration of all solutes. Because the concentrations are measured refractometrically it is convenient to insert the differential refractive increments R_j. This means only that the derivative describing the dependence of s_i on c_j is written as the product of two coefficients.

$$\left(\frac{\partial s_i}{\partial c_j} \right)_{c_k (k \neq 0)} = -a_j R_j \tag{68a}$$

Equation 66 can be integrated when a_j and a_{jk} have the same values (a_1 and a_2) for all solutes.

$$s_i = s_{i,0} - a_1 n_c - a_2 n_c^2 \tag{68b}$$

The result is analogous to equation 60.

$$\ln (r_b/r_0) = \bar{s}^\dagger \omega^2 t + \bar{s}^\dagger (\omega^2 t)^2 [a_1 n_c^\circ + 2a_2 (n_c^\circ)^2 - (p^2/\bar{s}^\dagger)] + \cdots \tag{69}$$

In addition to the constants a_1 and a_2 the term in $(\omega^2 t)^2$ of equation 69 contains p, the standard deviation of the distribution of s_0. If one defines a fraction α, based on refractive increment,

$$\alpha_i = R_i c_i^\circ / n_c^\circ \tag{70}$$

then p and $\bar{s}_0$ are defined by

$$\bar{s}_0 = \sum_i \alpha_i s_{i,0} \tag{71}$$

$$p^2 = \sum_i \alpha_i (s_{i,0} - \bar{s}_0)^2 \tag{72}$$

The mean sedimentation coefficient $\bar{s}^\dagger$ which appears in equation 69 is related to $\bar{s}_0$ by

$$\bar{s}^\dagger = \bar{s}_0 - a_1 n^\circ - a_2 (n^\circ)^2 \tag{73}$$

Measurement of p is discussed briefly under "tests of homogeneity," and methods of computing both $\bar{s}^\dagger$ and p have been given (10).

Experimentally it is simpler to measure the position of the maximum gradient, r_H, than it is to find r_b from equation 65. Also r_H can usually be measured more precisely than r_b. However, with a polydisperse system one wishes to measure either a well-defined average sedimentation coefficient or some definite property of $g(s)$ (the distribution of s) such as the position of the median or of the maximum ordinate. To a first approximation r_H corresponds to the maximum ordinate of $g(s)$ (see equation 86b). However, there is an exact correspondence only at zero time and zero concentration. The variation with time may be seen readily from equation 86b, which relates $\partial c/\partial r$ to $g(s)$ for the case in which both diffusion and the dependence of s on c are negligible. This has been discussed by Kinell (52). The variation with concentration is the result of the Johnston–Ogston and boundary-sharpening effects (see the section on the measurement of $g(s)$). Thus the sedimentation coefficient measured for a polydisperse system from $\mathrm{d}r_H/\mathrm{d}t$ corresponds approximately to that of the maximum ordinate of $g(s)$ once the results are extrapolated to zero concentration, but the slope of this extrapolation has not been analyzed in terms of dependence of the sedimentation coefficients on concentration.

D. THEORY FOR BOUNDARY-FORMING CELLS

With the cells developed by Kegeles (49) and by Pickels, Harrington, and Schachman (75) one can form boundaries between any two solutions, provided only that the outer solution is more dense than the inner one. These cells have been used to measure the sedimentation coefficients of small molecules (81), to find the rate of movement of a boundary formed between two different concentrations of the same solute (42), to measure the refractive increment of a solution (53), to study associating systems (81), and to find the rate of movement of a boundary formed by one solute when a more rapidly sedimenting solute is present on both sides of the boundary (43, 81).

Consider first the equations for a boundary formed between two solutions of the same solute (figure 3). The boundary position is defined in the same manner as in equation 44.

$$2\int_{r_v}^{r_w} rc\,\mathrm{d}r = (r_w^2 - r_b^2)c^\gamma + (r_b^2 - r_v^2)c^\beta \tag{74}$$

Differentiation with respect to time, followed by use of equations 41, 42a, and 45, yields an expression for $\mathrm{d}r_b/\mathrm{d}t$.

$$\frac{1}{\omega^2 r_b}\frac{\mathrm{d}r_b}{\mathrm{d}t} = \frac{(c^\gamma s^\gamma - c^\beta s^\beta)}{(c^\gamma - c^\beta)} \tag{75}$$

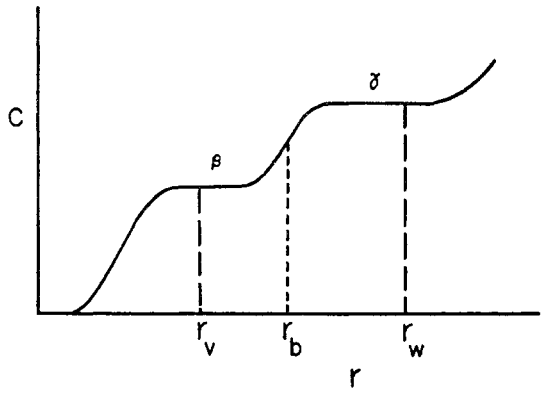

FIG. 3. Diagram of concentration versus distance after a boundary has been formed between two solutions (β and γ) containing different concentrations of the same solute, and sedimentation has proceeded for a time.

Thus the rate of movement of such a boundary does not give the sedimentation coefficient of the solute, as analogy with diffusion experiments might lead one to expect, but rather a function of s and c. Combination of equations 75 and 45, followed by integration, gives another instance of the radial dilution rule (96).

$$r_b^2(c^\gamma - c^\beta) = \text{constant} = [r_b^2(c^\gamma - c^\beta)]_{t=0} \tag{76}$$

Equation 75 can be integrated to give r_b as a function of time by the same procedures used when $c^\beta = 0$. Hersh and Schachman (42) have done this and have found good agreement between theory and experiment even for such a markedly concentration-dependent substance as deoxyribonucleic acid.

Use of the boundary-forming cell to measure sedimentation coefficients of small molecules is based on equations which have been discussed in Sections III,A and III,B. The advantage of the boundary-forming cell is convenience: the boundaries are of the type shown in figure 2b, while with a conventional cell they are of the type in figure 2c, and it is easier to measure r_H than to find r_b from equation 50 by numerical integration. However, the zero-gradient region disappears more quickly than when one uses a conventional cell. This results in less precision because the boundary has moved a smaller distance by the end of the experiment. Also equation 56b shows that the error in measuring s from r_H becomes serious for solutes of molecular weight less than 6000.

Measurement of the sedimentation coefficient of a slow component in the presence of a faster one is complicated by accumulation of the fast component in the boundary region. This effect, caused by a dependence of the sedimentation coefficient of the fast component on the concentration of the slow component, is likely to cause convection and spurious values for s (13). Hersh and Schachman (43) have obtained experimental evidence for this effect, and they suggest that it can be overcome by increasing the concentration of the fast component in the outer solution.

E. CONVERSION OF SEDIMENTATION COEFFICIENTS TO STANDARD CONDITIONS

In order to compare measurements it has been customary to refer values of s to a standard choice of temperature and solvent by the approximate relation:

$$s\eta/(1 - \bar{v}\rho) = \text{constant} \tag{77}$$

Equation 77 is based on the expression for s in two-component systems (equation 33a) with the assumption that f is proportional to the viscosity of the solvent, η. Such an assumption can be made only for rigid macromolecules and is known to be quite incorrect for synthetic high polymers. Proteins, on the other hand, are studied in systems of three components and equation 77 can only be an approximation for such systems. Studies by Longsworth (61) of the diffusion of bovine plasma albumin show a slight but significant dependence of (f/η) on temperature. Thus when measurements of s are referred to standard conditions by equation 77 it is important that the actual conditions of measurement be given, as well as the values of $\bar{v}$, η, and ρ used in the computation.

IV. Measurement of Heterogeneity

The most striking feature of sedimentation velocity experiments is their sensitivity to heterogeneity. This is a property shared by other moving boundary methods: for example, electrophoresis and chromatography by frontal analysis. A simple transformation of the boundary curves gives a complete description of a sample's heterogeneity with respect to s for the limiting case in which spreading of the boundaries by diffusion is negligible and the sedimentation coefficients can be treated as constants. This transformation yields the *distribution* of sedimentation coefficient; figure 4 shows diagrammatic distributions of s for the types of systems commonly encountered. The integral distribution, denoted here by $G(s)$, can be used to describe either discrete mixtures or samples with a continuous distribution of s. The differential distribution, $g(s)$, is used only for continuous distributions.

A. MEASUREMENT OF SEDIMENTATION COEFFICIENT DISTRIBUTIONS

The integral distribution gives the fraction of the sample having sedimentation coefficients less than or equal to a specified value of s. Fractions based on the refractive increment (see equation 70) will be used here. When the sample contains a discrete mixture of solutes (e.g., figure 4b), $G(s)$ is simply a summation.

$$G(s_j) = \sum_i \alpha_i \qquad (s_i \leq s_j) \tag{78a}$$

or

$$G(s_j) = (n_c'/n_c^\circ)_j \tag{78b}$$

where $(n_c'/n_c^\circ)_j$ is that fraction of the refractive increment of the original sample which includes solutes with $s \leq$ the specified value s_j. (The symbol n_c' will be used to denote a portion of the refractive increment of the original sample, while n_c refers to the total refractive increment at a position in the cell, r.) When the

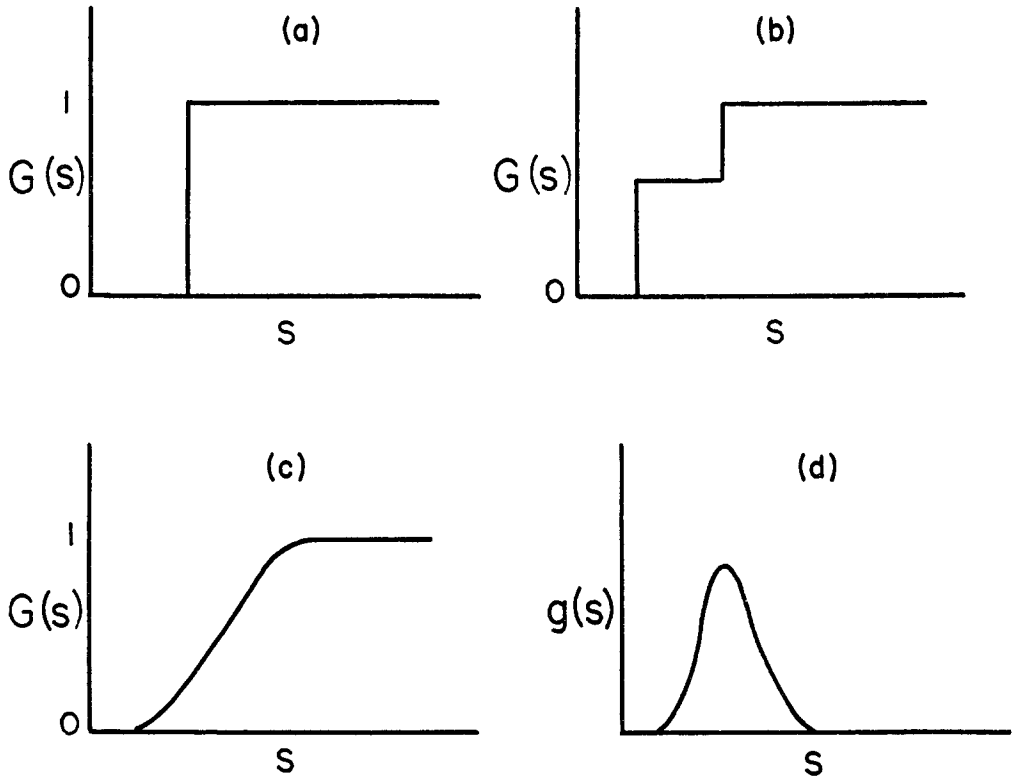

FIG. 4. Diagrams showing how the heterogeneity of a sample is described by its distribution of sedimentation coefficient: (a) one solute; (b) two solutes; (c, d) a continuous distribution of s.

sample contains a continuous distribution of s, $G(s)$ is given by the integral which corresponds to equation 78b.

$$G(s_j) = \int_0^{s_j} \frac{dn_c'}{ds} ds/n_c^\circ \tag{79}$$

The differential distribution is simply the derivative of $G(s)$ with respect to s.

$$g(s_j) = \left[\frac{dG(s)}{ds}\right]_{s=s_j} \tag{80a}$$

$$g(s_j) = \frac{1}{n_c^\circ}\left(\frac{dn_c'}{ds}\right)_{s=s_j} \tag{80b}$$

The sedimentation coefficients in equations 78–80 are those at zero concentration of all solutes; when the context might cause confusion on this point, the quantities in these equations will be denoted as s_0, $g(s_0)$, and $G(s_0)$.

Consider first the transformation which yields $G(s)$ or $g(s)$ in the limiting case when diffusion is negligible and the sedimentation coefficients can be treated

as constants. When the sample contains a discrete mixture of solutes each one will form a boundary at a position given by

$$s_i = [\ln (r_b/r_0)_i]/\omega^2 t \tag{81a}$$

or

$$(r_b)_i = r_0 e^{s_i \omega^2 t} \tag{81b}$$

The change in refractive increment $(\Delta n_c)_i$ across each boundary is simply the refractive increment $(R_i c_i)$ of the solute which forms this boundary, and is found by integrating equation 45 for s_i = constant.

$$(\Delta n_c)_i = R_i c_i^\circ e^{-2 s_i \omega^2 t} \tag{82}$$

Combination of equations 70, 81b, and 82 gives an experimental equation for α_i.

$$\alpha_i = (r_b^2 \Delta n_c)_i / r_0^2 n_c^\circ \tag{83}$$

This, together with equation 78a, gives $G(s)$.

$$G(s_j) = \sum_i (r_b^2 \Delta n_c)_i / r_0^2 n_c^\circ \qquad (s_i \leq s_j) \tag{84}$$

When there is a continuous distribution of s this becomes

$$G(s_j) = \int_{r_0}^{r_j} r^2 \frac{\partial n_c}{\partial r} \, dr / r_0^2 n_c^\circ \tag{85}$$

where r_j and s_j are related by equations 81a and 81b. Differentiation with respect to s gives

$$g(s_j) = \frac{\left[r^2 \left(\frac{\partial n_c}{\partial r} \right)_t \left(\frac{\partial r}{\partial s} \right)_t \right]_{r=r_j}}{r_0^2 n_c^\circ} \tag{86a}$$

or

$$g(s_j) = \omega^2 t \left(r^3 \frac{\partial n_c}{\partial r} \right)_{r=r_j} \Big/ r_0^2 n_c^\circ \tag{86b}$$

since equation 81b shows that

$$\left(\frac{\partial r}{\partial s} \right)_t = \omega^2 t r \tag{87}$$

These equations were understood in the earliest days of research with the ultracentrifuge (*cf.* Rinde (78, 90) and Signer and Gross (85)).

In order to use this approach it is necessary to take two factors into account. One is spreading of the boundaries by diffusion, and the other is sharpening of the boundaries by the dependence of s on c. (In some cases dependence of s on pressure is also a serious problem.) As a starting point, it is convenient to define experimental quantities which have the units of s, $g(s)$, and $G(s)$, and which

become identical with them once the effects of diffusion and concentration dependence have been removed.

$$\mathbb{s} \equiv [\ln (r/r_0)]/\omega^2 t \tag{88a}$$

$$g^*(\mathbb{s}) \equiv \frac{\omega^2 t r^3 \frac{\partial n_c}{\partial r}}{r_0^2 n_c^\circ} \tag{88b}$$

$$G^*(\mathbb{s}) \equiv \int_0^{\mathbb{s}} g^*(\mathbb{s})\, d\mathbb{s} \tag{88c}$$

When the effects of diffusion have been taken into account, but not those of concentration dependence, the last two quantities will be written $g(\mathbb{s})$ and $G(\mathbb{s})$, without the asterisk.

1. Effects of diffusion

In this section the effects of concentration dependence will be neglected and only those of diffusion discussed. From a study of the expression for σ^2, the second moment about the mean of the boundary gradient curve (*cf.* equation 52a), Baldwin and Williams (15) observed that extrapolation of $g^*(\mathbb{s})$ to infinite time should yield $g(s)$, when the sample contains a continuous distribution of s, because spreading of the boundary from diffusion is proportional to a lower power of t (roughly $t^{1/2}$) than that from heterogeneity (roughly proportional to t). They suggested extrapolating $g^*(\mathbb{s})$, at fixed values of $\mathbb{s}$, versus $(1/t)$ to $(1/t) = 0$. They also pointed out that p, the standard deviation of $g(s)$ (*cf.* equation 72), could be obtained from the variation of σ^2 with t.

Gosting (35) made a thorough mathematical study of the extrapolation and concluded that there is a range of time in which a linear extrapolation yields the desired result. However, this range of time is not always available to the experimentalist because the length of the cell limits the duration of the experiment, and there is also an experimental limitation on ω (at present corresponding to a rotor speed of 1000 R.P.S.). In order to see how well the extrapolation works when one cannot reach the linear region, an expression was obtained (9) for $g^*(\mathbb{s})$ when $g(s)$ can be represented by the normal error, or Gaussian, function.

$$g(s) = \frac{1}{p\sqrt{2\pi}} e^{-(s-\bar{s})^2/2p^2} \tag{89}$$

The resulting equation is simpler, and serves equally well to illustrate the nature of the problem, when $g^*(\mathbb{s})$ is obtained for the case of a rectangular cell and constant field, although an expression for the actual case of a sector cell and changing field has been given (9). For the former case

$$g^*(\mathbb{s}) = \frac{1}{p^*\sqrt{2\pi}} e^{-(\mathbb{s}-\bar{s})^2/2p^{*2}} \tag{90}$$

$$p^{*2} = p^2 + 2D/\omega^4 r_0^2 t \tag{90a}$$

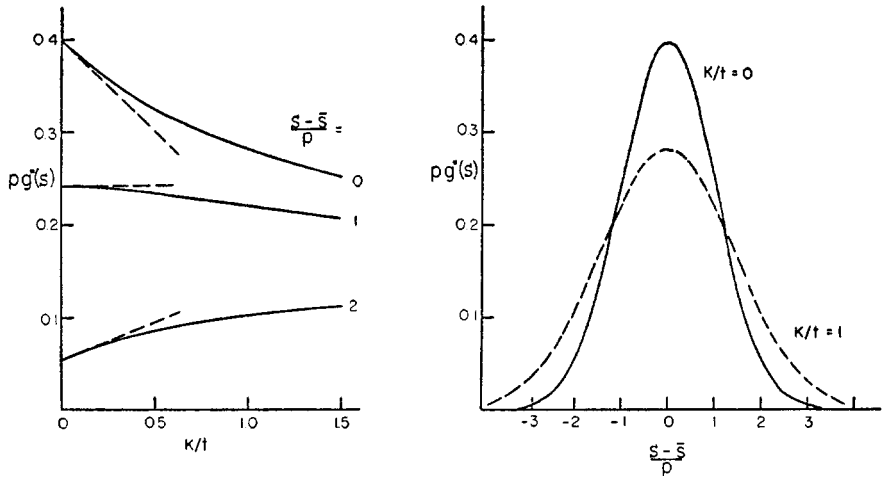

FIG. 5. Elimination of the effects of diffusion in finding distributions of sedimentation coefficient by an extrapolation to infinite time. The curves were computed by equation 90 for the case in which $g(s)$, the distribution of s, is Gaussian. The "apparent distribution of s" at any time is denoted by $g^*(\mathcal{S})$, where $\mathcal{S}$ is a reduced coördinate with the units of s (equation 88); p is the standard deviation, and $\bar{s}$ the mean, of $g(s)$.

where the (constant) centrifugal field strength has been represented by $\omega^2 r_0$ and, for simplicity, it has been assumed that all solutes have the same diffusion coefficient D. This equation, in a slightly different form, was derived by Alberty (2) in his studies on boundary spreading in electrophoresis and has been used (14) to study the feasibility of finding mobility distributions by extrapolation.

The extrapolations to infinite time are shown in figure 5a as a plot of $pg^*(\mathcal{S})$ vs. (K/t), where $K = 2D/p^2\omega^4 r_0^2$, and the apparent distributions are shown in figure 5b as $pg^*(\mathcal{S})$ vs. $(\mathcal{S} - \bar{s})/p$. In this way one can represent the behavior of all systems with a Gaussian $g(s)$, regardless of their values of p and D. The limiting slopes for the extrapolations, which coincide with the actual graphs in the linear region of the plot, are shown as dashed lines. These were found from Gosting's equation 19 (35), which applies regardless of the form of the distribution. One can see that there is only a very small region ($0 < K/t < 0.1$) in which the extrapolation is strictly linear but that there is a larger region ($0.1 < K/t < 0.8$) in which choosing the best straight line will yield values of $g(s)$ which are correct within present experimental error. In the linear region $g^*(\mathcal{S})$ should be extrapolated against a function of t (35) which can be approximated by $(1/rt)$. However, in this region $g^*(\mathcal{S})$ differs from $g(s)$ by little more than experimental error, and in the approximately linear region the simpler plot of $g^*(\mathcal{S})$ vs. $(1/t)$ serves equally well (9).

2. Effects of concentration dependence

In this section only the effects of concentration dependence will be considered, and those of diffusion will be treated as negligible. A rigorous treatment of the problem is not available, but one can achieve an understanding of its nature by

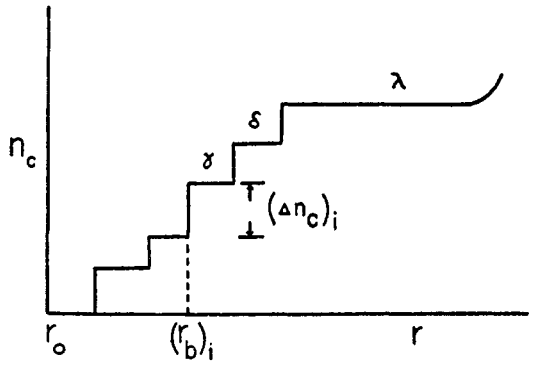

FIG. 6. Sketch of the boundary curve of n_c vs. r for a multicomponent system when diffusion is negligible.

considering simplified cases. The chief effect of concentration dependence is to cause a sharpening of the boundary when $G(s)$ is continuous, or to compress the system of boundaries if $G(s)$ represents a discrete mixture. In treating the latter case, which is sketched in figure 6, two simplifying assumptions will be made. First it is assumed that the boundary position $(r_b)_i$ is related to the sedimentation coefficient of solute i in the region (γ) just ahead of the boundary by

$$\ln (r_b/r_0)_i = s_i^\gamma \omega^2 t + \cdots \tag{91}$$

This neglects terms of order $(\omega^2 t)^2$ (see equation 60) and assumes that transport of solute i in the γ region occurs only by sedimentation. The second assumption is that α_i still is related to $(r_b^2 \Delta n_c)_i$ by equation 83. This neglects the Johnston–Ogston effect (47), which will be discussed shortly. Equations 84 and 85 follow from equation 83, but differentiation of equation 85 with respect to s_0 now yields

$$g(s_0) = \frac{r^2 \left(\frac{\partial n_c}{\partial r}\right)_t \left(\frac{\partial r}{\partial \mathcal{S}}\right)_t \left(\frac{\partial \mathcal{S}}{\partial s_0}\right)_t}{r_0^2 n_c^\circ} \tag{92a}$$

or

$$g(s_0) = g(\mathcal{S}) \Big/ \left(\frac{\partial s_0}{\partial \mathcal{S}}\right)_t \tag{92b}$$

since $(\partial s_0/\partial \mathcal{S})_t$ is the reciprocal of $(\partial \mathcal{S}/\partial s_0)_t$.

The derivative $(\partial s_0/\partial \mathcal{S})_t$ is given by the relation between $s_{i,0}$ and s_i^γ, since a comparison of equations 88a and 91 shows that $s_i^\gamma = \mathcal{S}$. Thus when s_i^γ is expressed as a function of all the solute concentrations in the γ region

$$s_i^\gamma = s_{i,0} - f(c_1^\gamma, c_2^\gamma, \cdots, c_i^\gamma) \tag{93}$$

then $(\partial s_0/\partial \mathcal{S})_t$ is given by

$$\left(\frac{\partial s_0}{\partial \mathcal{S}}\right)_t = 1 + \left(\frac{\partial f}{\partial \mathcal{S}}\right)_t \tag{94}$$

One can express f as a Taylor's series (equation 68), but in practice the relationship between $s_{i,0}$ and s_i^γ rarely is known accurately in a complex system such as this, and one must rely on approximations such as equation 68b, in which s_i is treated as a function of n_c only. Then

$$\left(\frac{\partial s_0}{\partial \mathcal{S}}\right)_t = 1 + a_1\left(\frac{\partial n_c}{\partial \mathcal{S}}\right)_t + 2a_2 n_c\left(\frac{\partial n_c}{\partial \mathcal{S}}\right)_t \tag{94a}$$

When $a_2 = 0$ this may be expressed as

$$\left(\frac{\partial s_0}{\partial \mathcal{S}}\right)_t = 1 + a_1 n_c^\circ g(\mathcal{S}) e^{-2\mathcal{S}\omega^2 t} \tag{94b}$$

with the aid of equations 88a and 88b. Jullander (48) introduced this approach to the problem of concentration dependence effects in finding $g(s_0)$; he made use of an empirical relation between s_0 and M to find $g(M)$.

The boundary-sharpening effect is shown in figure 7 by a family of curves, $g(\mathcal{S})$ vs. $\mathcal{S}$, corresponding to the same $g(s_0)$ but to different concentrations. These were computed from equations 92b and 94b for the case of a Gaussian $g(s_0)$; equation 68b was used to relate s_i to $s_{i,0}$ with $\bar{s}_0 = 4 \times 10^{-13}$, $a_1 = 0.4 \times 10^{-10}$, and $a_2 = 0$. The curves coincide for small values of $\mathcal{S}$ where the concentrations are low, but the maximum ordinate appears at lower values of $\mathcal{S}$ as the concentration is increased and the curves diverge for large $\mathcal{S}$. The behavior shown in figure 7 is typical of experimental data (for example, see figure 2 of Williams and Saunders (98)). Occasionally this has been interpreted to mean that solutes of smaller s show less dependence on concentration than those with larger values of s. The example given in figure 7, in which all s_i have been assigned the same dependence on concentration, shows that such an interpretation is not warranted.

A second effect of dependence on concentration is the Johnston–Ogston effect. Johnston and Ogston (47) found that c_i will differ on the two sides of a boundary if s_i differs. Their work explained why the apparent analysis for a system (by equation 83) gave results which varied with the total concentration. The derivation used in finding equation 75 is directly applicable to this problem if one assumes again that transport occurs only by sedimentation. Then equation 75 can be rearranged to give the difference in c_i between the regions δ and γ (figure 6), on either side of the boundary formed by solute j.

$$\Delta c_i = c_i^\gamma \Delta s_i / (s_j^\delta - s_i^\delta) \tag{95}$$

In equation 95 Δc_i and Δs_i are abbreviations for $(c_i^\delta - c_i^\gamma)$ and $(s_i^\delta - s_i^\gamma)$; equation 46 has been used to identify $(dr_b/dt)/\omega^2 r_b$ with s_j^δ. The equations derived by Johnston and Ogston (47) apply to a rectangular cell and constant field, but nevertheless their result agrees with equation 95. Some of the complications which result from use of a sector-shaped cell and changing field are discussed under "Analysis for Two Solutes," Section IV,C (page 773).

By means of equation 95 one can proceed from left to right through the system of boundaries sketched in figure 6, if the dependence of s_i on c_1, c_2, $\cdots$ is known (equation 68), and find the concentration of each solute in the λ region ahead of

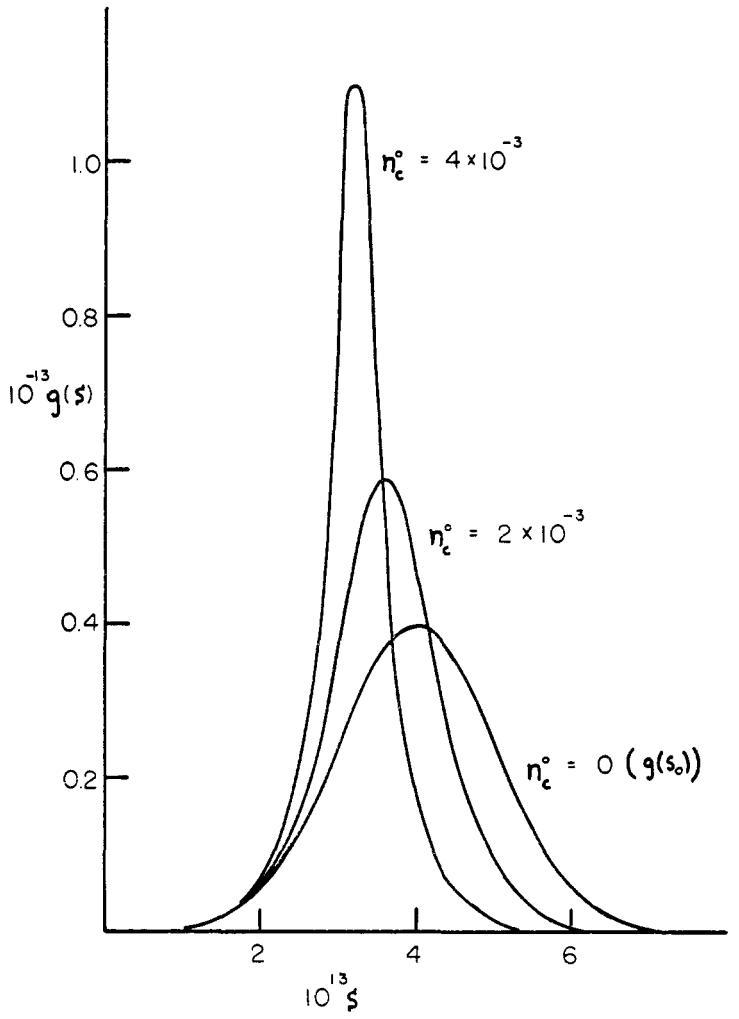

FIG. 7. Theoretical curves for the boundary-sharpening effect when there is a continuous distribution of sedimentation coefficient. The refractive increment n_c° is a measure of the total concentration; when $n_c^\circ = 0$ the curve of $g(\mathcal{S})$ vs. $\mathcal{S}$ yields the distribution of sedimentation coefficient, $g(s_0)$ vs. s_0. The curves were calculated for a Gaussian $g(s_0)$ by means of equations 92b, 94b, and 68b.

the boundary system. The concentrations in the λ region are related to those in the initial solution by an integrated form of equation 45. If the concentration dependence of s_i can be represented by equation 68b, then

$$\ln (c_i^\lambda/c_i^\circ) = -2\omega^2 t[s_{i,0} - a_1 n_c^\circ - a_2(n_c^\circ)^2] - 2(\omega^2 t)^2 \bar{s}^\dagger [a_1 n_c^\circ + 2a_2(n_c^\circ)^2] + \cdots \quad (96)$$

From c_i° one can find α_i and this, together with the computation of $s_{i,0}$ from s_i^λ, is a solution to the problem of finding $G(s_0)$ (8, 54). An approximate procedure has been given (8) for performing these computations when $g(s_0)$ is continuous.

and Larner, Ray, and Crandall (58) have programmed the calculations for a computing machine. For a continuous distribution, the shape of the boundary is much more dependent on the boundary-sharpening effect than on the Johnston–Ogston effect (see figure 5 of Baldwin (8)), although the latter influences the position of the boundary.

An alternative approach to the problem of concentration dependence effects is the extrapolation of curves of $g(\mathcal{S})$ vs. $\mathcal{S}$ to zero concentration (24, 98, 99). The theory has not been studied, although the results of extrapolation and of computation have been compared (8) and found to agree reasonably well. Methods which seem to work fairly well are the extrapolation of $\mathcal{S}$ *vs.* n_c° at fixed values of $G(\mathcal{S})$ (24) and extrapolation of $\mathcal{S}$ vs. n_c° at fixed values of $g(\mathcal{S})/g(\mathcal{S})_{max}$ (8). In order to succeed in finding $g(s_0)$, by either extrapolation or computation, one must be able to measure curves of $g(\mathcal{S})$ vs. $\mathcal{S}$ at concentrations where the sedimentation coefficients are within 10 per cent of their values at infinite dilution ($s/s_0 \geq 0.9$). Thus it has been possible to measure the heterogeneity of deoxyribonucleic acid (DNA) (83, 84) only by using a light-absorption optical system which allows measurements to be made on deoxyribonucleic acid at concentrations as low as 0.001 g./100 ml.

Effects of pressure dependence on finding $g(s_0)$ have been discussed recently by Eriksson (24), who made a very careful comparison of the size distributions found for polymethyl methacrylate samples by sedimentation analysis and by fractionation.

B. TESTS OF HOMOGENEITY

When a single, symmetrical boundary curve is observed the most useful test of homogeneity is Fujita's equation (28), which was obtained by solving the continuity equation for the case of a single solute whose sedimentation coefficient varies linearly with c. This equation is discussed in Part III of this review. To use it as a test of homogeneity one needs the diffusion coefficient D, the dependence of s on c, and the area and maximum height of the boundary gradient curve as a function of time. Methods of computing the results have been discussed (11) and applied to a study of the homogeneity of bovine plasma albumin. The boundary-sharpening effect is quite large even for a globular protein such as bovine albumin, which shows only a mild dependence of s on c (see figure 8).

A different approach is required to look for the presence of small amounts of other components. If one could neglect dependence on concentration, then Faxén's equation (25, 89) could be used as the basis for plotting ln $(\partial c/\partial r)$ vs. $(r - r_H)^2$; according to his equation this graph should be quite linear and its slope should be $s\omega^2/2D(e^{2s\omega^2 t} - 1)$. The presence of additional components is shown readily by a plot of this kind. However, because the boundary-sharpening effect is usually large (*cf.* figure 8), one should use Fujita's equation to study this problem. It predicts that the boundary curves will be fairly symmetrical, but the equation is rather complicated for use in comparing the shape of the boundary with theory.

An alternative approach to this problem is the use of moments of the boundary gradient curve. An equation can be written directly for the derivative with

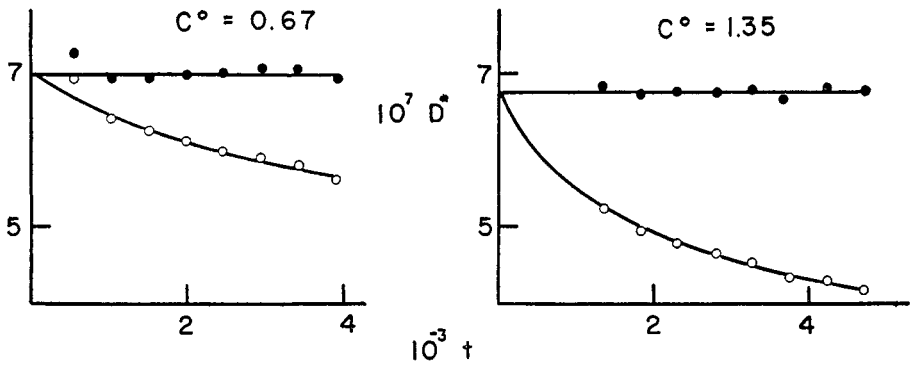

FIG. 8. The apparent diffusion coefficient versus time for bovine plasma albumin at two different concentrations (c° = 0.67 and 1.35 g./100 ml.). Filled circles show results calculated by Fujita's equation; open circles show results calculated by the older equation of Faxén, which does not take into account the dependence of s on c. These graphs show the behavior of the main component; a small amount of more rapidly sedimenting material was also present. Heterogeneity of the main component would result in an upward slope of the line through the filled circles. (Taken from Baldwin (11).)

respect to time of any moment and this equation can be integrated without knowing the form of the boundary curve, provided that the concentration is zero at r_0. This is a very general approach, since one can take account simultaneously of heterogeneity, diffusion, and dependence of sedimentation and diffusion coefficients on concentration, to the extent that s_i and D_i are expressed as functions of n_c. The resulting equation (equation 20 of Baldwin (10)) relates p, the standard deviation of the distribution of s_0, to σ^2 by measurable quantities. Since $p = 0$ if only a single solute is present, this is a useful test for the presence of additional components. Measurement of p from the variation of σ^2 with t also is useful in checking the accuracy of $g(s_0)$, since p can be computed directly from $g(s_0)$.

C. ANALYSIS FOR TWO SOLUTES

When effects of dependence on concentration can be neglected equation 83 gives the fractional amount of each solute. The dependence of s on c was taken into account in deriving equation 95; the only assumptions were that transport occurs solely by sedimentation in the regions on either side of the boundary and that the rate of movement of the boundary gives the sedimentation coefficient of the solute which disappears in the boundary. However, Harrington and Schachman (41) found that equation 76, which is a corollary of equations 75 and 95, is not obeyed by strongly concentration-dependent systems. A typical boundary curve is sketched in figure 9; both solutes 1 and 2 are present in the γ region, while only the more slowly sedimenting solute 1 is present in the β region. Harrington and Schachman concluded that transport by convection occurs in the β region as a result of solute 1 being left behind the $\beta\gamma$ boundary at a lower concentration than the existing c_1^β; the negative density gradient then results in convection.

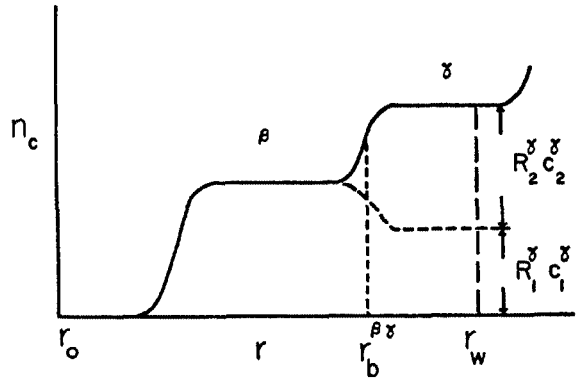

FIG. 9. A sketch showing the Johnston–Ogston effect in a system containing two solutes (1 and 2). The boundary curve of refractive increment (n_c) against distance (r) is shown.

A very interesting solution to the problem of finding α_1 and α_2 under these circumstances was worked out by Trautman, Schumaker, Harrington, and Schachman (97). Granted the existence of convection in the β region but not in the γ region, one can measure the following quantities, provided the concentrations are zero at r_0.

$$n_c^\gamma = \int_{r_0}^{r_w} \frac{\partial n_c}{\partial r}\, dr = R_1 c_1^\gamma + R_2 c_2^\gamma \tag{97}$$

$$n_c^\circ = \int_{r_0}^{r_w} r^2 \frac{\partial n_c}{\partial r}\, dr = R_1 c_1^\circ + R_2 c_2^\circ \tag{98}$$

(See equations 65 and 67 for the derivation of equation 98.) Since there is assumed to be no convection in the γ region, one can measure s_2^γ from the rate of movement of the $\beta\gamma$ boundary provided the boundary positions for solute 2 and solute 1 are coincident in the $\beta\gamma$ boundary. (As can be seen in figure 9, solute 1 contributes to $\partial n_c/\partial r$ in the $\beta\gamma$ boundary region and thus influences the position of $r_b^{\beta\gamma}$.) Then, according to equations 45 and 46,

$$\ln (c_2^\gamma/c_2^\circ) = -2\omega^2 \int_0^t s_2^\gamma\, dt = -2 \ln (r_b^{\beta\gamma}/r_0) \tag{99}$$

$$R_2 c_2^\circ = R_2 c_2^\gamma (r_b^{\beta\gamma}/r_0)^2 \tag{99a}$$

One more equation is needed in order to solve for $R_1c_1^\circ$ and $R_2c_2^\circ$, and so it was assumed (97) that

$$s_1^\gamma/s_2^\gamma = \kappa \tag{100}$$

where κ is a constant, and therefore given by $s_{1,0}/s_{2,0}$. Substitution of equation 100 into equation 45 gives

$$\ln (c_1^\gamma/c_1^\circ) = -2\omega^2\kappa \int_0^t s_2^\gamma\, dt \tag{101}$$

and combination with equation 99 yields

$$R_1c_1^\circ = R_1c_1^\gamma(r_b^{\beta\gamma}/r_0)^{2\kappa} \tag{101a}$$

One can solve equations 97, 98, 99a, and 101a for either $R_1c_1^\circ$ or $R_2c_2^\circ$; thus

$$R_1c_1^\circ = [n_c^\gamma(r_b^{\beta\gamma}/r_0)^2 - n_c^\circ]/[(r_b^{\beta\gamma}/r_0)^{2(1-\kappa)} - 1] \tag{102}$$

These equations were found (97) to give an accurate analysis of a highly concentration-dependent system.

V. Study of Interacting Systems

When studying a system in which A reacts with B to form AB, or A reacts with itself to form polymers, one would like to determine the composition of each complex and the equilibrium and rate constants for the reactions. Little work has been done in adapting the ultracentrifuge for any of these measurements, but there are clear indications that it will be a useful tool for studying reactions of macromolecules, and it is likely that developments will come rapidly in this field. The theory for chemically reacting systems is important also for the analysis of mixtures, since misinterpretations can result if one ignores the occurrence of chemical reactions. This point has been strikingly demonstrated by recent work of Gilbert (31), Gilbert and Jenkins (32) and Cann, Kirkwood, and Brown (18).

A. Constituent Sedimentation Coefficients

When chemical reactions occur one must reëxamine the basic theory for measuring concentrations and sedimentation coefficients. The conservation of mass applies to the sum of all forms in which a component exists, and one can write equations analogous to 6, 7, and 42a in which the concentrations and transport coefficients are replaced by *constituent* concentrations and *constituent* sedimentation and diffusion coefficients. Tiselius (89, 92) introduced this concept, and Alberty (3) has used constituent mobilities to study the electrophoresis of chemically reacting systems. His notation will be followed here in writing a bar above a constituent quantity. If constituent A exists in m forms, then

$$\bar{J}_A = \sum_{i=1}^{m} (J_A)_i = \bar{c}_A \bar{s}_A \omega^2 r - \bar{D}_A \left(\frac{\partial \bar{c}_A}{\partial r}\right)_t \tag{103}$$

$$\bar{c}_A = \sum_{i=1}^{m} (c_A)_i \tag{103a}$$

$$\bar{s}_A = \sum_{i=1}^{m} (c_A)_i (s_A)_i / \bar{c}_A \tag{103b}$$

$$\bar{D}_A = \sum_{i=1}^{m} \left(\frac{\partial c_A}{\partial r}\right)_i (D_A)_i \Big/ \frac{\partial \bar{c}_A}{\partial r} \tag{103c}$$

In these equations $(c_A)_i$ is the number of grams of *constituent* A in form i, per unit volume.

The statement of conservation of mass takes the following forms:

$$(r\bar{J}_A)_{r_1} - (r\bar{J}_A)_{r_2} = \frac{\partial}{\partial t}\left(\int_{r_1}^{r_2} r\bar{c}_A \, dr\right) \tag{104a}$$

$$\left(\frac{\partial \bar{c}_A}{\partial t}\right)_r = -\frac{1}{r}\left[\frac{\partial(r\bar{J}_A)}{\partial r}\right]_t \tag{104b}$$

The reader can verify that equation 104b is consistent with the continuity equation given earlier for each species of a chemically reacting system (equation 9). It is useful to have an equation relating boundary velocity to constituent concentrations and sedimentation coefficients, and the equation analogous to 75 will be given next. Suppose that constituent A is found in two adjacent regions (β and γ) on either side of a boundary, and that transport occurs only by sedimentation in these regions. Then the boundary position of constituent A is defined by

$$(r_b^2)_A^{\beta\gamma} = \int_{r_v}^{r_w} r^2 \frac{\partial \bar{c}_A}{\partial r} \, dr/(\bar{c}_A^{\gamma} - \bar{c}_A^{\beta}) \tag{105}$$

and it follows from equations 104a, 104b, and 103a that

$$\left(\frac{1}{\omega^2 r_b}\frac{dr_b}{dt}\right)_A^{\beta\gamma} = \frac{\bar{s}_A^{\gamma}\bar{c}_A^{\gamma} - \bar{s}_A^{\beta}\bar{c}_A^{\beta}}{\bar{c}_A^{\gamma} - \bar{c}_A^{\beta}} \tag{106}$$

Thus if constituent A disappears across the $\beta\gamma$ boundary, $\bar{s}_A^{\gamma}$ is given by the rate of movement of the boundary position defined by equation 105. Equation 106 shows the experimental significance of constituent sedimentation coefficients.

One can use values of $\bar{s}$ to study complex formation (*cf.* Alberty and Marvin (5)). Suppose that a macromolecule P forms a series of complexes with a smaller molecule A. One would like to measure y, the average number of molecules of A bound per molecule of P. The equilibrium constant for the formation of complex PA_i can be written as:

$$c_{PA_i} = K_i c_P c_A^i \tag{107}$$

If m is the maximum number of molecules of A bound, then the constituent sedimentation coefficients of P and A are

$$\bar{s}_P \bar{c}_P = \sum_{i=0}^{m}\left(\frac{M_P}{M_P + iM_A}\right) c_{PA_i} s_{PA_i} \tag{108}$$

$$\bar{s}_A \bar{c}_A = s_A c_A + \sum_{i=1}^{m}\left(\frac{iM_A}{M_P + iM_A}\right) c_{PA_i} s_{PA_i} \tag{109}$$

where $M_P c_{PA_i}/(M_P + iM_A)$ is the weight concentration of P in the form PA_i. The definition of y (*moles* of A bound per *mole* of P) gives

$$y = \sum_{i=0}^{m}\left(\frac{iM_P}{M_P + iM_A}\right) c_{PA_i}/\bar{c}_P \tag{110}$$

Substitution of equation 107 into 108, followed by differentiation with respect to c_A at constant $\bar{c}_P$ gives, after multiplying by c_A and making use of equations 109 and 107:

$$\bar{c}_P \frac{d(\bar{s}_P)}{d\ln c_A} = \frac{M_P}{M_A}(\bar{s}_A \bar{c}_A - s_A c_A) + \bar{s}_P \bar{c}_P \frac{d \ln c_P}{d \ln c_A} \tag{111}$$

Next differentiation with respect to c_A of the expression for $\bar{c}_P$ (again at constant $\bar{c}_P$)

$$\bar{c}_P = c_P \left[1 + \sum_{i=1}^{m} \left(\frac{M_P}{M_P + iM_A}\right) K_i c_A^i\right] \tag{112}$$

shows that (*cf.* Linderstrøm-Lang (59)):

$$\frac{d \ln c_P}{d \ln c_A} = -y \tag{113}$$

(It is assumed in equations 111 and 113 that the K_i can be treated as constants independent of c_A.) Combination of equations 111 and 113 gives an expression for y in terms of constituent sedimentation coefficients and concentrations.

$$y = \frac{\bar{c}_A M_P (\bar{s}_A - s_A)}{\bar{c}_P M_A (\bar{s}_P - s_A)} - \frac{1}{(\bar{s}_P - s_A)} \frac{d(\bar{s}_P)}{d \ln c_A} \tag{114}$$

where use has been made of the relation

$$\bar{c}_A - c_A = y\bar{c}_P M_A / M_P \tag{115}$$

One can find c_A, for use in the right-hand term of equation 114, by successive approximations.

Comparison of equation 114 with the equations derived by Alberty and Marvin (5) for electrophoresis shows that (*a*) for a Langmuir distribution of complexes with $(m/m - 1) \simeq 1$

$$\frac{d(\bar{s}_P)}{d \ln c_A} = \bar{s}_P - s_P \tag{116}$$

and (*b*) for the case in which only the single complex PA_i is formed

$$\frac{d(\bar{s}_P)}{d \ln c_A} = (i - y)(\bar{s}_P - s_P) \tag{117}$$

(In making this comparison the reader should note that Alberty and Marvin (5) use molar concentrations in their definitions of constituent concentrations and mobilities.) Richards and Schachman (77) have quoted an equation which includes m as a parameter and which implicitly relates y to the constituent concentrations and sedimentation coefficients. (Compare equations 19 and 21 of Alberty and Marvin (5).)

B. MONOMER–POLYMER EQUILIBRIA

Theoretical treatments of this case have been given by Steiner (87), Field and Ogston (26), and Gilbert (31). When the reactions are sufficiently rapid so

that the concentrations may be treated as if the system were at equilibrium, one can describe the experiment as if there were only a single solute with an unusual dependence of s and D on concentration. Thus if

$$mA \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A_m \tag{118}$$

and one can assume that the equilibrium relation between the concentrations is always a good approximation

$$c_A^m = Kc_{A_m} \tag{119}$$

then $\bar{s}_A$ and $\bar{D}_A$ are functions of $\bar{c}_A$. Steiner (87) has expressed $\bar{s}$ in terms of the sedimentation coefficients of the individual species and the equilibrium constants for the reactions.

Gilbert (31) has solved the continuity equation to give a description of the shape of the boundary curve for the case in which equilibrium is rapid, and equation 119 is therefore a good approximation. The concentrations of intermediate polymers, containing less than m monomers, are assumed to be negligible. He treats the case of sedimentation in a rectangular cell, with a constant field, when diffusion is negligible and s for each species is a constant. For $m \geq 3$, the concentration gradient curve shows two maxima in the boundary region. Since $\partial c/\partial r$ does not fall to zero between the two maxima, one has a simple experimental criterion for distinguishing this case from that of a mixture of two nonreacting solutes. However, it is often difficult to obtain complete resolution of the boundary curves, so that this distinction may not always be useful. Gilbert concluded that if one treats such a chemically reacting system as if it were a simple mixture, both the apparent sedimentation coefficient and the apparent fraction of the faster component would increase with the total concentration, while the apparent sedimentation coefficient of the slower component would not change. He illustrated the possible application of his theory to data of Massey, Harrington, and Hartley for the polymerization of a chymotrypsin (65). There are many protein systems known to undergo association-dissociation reactions: for example, insulin, hemoglobin, casein, and many proteolytic enzymes.

Since the case of rapid equilibrium between monomer and polymer is phenomenologically equivalent to the case of a single, concentration-dependent solute, one can use existing theories to study this problem. When the dependence of $\bar{s}$ on $\bar{c}$ is linear, and the variation of $\bar{D}$ with $\bar{c}$ can be neglected, Fujita's equation (28) describes the shape of the boundary. The result of an increase of $\bar{s}$ with $\bar{c}$ is a boundary-spreading effect and one can expect associating systems to show rapidly spreading boundaries. If the dependence of $\bar{s}$ on $\bar{c}$ is found to be linear, one can expect rather symmetrical boundaries (28). For nonassociating systems a rapidly spreading boundary generally means heterogeneity. Provided the equilibrium is rapid one can tell whether or not heterogeneity is present, in addition to association, by means of the equation for measuring p (10); the boundary spreading which results from association is taken care of by terms involving the dependence of $\bar{s}$ on $\bar{c}$. If the monomer is homogeneous p should be found to equal zero.

Schachman and Harrington (81) have suggested using the boundary-forming cell to see whether or not the equilibrium is rapid. When a boundary is formed between two solutions (β and γ) of an associating solute, $\bar{s}^{\gamma} > \bar{s}^{\beta}$ if γ is the outer solution. As the solute in the γ region sediments away from that in β region, convection should result if equilibrium is reached slowly.

C. COMPLEX FORMATION

Formation of a corticotropin-bovine albumin complex has been studied by Brown, Moyer, Davies, and Cox (17), who measured the constituent sedimentation coefficient of corticotropin in the presence of bovine albumin. The interpretation of their measurements is made somewhat uncertain, as they point out, by the possibility of convection resulting from accumulation of albumin in the corticotropin boundary (see Section III,D on page 762). Richards and Schachman (77) have recently demonstrated the possibility of measuring ($\bar{s}_P - s_P$) directly, by use of a double cell and a Rayleigh optical system (74).

Gilbert and Jenkins (32) have solved the continuity equations for the case in which A and B react to give AB and the concentrations are always related by the equilibrium constant. They consider the case of sedimentation in a rectangular cell and constant field, with negligible diffusion and constant sedimentation coefficients. In the numerical example with which they illustrate their equations, the boundary positions of the A and B constituents are not coincident in the $\beta\gamma$ boundary across which A disappears. The difference between $\bar{s}_A^{\gamma}$ and the velocity of the boundary, per unit field strength, is fairly small and dependent on M_B/M_A; when $M_B/M_A = 0$ the two are the same. This poses a problem in using equation 106 to measure $\bar{s}_A$, since usually one cannot measure the boundary position for constituent A alone (equation 105), but instead must use a curve of $\partial n_c/\partial r$ vs. r to find r_b.

Complex formation between P and A sometimes is studied by means of a partition cell and the results are interpreted by the equations used for dialysis equilibrium experiments. This is based on a simple physical picture: the macromolecule binds a certain number of molecules of A and carries these with it. To find this number (y) one uses equation 115 with $\bar{c}_A$ and $\bar{c}_P$ equal to the initial concentrations and c_A equal to the concentration of A remaining after the macromolecule has sedimented past the partition. Such a treatment assumes, among other things, that $s_A = 0$; it is useful only for studying the binding of quite small molecules by a macromolecule.

D. INTERCONVERSION OF ISOMERS

When there is an equilibrium between two isomeric forms of a solute whose sedimentation coefficients differ, two moving boundaries will be observed if the reaction is slow. The $N \rightleftharpoons \alpha$ reaction of thyroglobulin, studied by Lundgren and Williams (63), is believed to be such a case. The problem of how the half-time of the reaction determines the resolution of the boundaries has been solved recently by Cann, Kirkwood, and Brown (18) for the case of a rectangular cell and constant field. (They were interested in the application to electrophoresis experiments.) For the special case in which the rates of the forward and reverse

reactions are the same, their calculations show that two maxima will be resolved in the boundary gradient curve when the length of the sedimentation experiment is less than the half-time of the reactions. Although the apparent amounts of the two forms do not depend on the degree of resolution, the apparent sedimentation coefficients or mobilities do depend upon the half-time of the reaction and upon the time of sedimentation or electrophoresis.

When the rates of reaction are zero, the standard equations for analysis of two solutes apply. When the rates of reaction are infinitely fast, the system behaves as if it contained only one solute. To find out whether an experimental system falls into the intermediate class in which effects of finite rates of reaction are significant, one can perform experiments at different field strengths (i.e., at varing ω) and observe whether the apparent sedimentation coefficients vary with ω.

VI. Measurement of Molecular Weights During the Approach to Equilibrium

Archibald (6) observed that one can measure the molecular weight of a solute by use of the boundary condition of zero flow at either end of the cell. Thus for a two-component system equations 29 and 43 give

$$s_1 c_1 \omega^2 r = D \frac{\partial c_1}{\partial r} \qquad (r = r_0, r_n) \tag{120}$$

when the two ends of the cell are denoted by r_0 and r_n. An expression for M_1 is obtained by substituting equations 33 and 34 for s_1 and D; the result is of course limited to incompressible systems.

$$\left(\frac{1}{\omega^2 r c_1}\frac{\partial c_1}{\partial r}\right)_{r=r_0, r_n} = \frac{s_1}{D} = \frac{M_1(1 - \bar{v}_1 \rho)}{RT\left(1 + c_1 \dfrac{\partial \ln y_1}{\partial c_1}\right)} \tag{121}$$

At equilibrium equation 121 holds at every point in the cell; this is not surprising, since the flow is zero in both cases. However at equilibrium not only J but also $\partial J/\partial r = 0$, and one might inquire whether the equilibrium relationship between the concentration and concentration gradients is established at the cell boundaries for the more complicated case of a multicomponent system.

It can be shown in the following way that the equilibrium condition does hold at the cell boundaries during the approach to equilibrium. The equations for flow relative to the cell (equations 27) can be inverted by the use of determinants, yielding

$$\left(\frac{\partial \bar{\mu}_k}{\partial r}\right)_t = -\sum_{i=1}^{q} R_{ki} J_i \qquad (k = 1, \cdots, q) \tag{122}$$

where the R_{ki} are a new set of phenomenological coefficients. This form of the equations finds several uses in thermodynamics of irreversible processes (see Onsager (70, 71)). One can see from equation 122 that when all $J_i = 0$

$$\left(\frac{\partial \bar{\mu}_k}{\partial r}\right)_t = 0 \qquad (r = r_0, r_n, k = 1, \cdots, q) \tag{123}$$

This is the basic differential equation for sedimentation equilibrium and leads directly to the result that, for incompressible systems:

$$M_i(1 - \bar{v}_i\rho)\omega^2 r = \frac{RT}{c_i}\left(\frac{\partial c_i}{\partial r}\right)_t + RT\sum_{j=1}^{q}\left(\frac{\partial \ln y_i}{\partial c_j}\right)_{\substack{T,\ P,\ c_k \\ (k\neq 0)}}\left(\frac{\partial c_j}{\partial r}\right)_t \qquad (124)$$

The same conclusion was reached by Kegeles, Klainer, and Salem (51) on the basis of a slightly different derivation. They have shown also (51) that, if all solutes have the same $\bar{v}_i$ and R_i, one can obtain the weight-average molecular weight for a nonideal, polydisperse system. Only the limiting equation for infinite dilution of all solutes will be given here. Then, at zero time, equation 124 reduces to

$$\frac{\partial c_i}{\partial r} = \omega^2 r M_i(1 - \bar{v}_i\rho)c_i^\circ/RT \qquad (r = r_0, r_n, t = 0) \qquad (124a)$$

since $c = c^\circ$ everywhere in the cell at zero time. Multiplication by R_i, followed by summation over all solutes, gives (6):

$$\frac{1}{n_c^\circ}\frac{\partial n_c}{\partial r} = \frac{\omega^2 r}{RT}\sum_{i=1}^{q}\alpha_i M_i(1 - \bar{v}_i\rho) \qquad (r = r_0, r_n, t = 0) \qquad (125)$$

In using the Archibald method it is convenient to have an expression for c or n_c at r_0. Combination of equations 65 and 67 shows that

$$(n_c)_{r_0} = n_c^\circ = \int_{r_0}^{r_w}\left(\frac{r}{r_0}\right)^2\frac{\partial n_c}{\partial r}\,dr \qquad (126)$$

so long as there remains a zero-gradient region in the center of the cell (see 7, 51, 53). Trautman (94) has pointed out that one can use this equation to find M without knowing n_c°. When there is only one solute, substitution of equation 126 into 121 yields

$$\left(\frac{1}{r}\frac{\partial c_1}{\partial r}\right)_{r=r_0} = \frac{\omega^2 s_1}{D}\left\{c_1^\circ - \int_{r_0}^{r_w}\left(\frac{r}{r_0}\right)^2\frac{\partial c_1}{\partial r}\,dr\right\} \qquad (127)$$

A plot of $(\partial c_1/\partial r)/r$ at r_0 against the integral on the right gives a straight line with slope $\omega^2 s_1/D$, to the extent that (s_1/D) is independent of c.

Several studies of the Archibald method have been reported; for example, see Klainer and Kegeles (53), Ginsburg, Appel, and Schachman (33), and Smith, Wood, and Charlwood (86).

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