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Bioseparation by Zonal Centrifuges

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SYMPOSIUM ON NEW METHODS OF SEPARATION (continued)

Bioseparation by Zonal Centrifuges

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Abstract

A number of analyses relating to prediction of instantaneous particles position in a gradient solution, gradient capacity and stability in isopycnic banding, ideal number of septa in a zonal rotor, and an optimal density gradient solution for a velocity-sedimentation, etc. has been studied. The purpose of this review is to summarize the present status in analyses and operations of zonal centrifugation and to indicate the introduction of newer techniques which may open up a large number of separation methods by zonal centrifuges.

METHODS OF CENTRIFUGATION

Three basic techniques generally used in centrifugal separations are (*8*, *35*, *42*, *43*): (a) the sedimentation-velocity method, (b) the approach-to-equilibrium method, and (c) the equilibrium method. With the introduction of combinations of these methods in various ways, a large number of methods has been developed (*1–10*) and also a variety of terms has been used to describe these centrifuge techniques (*11*).

Each centrifugation method is briefly discussed in the following.

Sedimentation-Velocity Method

For this method, a centrifugal field of specified force is chosen for the system under investigation. Because the rotor is operated at high speeds, the solute particles are forced to settle at appreciable rates toward the wall of the rotor. Thus the sedimentation-velocity technique is basically a transport method; the parameter used to describe the transport of particles is called the "sedimentation coefficient." It is simply a measure of velocity of sedimentation particles per unit centrifugal force field and defined by Svedberg as (*12*, *13*),

$$s = \frac{(dr/dt)}{(\omega^2 r)} = \text{Svedberg units} \equiv \sec(\times\ 10^{-13}) \tag{1}$$

The rate of particle movement is a function of molecular weight as well as particle size and shape, the difference in density between the particles and the local sustaining liquid density, the local sustaining liquid viscosity, and the centrifugal force field strength. In order to make the characteristics unique, the observed sedimentation coefficient is customarily converted to the sedimentation medium of water at 20°C for the particle. The conversion formula is

$$s_{\mathrm{H_2O,20^\circ C}} = s_{\mathrm{obs}}\left(\frac{\mu_{G,T}}{\mu_{\mathrm{H_2O,20^\circ C}}}\right)\left(\frac{1 - \bar{v}\rho_{\mathrm{H_2O,20^\circ C}}}{1 - \bar{v}\rho_{G,T}}\right) \tag{2}$$

in which $\bar{v} = 1/\rho_p$ is the specific volume of a particle; $\mu_{G,T}$ is the viscosity of a sustaining solution at temperature T; and $\rho_{G,T}$ is the density of a sustaining solution at temperature T.

Sedimentation-Equilibrium Method (14)

Using the sedimentation-equilibrium method, the concentration distribution of particles at equilibrium positions (isopycnic banding) is usually studied. The banding solutes move outward from the center of the rotor with the velocity V_r until the solutes reach their respective locations of isodensity in the sustaining solution, then V_r vanishes and an equilibrium state is established. There are many observable properties which are characteristic of systems in a state of internal equilibrium. The effects of nonideality in any real system are so significant that the thermodynamic approach is usually used in its study.

The mathematical relationship describing sedimentation equilibrium can be obtained from equations which express the criteria of equilibrium in the system. Thus the general conditions for sedimentation equilibrium in a continuous system are:

(1) Thermal equilibrium

$$\text{Grad } T = 0 \tag{3a}$$

(2) Mechanical equilibrium

$$\text{Grad } P = 0 \tag{3b}$$

(3) Equilibrium distribution of concentration of various components

$$M_i(1 - \bar{v}_i^{(r)}\rho^{(r)})\omega^2 r = \sum_{k=1}^{N} \mu_{ik} \text{ Grad } C_k^{(r)}|_{T,P} \tag{3c}$$

where M_i and $\bar{v}_i$ are the molecular weight and partial specific volume of component i, ρ is the density of the system, ω is the angular velocity, r is the distance from the center of rotation, superscript (r) is for the values evaluated at this level, subscript T and P are for constant temperature and pressure, C_k is the molar volume concentration (component 0 is the principal solvent), $\mu_{ik} = (\partial\mu_i/\partial C_k)_{P,T,C_l}$ where μ_i is the chemical potential of component i and the subscript C_l signifies constancy of all molarities except that indicated in the differentiation, furthermore, $\mu_{ik} = \mu_{ki}$ due to the Maxwell relation.

It is important to note that the molecular weight of any component depends on all components in the system as expressed by the term μ_{ik}. In addition, the density ρ, which is introduced through the dependence of pressure, is clearly the density of the solution and is a function of position in the rotor. Most of the studies on the sedimentation-equilibrium method assume that $\bar{v}_i$ and ρ are independent of position in the rotor. Density changes both because of the variation in the concentration throughout the rotor and because of the increase in pressure with distance. But the dependence of the partial specific volume on concentration is often negligible (particularly for proteins). One major disadvantage in the equilibrium method is the time required to attain equilibrium. For example, in work with certain proteins of very high molecular weight, the time required to reach equilibrium might involve several weeks. And, during such long intervals, certain proteins and enzymes can be denatured.

Approach-to-Equilibrium Method (15)

The third centrifugal method, approach-to-equilibrium, preserves several advantages of the equilibrium method while eliminating the excess time consumption. This technique employs the principle that in a closed system the conditions for equilibrium are fulfilled at all times during the run at the meniscus and at the bottom of the rotor. Thus, if the intermediate stages of the sedimentation equilibrium run or the very late states of a velocity run are analyzed, molecular weight can be determined by evaluating concentration and the concentration gradient at the meniscus and the rotor bottom, although these measurements at the meniscus and the rotor bottom for a zonal centrifugation are not yet available.

When the banding solutes move outward from the axis with the velocity V_r, before solutes reach their respective zone of isodensity in the gradient, diffusion and sedimentation by mutual interference produces a pseudo-equilibrium state in a nonuniform system, where the diffusional fluxes of solutes vanish. In this pseudo-equilibrium state, there always exists a relationship between transport coefficients essentially derivable from classical thermodynamics.

The simultaneous occurrence of sedimentation and diffusion in any isothermal fluid mixture, the flux of component i, may be described as (*13*):

$$J_i = C_i s_i \omega^2 r - \sum_{k=1}^{N} D_{ik} \operatorname{Grad} C_k; \qquad i = 1, 2, 3, \ldots, N \tag{4}$$

The set of N independent equations is nothing more than a concise statement of the linear phenomenological laws for sedimentation and diffusion obtained from the irreversible thermodynamics which includes the definitions of the sedimentation coefficient s_i and the multicomponent diffusivity D_{ik}. After a certain period of time of centrifugation, concentration gradients have been built up, and diffusion processes occur which counteract the separation by sedimentation. All irreversible processes stop when, by counterbalance of the two types of transport phenomena, the flux of solutes vanishes everywhere. This is equivalent to the attainment of sedimentation pseudo-equilibrium. Thus one has

$$J_i = 0; \qquad i = 1, 2, 3, \ldots, N \tag{5a}$$

Therefore, the pseudo-equilibrium conditions may be written, according to Eqs. (4) and (5a),

$$C_i s_i \omega^2 r = \sum_{k=1}^{N} D_{ik} \operatorname{Grad} C_k; \qquad i = 1, 2, 3, \ldots, N \tag{5b}$$

On the other hand, thermodynamics for sedimentation equilibrium given in Eq. (3c) must also hold. One defines $|\mu_{ik}|$ as the determinant of all μ_{ik} and the following determinant,

$$\Gamma_k = \begin{vmatrix} \mu_{11} \cdots & \mu_{1\,k-1} & M_1(1-\bar{v}_1\rho) & \mu_{1\,k+1} & \mu_{1N} \\ \mu_{21} \cdots & \mu_{2\,k-1} & M_2(1-\bar{v}_2\rho) & \mu_{2\,k+1} & \mu_{2N} \\ \cdots & & & & \cdots \\ \cdots & & & & \cdots \\ \cdots & & & & \cdots \\ \cdots & & & & \cdots \\ \mu_{N1} \cdots & \mu_{N\,k-1} & M_N(1-\bar{v}_N\rho) & \mu_{N\,k+1} & \mu_{NN} \end{vmatrix} \tag{6}$$

and solves the set of Eqs. (3c) with respect to Grad C_k ($k = 1, 2, 3, \ldots, N$). The expressions thus obtained are to be inserted into Eq. (5b). One then obtains

$$s_i = (C_i|\mu_{ik}|)^{-1} \sum_{k=1}^{N} \Gamma_k D_{jk}, \qquad i = 1, 2, 3, \ldots, N \tag{7}$$

This is the general relationship between sedimentation coefficients s_i and multicomponent diffusion coefficients D_{ik} for any fluid mixture (since D_{ik} and s_i depend only on the local state variables, the conclusion, though derived from equilibrium conditions, remains valid for any state outside equilibrium).

Equation (7) shows that the sedimentation coefficient depends on the concentration and the diffusivity of the sedimenting materials. The degree of departure, however, varies markedly from one substance to another. This dependence, of itself, can provide some information about the physical properties of the macromolecules under investigation. Furthermore, the multicomponent diffusivity of the sedimenting materials, of itself, is highly concentration dependent. Because of this dependence of the sedimentation coefficient on concentration, three complications may arise:

(1) The sedimentation coefficient may not be constant throughout the experiment.

(2) The sedimentation coefficient may have to be determined at infinite dilution.

(3) The dependence of the sedimentation coefficient on concentration may cause distortion in the shape of the boundary.

These three phenomena must be taken into consideration if experimental data are to be interpreted with significance. The most serious consequence of the dependence of the sedimentation coefficient on concentration is its effect on the shape of the boundary. Diffusion will cause band broadening, which inevitably affects all systems. Another consequence from diffusion is that an initially stable band may rapidly develop instability at its lower boundary if a density inversion is created there by diffusion of a gradient material (*16*, *17*). When an equilibrium band is broad, the effective density gradient is more likely to depart from linearity. Consequently, the resolution on separation is greatly reduced by this effect, unless there is a measure to calibrate the exact density gradient. In order to facilitate the calibration, one has to know all the diffusivities of the sedimenting materials in the density gradient. Those diffusivities are highly concentration-dependent, and usually none of them is available in the literature.

ZONAL CONTRIFUGATION

A zonal centrifuge rotor is basically a cylindrical pressure vessel which spins about its own axis with a means of introducing and recovering a liquid density gradient and samples either dynamically (while rotating) or statically (at rest); the mechanical structure is such that measurements of quantities such as concentration gradient and sedimentation velocity bands, which are measured by an optical method in an ultra-analytical centrifuge run, are not easily made during zonal centrifuge runs. It is thus necessary to rely on a refined theory of mathematical modeling for prediction of such quantities.

Since the first zonal centrifuge was built by Anderson (*18*) in 1954, over 50 different zonal centrifuge designs have been evaluated. These have been grouped into a series of classes according to the range of speed, the material of construction, etc. A detailed summary of classification appears elsewhere (*5*).

Loading

Dynamic Loading

To load a zonal rotor during rotation requires fluid lines connecting to both the center and the edge of the rotor. Hydrostatic pressure is equalized by leading both lines back very close to the axis of rotation. When a single seal is used the two lines are coaxial, with the line connected to the rotor center in the center of the seal. The edge line therefore comes into the seal a few millimeters from the axis, thereby creating a small difference in hydrostatic pressure between the two lines during rotation. This difference is largely equalized by the presence of fluid in the edge lines, which is denser than that found in the center line—the density difference being that between the underlay or cushion under the gradient and the overlay solution over the sample.

When loading starts, the light-end of the gradient is introduced to the edge of the rotor during rotation. Centrifugal force at this state in the loading cycle makes the rotor act as a pump which draws the gradient in. Since most gradient engines pump at a constant rate, dissolved air in the gradient tends to form air bubbles in the edge line due to the negative pressure in this line. The air bubbles cause little difficulty until the rotor is nearly full. At that time the hydrostatic pressure due to the fluid in the main rotor chamber is only partially balanced by fluid in the edge line, resulting in a high back-pressure. To solve this problem, a gradient-producing device which forms the gradient as fast as it is drawn into the rotor is required. When the gradient is in the rotor, additional dense fluid, termed the underlay or "cushion," is added to the edge until the rotor is full. Flow through the rotor is now reversed and the sample layer is introduced through the center line, displacing an equal volume of the underlay.

In all dynamically loading rotors, tapered surfaces exist which serve to funnel particle zones into the center exit line during the unloading and also to minimize mixing of the sample with the gradient and the overlay during loading. To leave the sample in contact with these slanted surfaces would result in a starting sample of uneven thickness which is too close to the axis of rotation, i.e., in a low centrifugal field. Hence a solution having a density slightly less than that of the sample layer, termed the "overlay," is added to move the sample layer out into the rotor and free of the core surface. At this juncture, flow is reversed several times and small volumes of fluid are run alternately to the center and the edge to ensure that all entrapped air has been expelled. This illustrates an additional function of the overlay and the underlay, which is to provide the fluid volume necessary to allow the sample and gradient to be moved back and forth radially in the spinning rotor.

The rotor is now accelerated to operating speed. With some rotors the seals are left attached during the entire run. It should be noted that the centrifugal force field has a very considerable stabilizing effect on a liquid density gradient.

Static Loading

Static loading is generally done through a tube connected to the bottom of the centrifuge rotor. The overlay, sample, and gradient may be introduced in that order, or the sample and overlay may be carefully added from the top after the gradient is in position. An alternate method is to fill the rotor with dense underlay and then, by withdrawing part of the underlay from the bottom, draw in the gradient, sample layer, and overlay from the top. During acceleration and reorientation these isodensity levels become paraboloids of rotation described by the equation

$$Z = r^2\omega^2/2g + Z_0 \tag{8}$$

where r is the distance from the axis of rotation, Z is the vertical distance from the bottom of the rotor, and Z_0 is the minimum of the vertical distance of the paraboloid which is a function of the angular velocity ω (in radians per second) and can be above the bottom or below the bottom (*20*, *21*).

In an earlier work (*20*) we concluded that one has to bring the rotor as slowly as possible up to a pseudo-steady-state rpm, the minimum rpm at which the isodense paraboloid will stop changing its shape with an increase of rpm; then, after this speed is reached, the rate of acceleration of the rotor does not appreciably affect the shearing forces in the liquids. Because no further variation of interfacial area occurs, dispersion due to the shearing forces in each layer disappear. However, our theoretical and experimental studies (*22*) later indicated that the previous conclusion was incorrect. The dispersion of sample layers in centrifugation is independent of the rate of acceleration in the startup of a rotor. The dispersion of sample layers (the resolution) depends on the configuration of a rotor and loading levels. With a given rotor at a given loading level, a loss of resolution due to dispersion from the changes of interfacial area is a constant.

During the gradient reorientation, from rest to a stable orientation in a high centrifugal force field, the shearing forces occurring in a liquid confined in a closed cylinder will cause an increase in dispersion of a reorienting gradient system. The dispersion coefficient contributed from reorientation, Δ, may be written as

$$\Delta = \frac{dS}{dt}\left[\frac{\text{area}}{\text{time}}\right] \tag{9}$$

In a given time period, the total dispersion due to reorientation shearing forces by changing in isodensity interfacial area is

$$\sigma = \int_0^t \Delta\, dt = \int_0^t \frac{ds}{dt}\, dt = S(t) - S(0) \tag{10}$$

From Figs. 1 and 2 the paraboloid interfacial area as a function of speeds of revolution and the loading levels of liquid for K-III and J-I rotors, one finds that an isodensity interfacial areas approach a constant value

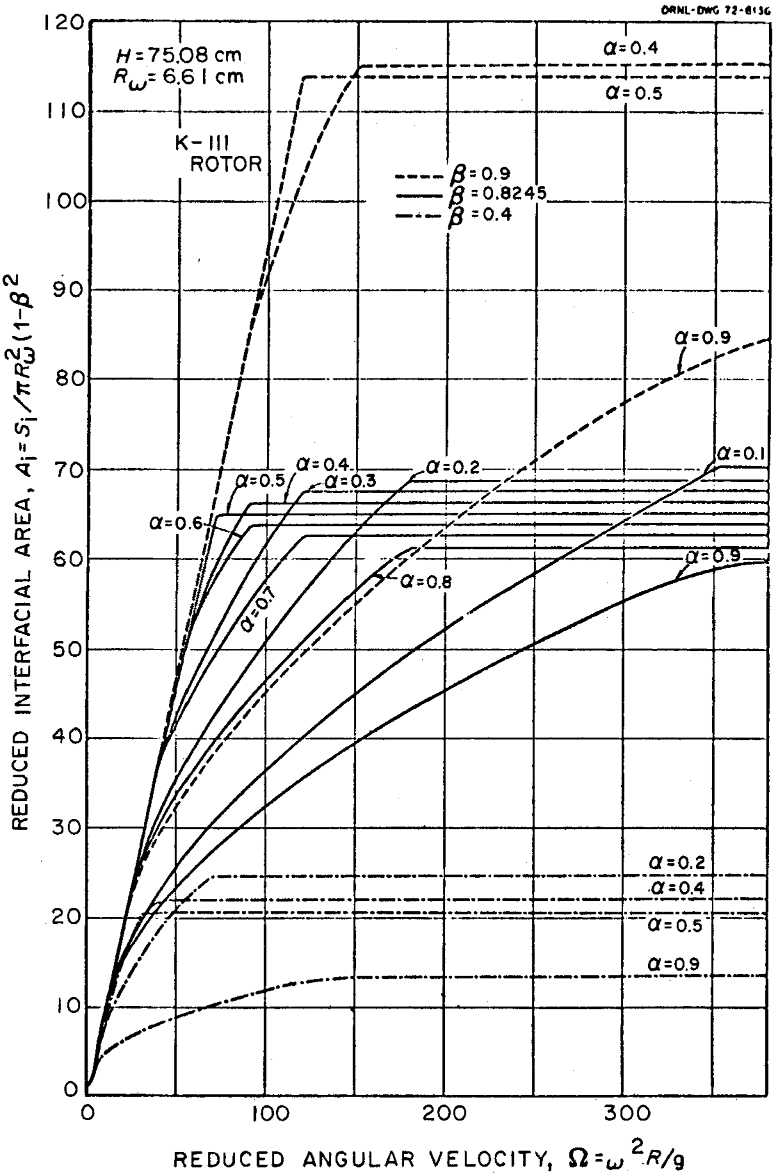

FIG. 1. Variation of reduced interfacial area with respect to speeds of revolution for a K–III rotor. α = reduced liquid loading level, β = ratio of core radius to rotor wall, H = height of rotor, and R_w = inside radius of rotor.

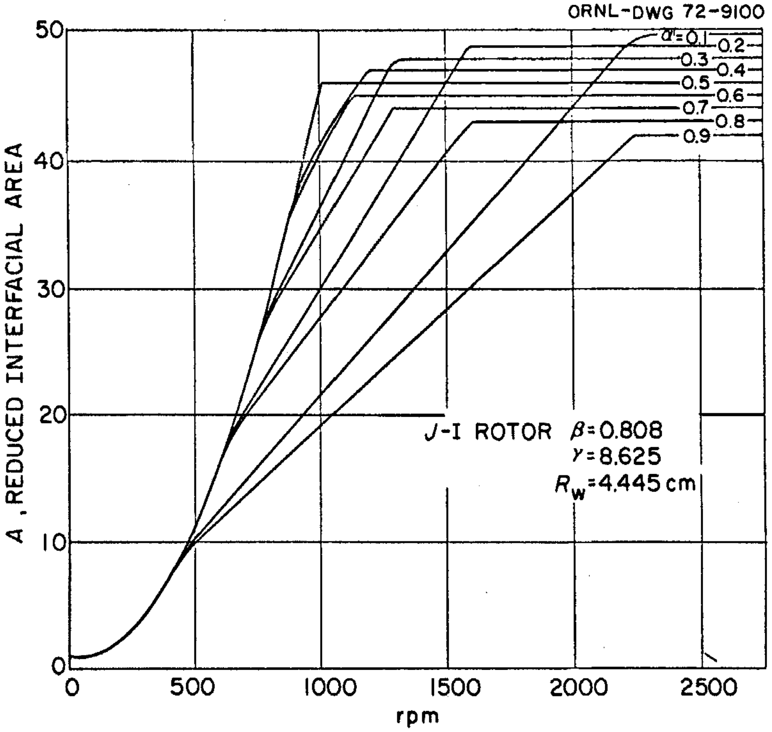

FIG. 2. Variation of reduced interfacial area with respect to speeds of revolution for a J–I rotor. γ = ratio of height to rotor wall.

(completely oriented) after a certain rotational speed for a given liquid loading level. A rotational speed, rpm $= 60\omega/2\pi$, is directly proportional to an angular velocity $\omega = \text{sec}^{-1}$. Thus Eq. (10) may be rewritten

$$\sigma = \int_0^t \frac{ds}{dt}\,dt = \int_0^{t_c} \frac{dS}{dt}\,dt + \int_{t_c}^t \frac{ds}{dt}\,dt = S(t_c) - S(0)$$

$$= \int_0^{\omega} \frac{ds}{d\left(\frac{1}{\omega}\right)}\,d\left(\frac{1}{\omega}\right) + \int_{\omega_c}^{\omega} \frac{ds}{d\left(\frac{1}{\omega}\right)}\,d\left(\frac{1}{\omega}\right) = S(\omega_c) - S(0) \qquad (11)$$

in which t_c is the time required to reach a completely reoriented paraboloid configuration, and ω_c is the angular velocity at which the paraboloid configuration is completely reoriented. From Eq. (11), one may see that $S(\omega_c)$ is a function of rotor configuration and liquid loading level only.

This finding is in agreement with our experimental investigation (*22*) on two-dimensional transient flow patterns and shear stress distributions, in which we have found that changing the rate of acceleration produces no noticeable effect in any flow patterns, shear stress distributions, or their magnitudes during transient periods for a given rotor.

From the foregoing analysis, we would like to make the following suggestions: One should accelerate a rotor quickly to reduce total operation time, so that a dispersion due to a molecular diffusion will be minimized. Control of rotor acceleration or deceleration is a very difficult task. Therefore, the control is unnecessary. Furthermore, at high speeds the fluctuation of a rotor speed is less than that at lower speeds and a smooth operation is easier to obtain.

We feel that the improvement of zonal centrifugation should be directed to an improvement in loading and unloading methods to reduce the dispersion from these operations. The next step will be to improve rotor configuration so that dispersion from shearing force by the reorienting gradient can be minimized.

To date the practical use of gradient reorientation has been chiefly continuous-sample-flow-with-banding centrifugation. However, it is a potentially useful method for large-scale, low-speed gradient centrifugation and also for centrifugation at very high speeds where continuously attached seals cannot be used. Use of detachable seals for high-speed rotors also presents problems because they are light in weight and have insufficient rotational momentum to allow seals to be attached without creating turbulence. As discussed below, the turnover effect can markedly decrease resolution in rotors loaded at rest. However, it does not affect gradients after a separation has been made. Simple methods for inserting the sample layer during rotation in rotors otherwise loaded and unloaded statically are required.

Gradient Runs

In order to design experimental runs, one needs to know the exact position of each particle as a function of time in a given gradient solution. A mathematical analysis for prediction of instantaneous positions and sedimentation coefficients of each particle was made by Hsu (*23*). His analysis is as follows:

The sedimentation of a particle which does not behave osmotically in a gradient density is a function of the following: (a) the amount and duration of the applied centrifugal force field, usually indicated by $\omega^2 t$; (b) the density and viscosity of the gradient solution medium; and (c) the size, shape, and density of the particles.

In expressing profiles of a gradient solution whose viscosity and density increase with radial distance from the axis by the polynomials

$$\mu = \mu_0(1 + \lambda_1'r + \lambda_2'r^2 + \lambda_3'r^3 + \cdots) \tag{12a}$$

$$\rho = \rho_0(1 + \varepsilon_1'r + \varepsilon_2'r^2 + \varepsilon_3'r^3 + \cdots) \tag{12b}$$

Hsu (*24*) has obtained mathematical expressions for (a) a particle position as a function of reduced rotation time, (b) an instantaneous particle sedimentation velocity in a given gradient solution, (c) an instantaneous observed particle sedimentation coefficient in a given gradient solution, and (d) the shear-stress exerted by a particle during zonal centrifugation runs in a given gradient solution.

In his derivation, μ_0 and ρ_0 are the light-end of viscosity and density, respectively. The coefficients λ_i' and ε_i' are characteristic constants for viscosity and density profiles in a rotor as a gradient solution. The reduced rotation time $A\tau$ is defined in terms of (a) radius of a rotor R, (b) angular velocity ω, (c) the light-end of viscosity and density μ_0 and ρ_0, (d) size of a particle dp, (e) density ratio between particle and the light-end of a gradient solution ρ_0/ρ_p, and (f) the centrifugation time t. Thus the reduced rotation time is given as

$$A\tau = \left[\frac{1}{Q} - 1\right]\frac{N}{18}\tau \tag{13}$$

in which

$$N = \left[\frac{\omega\rho_0 dpR}{\mu_0}\right]^2 \tag{13a}$$

$$\tau = \left[\frac{\mu_0 t}{\rho_0 R^2}\right] \tag{13b}$$

$$Q = \rho_0/\rho_p \tag{13c}$$

Quantitative evaluations of properties of the mathematical expressions derived were then made by using the following hypothetical gradient solution profiles:

$$\mu/\mu_0 = 1 + \lambda\zeta + \frac{\lambda^2}{2}\zeta^2 + \frac{\lambda^3}{6}\zeta^3 \tag{14a}$$

$$\rho/\rho_0 = 1 + \varepsilon\zeta + \frac{\varepsilon^2}{2}\zeta^2 + \frac{\lambda^3}{6}\zeta^3 \tag{14b}$$

where $\lambda_i = \lambda_i'/R$, $\varepsilon_i = \varepsilon_i'/R$, and $\zeta = r/R$. The characteristic constants for the gradient solution used were $\lambda = 0.5$, 1.0 (0.5)* 3.0, and $\varepsilon = 0.1$ (0.1)* 1.0. The numerical studies indicate the following conclusions.

(1) If the viscosity of a gradient solution decreases while the density of a gradient solution increases, a particle sediments much faster than in a medium of constant density and viscosity. Therefore, one may conclude that the viscosity of a gradient solution is the controlling factor in the decision of length of time required for a zonal centrifugation run for isopycnic banding and also for velocity sedimentation separation.

(2) The variation of the viscosity profile indicates that the steeper the profile, the longer is the time required for a particle to reach a given position.

(3) The centrifugation time for a particle to reach a certain position in a rotor is inversely proportional to the square of the particle diameter and to the square of the rotor speed.

(4) The particle diameter and the rotor speed are also inversely proportional to each other. If the diameter of a particle is one-tenth that of a reference particle, an increase in rotor speed to 10 times the original speed will result in the particle reaching the same position at the same time as the reference particle traveling at the original speed.

(5) The variation of the density profile also indicates that the steeper the profile, the longer is the time required for a particle to reach a given position.

(6) The variation in the density ratio between a particle and the light-end of a gradient solution shows the smaller the ratio, the shorter is the time for a particle to reach its isopycnic position.

The mathematical expressions were also tested from the zonal experimental data, numbers 813 and 859, at the Molecular Anatomy Program, Oak Ridge National Laboratory. The mathematical prediction of a particle's position in a rotor for a given rotation time agrees excellently with experiments for both runs. It is anticipated that these expressions will be eventually used in conducting a zonal centrifugation for design and predictions.

The Turnover Effect

When a particle-rich suspension is layered over a homogenous solution made with a freely diffusible solute, particulate material is observed to

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*The number in parentheses indicates the interval for the increment.

stream to the bottom of the tube at normal gravitation force field at a velocity much greater than that exhibited by individual particles. This has been termed the "turnover effect" (*1*, *8*). With very large particles, statistical fluctuations in the particle concentration may play a minor role in producing these phenomena; however, as proposed by Brakke (*24*), differences in the diffusivity of the gradient solute and the sample particles appear to be the major cause. The effect is not prevented when the two-solution system is centrifuged at low speed. If a density gradient is employed, a marked decrease in this form of convective transport is seen (*25*). Since the shields used in high-speed swinging-tube centrifugation are opaque, the magnitude of the effect on published rate studies where no internal particle standard is used is not known. The effect has recently been examined by Schumaker (*11*) with no clear-cut decision as to the role of diffusion vs local density fluctuations. It is unfortunate that simple diffusion theory cannot be applied to the convective disturbances occurring at the interface between sample and gradient. The suggestion of Svensson, Hagdahl, and Lerner (*26*) that the sample be applied as a double gradient, positive with respect to the gradient solute (increasing in concentration in a downward or centripetal direction) and negative with respect to the sample, solves this problem at the beginning. However, as shown by Berman (*27*), an initially stable zone may become unstable as it moves down the gradient, either because the gradient changes slope and therefore capacity, or because the sedimenting zone becomes thinner at some point in the gradient and overloads it. Clearly the turnover effect is a serious problem which must be solved if effective use is to be made of rate-zonal centrifugation for preparative purposes.

There is no convincing evidence that the effect is not entirely due to differences in the diffusion coefficients of gradient solute and sample. It appears to involve three phases: (a) Diffusion which increases the density of the boundary zone in the sample layer and decreases the density in the gradient solution beneath. This double effect produces an instability. (b) Fluid movement occurs when a sufficiently large volume of unstable fluid exists to be moved by gravitational force. To a first approximation the size of the moving droplets will be inversely proportional to the gravitational field. (c) As the droplets move they gradually disappear as their contents mix with the surrounding fluid. The rate at which this occurs is inversely proportional to some function of droplet size and the diffusivity of their contents. The net effect, given a sufficiently large centrifugal field, is to move the *minimum particle mass the minimum distance required to restore stability.* The turnover effect is observed in dynamically loaded and unloaded zonal centrifuges, and would have been readily seen with those rotors of the A-series which have transparent end-plates. Autocompensation of overloaded zones in zonal centrifuges due to movement of very small droplets a short distance is a key process and is the compelling reason for employing dynamic loading where high resolution and capacity are required. Autocompensation appears to occur at any level of the gradient where overloading may occur.

In addition to autocompensation, another process occurs concurrently in dynamically loaded rotors. Large sample zone particles which diffuse very slowly begin to sediment at a rate which may move them into the gradient faster than gradient solutes diffuse into the sample. Diffusion and sedimentation may thus partially compensate for each other.

A further stabilizing effect is provided by the heterogeneity of most biological suspensions. The narrow initial sample zone is rapidly spread out through the gradient, decreasing the sample load at the sample–gradient interface.

Gradient Capacity and Gradient Shape

As first pointed out by Svensson et al. (*26*), the theoretical capacity of a gradient is a function of its density slope. Adaptations of their gradient capacity equations (*8*, *24*, *26–28*) have been presented which suggest that the zones would be more Gaussian and less wedge-shaped. Experimentally, Brakke (*29*, *30*) found that only a few percent of the theoretical load could be supported in swinging-bucket rotors. However, in a dynamically loaded zonal rotor, Spragg and Rankin (*31*) were able to demonstrate 60 to 70% of the theoretical capacity using shallow gradients.

It now appears that the problem of gradient capacity resolves itself into a very simple question; namely, what is the minimum gradient required to maintain gradient stability in the rotor? Whether the gradient is formed from sucrose, virus particles, subcellular components, or a mixture of these is apparently unimportant. This question remains to be answered experimentally for each class of rotor and condition of operation. From the data of Spragg and Rankin (*31*), the minimum gradient appears to be about 0.001 g/cc/cm for the B-XIV rotor. The value probably depends on rotor volume and configuration, temperature and temperature control, gradient viscosity, number of septa and the clearance between septa and the rotor wall, and the amount and frequency of vibrations originating in the drive system.

As noted by Berman (*27*), gradients can be constructed in which initially stable particle bands become unstable during sedimentation and broaden by autocompensation. The shape of gradients used for particle separation therefore deserves comment. A clear distinction should be made between the gradient as produced by the gradient engine (plotted as concentration vs volume) and the shape of the gradient as it exists in a spinning rotor (plotted as concentration vs radius). A gradient linear with respect to volume is concave when plotted as concentration vs radius because of the sector-shape of the zonal rotor compartments.

For the following reasons a gradient convex with respect to radius is desired: (a) greatest particle-supporting capacity is needed in the region of the sample; (b) as the difference between particle density and gradient density diminishes along the gradient, less gradient slope is required to support a given mass of particles; and (c) radial dilution in sector-shaped compartments decreases the particle concentration in a given zone, again requiring a gradient decreasing in slope with increasing radius.

Stability Analysis of Particle Sedimentation in Gradient Solutions

In studying the turnover effect, Sartory (*16*) has used a small perturbation analysis to determine whether the disturbance grows or decays in time for two layers of stationary diffusing solute in a common solvent in a gravitational field. His theory predicts that instability occurs under the much wider range of conditions $\bar{\rho}_2{}^0/\bar{\rho}_1{}^0 > (D_2/D_1)^{3/2}$ for sufficiently long times for thick upper layers, and under the conditions $\bar{\rho}_2{}^0/\bar{\rho}_1{}^0 > (D_2/D_1)^{5/2}$ for sufficiently long times for very thin upper layers. The quantities $\bar{\rho}_2{}^0$ and $\bar{\rho}_1{}^0$ are initial macromolecular (particle) density and initial gradient salt density, respectively. D_2 and D_1 are diffusivities of macromolecule in a gradient medium and gradient salt in the medium.

Recently, experiments have been conducted by Halsall and Schumaker (*32*) to determine the onset of turnover effect in diffusion experiments in zonal ultracentrifuge. They have found that the stability criteria for their experiment is $\bar{\rho}_2{}^0/\bar{\rho}_1{}^0 < (D_2/D_1)^{1.010}$. They concluded that their discrepancy from Sartory's prediction may be due to inhomogeneity or association–dissociation of the sample under study. Recently Meuwissen and Heirwegh (*33*) have shown experimentally that the stability of zones in liquid density gradient under a normal gravitational field depends on the strength of the supporting gradient.

Hsu (*34*) has made an analysis for stability of isopycnic banding in zonal centrifugation recently. Due to the isopycnic condition, the analysis can be made by the lumped parameter method and an analytical solution can be obtained. The result is summarized in the following.

A small pertubation analysis has been applied to the generalized Lamm sedimentation equation to determine whether the disturbance grows or decays in time for two stationary diffusing solutes [a gradient solute (1) and a macromolecule (2)] in a solvent (0) in a given centrifugal force field. The criterion for the stability has been obtained in terms of normal modes, so that the pertubations decay with time. It is found that the stability criterion is given by

$$\alpha r \leq \left\{1 - \frac{(D_{11} + D_{22})^2}{D_{11}D_{22} - D_{12}D_{21}}\left[\frac{D_{11} + D_{12}}{(D_{11} + D_{22})^2}\frac{\omega^2 d_2{}^2 r^2 \bar{\rho}_2}{18\eta}\left(3 - \frac{d \ln \eta}{d \ln r}\right)\right.\right.$$
$$\left.\left. + \frac{1}{4}\left(1 - \frac{1}{D_{11} + D_{22}}\frac{\omega^2 d_2{}^2 r^2 \bar{\rho}_2}{18\eta}\right)\right]\right\}^{\frac{1}{2}} \tag{15}$$

It is implied that the stability of an isopycnic banding is determined by the band width (αr), with the maximum band capacity as the right-hand of the equation.

The stability criterion given in the equation presents the unifying theory. The theory obtained from the previous observations during centrifugation, such as density inversion theory, and the theory from the infinitesimal pertubation analysis of diffusional phenomena under one gravitational field are inclusively represented.

It is interesting to note that Meuwissen and Heirwegh's conclusion (*33*), that stability depends on the shape or strength of the supporting gradient, is also represented by the terms of $[3 - (d \ln \eta)/(d \ln r)]$ and $\omega^2 d^2 r^2 \bar{\rho}_2/18\eta$ in Eq. (15). If the gradient increases with the cubic of the radius, the term $[3 - (d \ln \eta)/(d \ln r)]$ drops out. The shape of the gradient is not a factor for the stability. If the slope of a gradient viscosity is $(d \ln \eta)/(d \ln r) < 3$, the contribution of that term to the stability is negative, thus the maximum load capacity is reduced. If $(d \ln \eta)/(d \ln r) > 3$, the steeper the gradient solution the more the stability of a system. In this case the band width increases with the slope of a gradient solution, thus the range of the stable region increases. The same conclusion can also be drawn for the density inversion theory. If the shape of a gradient is $(d \ln \eta)/(d \ln r) < 3$, the second term on the right-hand side of Eq. (15) is positive. Therefore, the following situation has occurred in the gradient, $\bar{\rho}_2 < \rho_{\text{grad}}[3 - (d \ln \eta)/(d \ln r)]$, since at an isopycnic point $\bar{\rho}_2 = \bar{\rho}_{\text{grad}}$. Thus density inversion does take place in order to return to the physical stable situation. If

$(d \ln \eta)/(d \ln r) > 3$, i.e., $\bar{\rho}_2 > \bar{\rho}_{\text{grad}}[3 - (d \ln \eta)/(d \ln r)]$, the system remains unchanged with the region. If a system is under one gravitational field, i.e., the terms containing $\omega^2 d_2{}^2 r^2 \bar{\rho}_2/18\eta$ drop out, the stability criterion becomes

$$\alpha r \leq \left[1 - \frac{(D_{11} + D_{22})^2}{4(D_{11}D_{22} - D_{12}D_{21})}\right]^{1/2} \tag{16}$$

The criterion is similar to that obtained by Sartory (*16*) except for the powers on the diffusivities. The four diffusivities for three-component systems can be obtained by Fujita and Gosting's procedure (*38*).

An additional characteristic in addition to previous theories, which appears in Eq. (15), is that one would like to increase the right-hand side of Eq. (15) so that the stability range can be increased. In order to increase the right-hand side of Eq. (15) for a given system, one will set the term

$$\frac{\omega^2 d_2{}^2 r^2 \bar{\rho}_2}{(D_{11} + D_{22})\eta} < 1$$

After rearranging, this becomes

$$\omega < \frac{1}{d_2 r}\left[\frac{(D_{11} + D_{22})\eta}{\bar{\rho}_2}\right]^{1/2} \tag{17}$$

in which r is the position where ideal isopycnic banding takes place. Equation (17) shows that the increasing of angular velocity in a zonal run does not improve the resolution. The angular velocity has to be constrained by Eq. (17). The physical interpretation of this phenomena is that with too high an angular velocity, the banding solute penetrates too deeply into a gradient solution, therefore an instability due to a density inversion or lack of strength in a supporting gradient will take place. Therefore, the angular velocity also has to be constrained.

The stability criterion is such a complicated phenomena that one should consider all the means to increase the right-hand side of Eq. (15). Thus Eq. (15) shows the unification of all previous theories.

Dispersion Coefficient of Sample in Gradient Solutions

The use of zonal centrifuges has two primary objectives: (a) separation and purification of biological materials from sample mixtures, and (b) concentration of separated biological material from a dilute solution. These two objectives are related. An optimum separation and purification is achieved by having a high resolution in each separated zone or band, and concentration increases as density of separated biological material in a band increases, likewise to achieve a high resolution in a separated zone. A band broadening effect in zonal centrifugation is due to a complicated phenomena of interaction of diffusion and sedimentation. In our investigation (*36*) we lumped all the contributing factors together and called it dispersion coefficients. In the determination of parameters which affect dispersion between sedimenting macromolecules and the sustaining gradient solution, dispersion coefficients of two sizes of polystyrene latex beads (diameters 0.91 ± 0.0058 and 0.312 ± 0.0022 μ) and bovine serum albumin (BSA) in sucrose and Ficoll (polysucrose by Pharmacia Fine Chemicals, Sweden), 9.5 to 10% w/v step-gradient and 10 to 25% w/v linear-with-volume gradient solutions, have been measured in an Oak Ridge B-XV zonal centrifuge rotor at 20°C. Dispersion coefficients were obtained from experimental data on intensity of UV absorbance of macromolecule vs volume fractions using the moment analysis technique.

In order to make the experimental results general and more useful, dispersion coefficients were correlated in a dimensionless number, the Schmidt number ($\mathrm{Sc} = \mu_0/\rho_0 D$) as a function of various dimensionless parameters. Using results from step-gradient solution runs by a trial-and-error method, it is found that the following formula

$$\mathrm{Sc} = 105[30F^{1.03}(\mathrm{Ta}\cdot Q)^{-1} + 19Se^{0.19}\tau]^{1.02} \tag{18}$$

fits all the data points within an average deviation of 2.5% and a maximum deviation of 6.3%. Various quantities in Eq. (18) are

$\mathrm{Sc} = \mu_0/\rho_0 D$	Schmidt number
$F = \dfrac{\omega^2 t d_p{}^2 \rho_0}{\mu_0}$	reduced centrifugal force field strength
$\mathrm{Ta} = \dfrac{\omega d_p R \rho_0}{\mu_0}$	Taylor number
$Q = \rho_0/\rho_p$	density ratio
$Se = \dfrac{s\omega^2 R^2 \rho_0}{\mu_0}$	reduced sedimentation coefficient
$\tau = \mu_0 t/\rho R^2$	reduced time

The quantities μ_0 and ρ_0 are the viscosity and density of the gradient solution evaluated over the band-width values, respectively. D is the dispersion coefficient between the sample and a gradient solution, instead

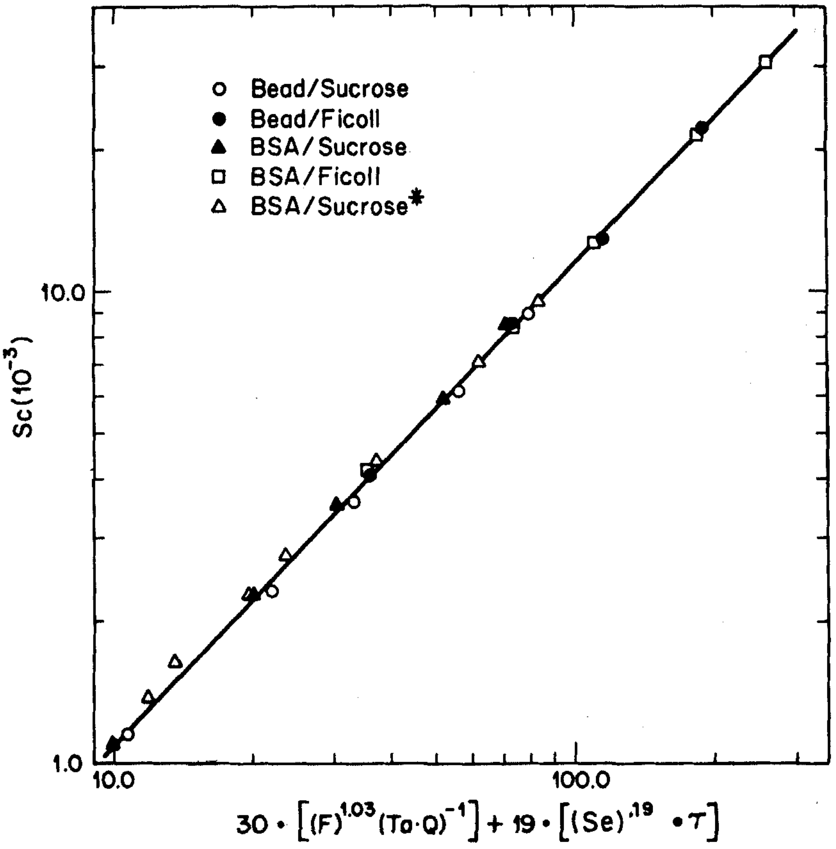

FIG. 3. A plot of Eq. (18) and general correlation of experimental data points. (Data points △ were not used in the correlation.)

of the customary term of the diffusion coefficient. If $Q < 1$, a sedimentation takes place until $Q = 1$ (isopycnic point).

The results obtained from the linear-with-volume gradient solution runs were checked against Eq. (18). A good agreement exists. All the data points were within an average of 3.5% deviation and a maximum of 8.9% deviation. They are assumed to be within the range of experimental errors, so that a further correlation to improve the results was not made. Equation (18) and both correlations from a step-gradient solution and a linear-with-volume gradient solution are presented in Fig. 3.

Equation (18) and Fig. 3 may be used to estimate a dispersion coefficient on new biomaterials for which sizes of particles and their sedimentation coefficients are available.

Optimal Gradient Solution for Velocity Sedimentation

For the velocity-sedimentation method, a centrifugal field of specified force is chosen for the system. Because the rotor is operated at high speeds, the solute particles are forced to settle at appreciable rates toward the wall of a rotor. Thus it is basically a transport method. As pointed out previously (*23*), the viscosity of a gradient solution is the major factor in controlling the rate of particle movement. The design of an optimal density gradient solution is of importance in the velocity-sedimentation method.

Recently Hsu (*37*) has obtained analytically the optimal profile of a density gradient solution characterized by viscosity and density profiles which are expressed as functions of radial distance of a zonal rotor, the estimation of an optimal centrifugation time, so that the resolution of separation by the velocity-sedimentation method will be the maximum, and also the prediction of each particle's position in a rotor when centrifugation is stopped, so that the separated particles can be collected adequately. Therefore, the analysis will permit the rational selection of optimal operating conditions for mass separation of new biological materials by the velocity-sedimentation method.

By using the maximum principle, a performance index for the velocity-sedimentation for Particle 1 and Particle 2 is defined by (*37*):

$$\max\left[\Delta R = \int_0^{t_f}\left(\frac{dr_1}{dt} - \frac{dr_2}{dt}\right)dt\right] \tag{19}$$

The problem is to find a density gradient solution characterized by viscosity $\eta(r)$ and density $\rho(r)$ which maximize the difference of instantaneous position of two particles in a rotor so that the resolution of a separation will be maximum. The constrain conditions associated with the performance index equation, Eq. (19), are the Lamm's sedimentation equations for Particle 1 and Particle 2 together with the following

$$\frac{dr_1}{dt} = \frac{k_1\omega^2 r}{\eta(r)}\frac{\rho_1 - \rho(r)}{\rho_1 - \rho(\mathrm{H_2O}, 20\,^\circ\mathrm{C})} \tag{20a}$$

$$\frac{dr_2}{dt} = \frac{k_2\omega^2 r}{\eta(r)}\frac{\rho_2 - \rho(r)}{\rho_2 - \rho(\mathrm{H_2O}, 20\,^\circ\mathrm{C})} \tag{20b}$$

Equations (20a) and (20b) are obtained by combining the definition of sedimentation coefficient by the Svedburg and the usual way of converting the instantaneous sedimentation coefficient to the standard sedimentation coefficient in a 20°C water medium, Eq. (2). The quantity K_i is given as

$$k_i = s_i^0(\mathrm{H_2O}, 20\,^\circ\mathrm{C})\eta(\mathrm{H_2O}, 20\,^\circ\mathrm{C}).$$

with the boundary conditions that the properties of the light-end density gradient solution at the rotor core r_c are

$$\rho(r_c) = \rho_0 \tag{21a}$$

$$\eta(r_c) = \eta_0 \tag{21b}$$

The optimal viscosity profile obtained is

$$\eta(r) = \eta_0\left(\frac{r}{r_c}\right)^{4/3} \tag{22a}$$

The optimal density profile is obtained by using the boundary condition, Eq. (21a), together with Eq. (22a), which gives

$$\rho(r) = (\rho_0 - \alpha)\left(\frac{r}{r_c}\right)^{2/3} + \alpha \tag{22b}$$

in which

$$\alpha = \frac{1 - \left(\dfrac{\rho_2}{\rho_1}\right)\left(\dfrac{s_2^0}{s_1^0}\right)\left(\dfrac{\rho_1 - \rho_\mathrm{H}}{\rho_2 - \rho_\mathrm{H}}\right)}{1 - \left(\dfrac{s_2^0}{s_1^0}\right)\left(\dfrac{\rho_1 - \rho_\mathrm{H}}{\rho_2 - \rho_\mathrm{H}}\right)} \tag{22c}$$

Equations (22) characterize the optimal density gradient solution for a velocity-sedimentation in terms of viscosity and density profiles.

Besides an optimal density gradient solution built in a rotor for a velocity-sedimentation, an important problem is that of when and where the maximum separation will take place, so that one can stop the centrifugation run and collect the separated fractions from a rotor either by draining the gradient out of the bottom of the rotor or by displacing it out of the top by using a heavier gradient solution. The final positions of Particles 1 and 2 are found to be

$$r_1(t_f) = r_c\left\{\frac{\dfrac{4}{6}\left[\dfrac{s_1^0(\rho_1 - \alpha)}{\rho_1 - \rho_\mathrm{H}} - \dfrac{s_2^0(\rho_2 - \alpha)}{\rho_2 - \rho_\mathrm{H}}\right] + \dfrac{s_1^0(\rho_2 - \rho_1)}{\rho_1 - \rho_\mathrm{H}}}{\left[\dfrac{k_1}{\rho_1 - \rho_\mathrm{H}} - \dfrac{k_2}{\rho_2 - \rho_\mathrm{H}}\right](\rho_0 - \alpha)}\right\}^{3/2} \tag{23a}$$

$$r_2(t_f) = r_c \left\{ \frac{-\dfrac{4}{6}\left[\dfrac{s_1{}^0(\rho_1 - \alpha)}{\rho_1 - \rho_H} - \dfrac{s_2{}^0(\rho_2 - \alpha)}{\rho_2 - \rho_H}\right] + \dfrac{s_1{}^0(\rho_2 - \rho_1)}{\rho_2 - \rho_H}}{\left[\dfrac{s_1{}^0}{\rho_1 - \rho_H} - \dfrac{s_2{}^0}{\rho_2 - \rho_H}\right](\rho_0 - \alpha)} \right\}^{3/2} \tag{23b}$$

The maximum separation ΔR thus obtained is

$$(\Delta R)_{\max} = r_c \left\{ \frac{\dfrac{4}{3}\left[\dfrac{s_1{}^0(\rho_1 - \alpha)}{\rho_1 - \rho_H} - \dfrac{s_2{}^0(\rho_2 - \alpha)}{\rho_2 - \rho_H}\right]}{\left(\dfrac{s_1{}^0}{\rho_1 - \rho_H} - \dfrac{s_2{}^0}{\rho_2 - \rho_H}\right)(\rho_0 - \alpha)} + \frac{(\rho_2 - \rho_1)}{(\rho_0 - \alpha)} \right\}^{3/2} \tag{23c}$$

The optimum centrifugation time thus obtained is

$$\tau_f = \frac{1}{AB} \sum_{n=0}^{\infty} \frac{3M^n}{2(n+2)} \zeta_c{}^{[2(n+2)/3]} \times \left[\left\{ \frac{\dfrac{4}{6}\left[\dfrac{k_1(\rho_1 - \alpha)}{\rho_1 - \rho_H} - \dfrac{k_2(\rho_2 - \alpha)}{\rho_2 - \rho_H}\right] + \dfrac{k_1(\rho_2 - \rho_1)}{\rho_1 - \rho_H}}{\left(\dfrac{k_1}{\rho_1 - \rho_H} - \dfrac{k_2}{\rho_2 - \rho_H}\right)(\rho_0 - \alpha)} \right\}^{n+2} - 1 \right] \tag{24}$$

Equation (24) implies that the length of centrifugation for a velocity-sedimentation is determined by this formula in which the density gradient solution is characterized by Eq. (22).

These results are based on the assumption that the particles are still far away from their respective isopycnic points, so that sedimentation is dominant in the mutual interference of diffusion and sedimentation. Hence changes of a density gradient solution profile due to diffusion are negligible. In a velocity-sedimentation, the centrifugation time is generally very short. Therefore, concentrations of macromolecules and a density gradient solution do not disperse appreciably by molecular diffusion. Also, the partial specific volumes of each component are assumed constant. This implies that the solution is regular. The solution may not be regular but can be corrected by an activity coefficient. These assumptions are considered to be reasonable, and they permit the development of an analytical solution with relative ease.

The estimates of an optimal centrifugation time and the positions of particles serve as a guide for designing velocity-sedimentation runs. It is perhaps very difficult to build an optimal density gradient solution as specified in Eq. (22) by a single gradient solute. A mixed solutes gradient solution probably can provide the viscosity and density profiles as designed.

INTRODUCTION OF NEWER TECHNIQUES (5)

The sedimentation rate of a particle, its banding density, or both of these properties may be altered in a density gradient in a manner useful for achieving separations. These factors involved are:

(1) Precipitation (increase in effective particle size) produced by diffusion of a precipitant into a sample zone.
(2) Resolubilization in a gradient negative with respect to the precipitating agent.
(3) Alteration in volume and density by change in osmotic pressure or biochemical environment.
(4) Specific binding to ions or substances, altering sedimentation rate or banding density.
(5) Sequential dissection by sedimentation through zones of immobilized reagents.

These techniques, singly or in combination, open up many new interesting avenues of research.

Precipitation by a Diffusion Precipitant

Soluble sample materials of high or of low molecular weight may be precipitated by the diffusion of a suitable precipitant into the sample zone. Inorganic salts in aqueous solution may be caused to precipitate, often in the form of small crystals, as an organic solvent miscible with water (and in which the salts are not soluble) is allowed to diffuse in. Similarly, organic substances insoluble in water may be precipitated out of alcohol or acetone by water diffusing into the sample zone. High-molecular-weight substances such as proteins and nucleic acids may be precipitated by water-miscible organic solvents or heavy metal salts. The precipitating agent may be in either the overlay, in the underlying gradient, or in both. In all instances care must be taken to ensure that the proper density increments are present to ensure stability from a gravitational viewpoint. When ethyl alcohol is used as a solvent, its density may be adjusted with 2-chloroethanol, for example.

The unique feature about precipitation in a centrifugal field is that precipitated particles sediment out of the sample zone as they increase in size. Since precipitation occurs in response to a diffusion gradient, different substances may precipitate at different times and levels in the diffusion gradient, tending to minimize cross-contamination in the particles formed. Particle size will be a function of the centrifugal field used, and with reasonably high forces only very tiny particles will be formed. If the particles are not soluble at any level as they sediment through the gradient, they may be fractionated by isopycnic banding. This method of preparative particle separation has not been widely applied to precipitates although the crystal densities of a large number of substances are known.

Gradient Resolubilization

As precipitated particles are sedimented through a gradient, the composition of the gradient may vary in such a way that the particles go back into solution (*4*, *41*, *42*). One of the advantages of this method is that the time during which a substance is precipitated is rather short, especially where a high centrifugal field is employed. The rate at which a sedimenting particle will dissolve increases as the size of the particle increases. Interest in these processes centers chiefly around the possibility of adapting them to continuous on-stream protein fractionation.

Alteration in Volume and Density by Change in Osmotic Pressure or Biochemical Environment

Gradients may be prepared having similar density slopes, but having osmotic pressure gradients which may vary, and in the case of specially prepared sucrose-dextran gradients, may approach zero (*40*). Where particles differ in their response to an osmotic pressure gradient, the gradient may be adjusted to maximize the separation. Mitochondria appear to shrink appreciably more than do peroxisomes in sucrose gradients. The mitochondria initially sediment ahead of the peroxisomes; however, the latter overtake them and band at a slightly denser level. For optimal rate separations it appears advantageous not to employ isotonic gradients, but rather to let the mitochondria shrink and sediment more rapidly ahead of the lysosomes. Sedimentation rates may also be affected by the biochemical environment quite apart from osmotic effects. The volume changes produced in isolated nuclei by small amounts of divalent cations (*41*, *42*) and in mitochondrial volume by ATP or thyroxine are examples of this.

Specific Binding to Ions or Substances Altering Sedimentation Rate or Banding Density

The banding density of smooth and rough endoplasmic reticulum fragments, and subfamilies of these, may be altered by small changes in the ionic environment (*40*). The use of uranium or ferritin-labeled antibodies to specifically alter the banding density of an organelle or fragment does not appear to have been explored. A simple and frequently used method for increasing the sedimentability of haemagglutinating viruses is to allow them to attach to red cells which are easily sedimented. As the virus desorb, they are easily separated from the red cells by centrifugation (*43*).

Sequential Dissection by Sedimentation through Zones of Immobilized Reagents

Reagents having low sedimentation coefficients may be incorporated at different levels in a density gradient to attack larger particles moving through the gradient. At the MAN Program the method was employed in early studies on the extraction of histones from nuclei sedimenting through gradients of increasing acidity, and more recently detergent gradients have been used to solubilize microsomes. As more interest develops in the dissection of membranes and ribosomes, these methods will probably be more widely employed.

DISCUSSION

Preparative zonal centrifugation is now in a state of very rapid growth, with new rotor systems and separative techniques appearing at frequent intervals. The evaluation of these methods and comparison with other methods in a variety of specific instances will require a period of several years.

We conclude the rotor development should proceed in those directions:

(1) The limits of speed, capacity, volume, and resolution should continue to be explored by using systems which exhaust presently available technology.

(2) The improvement of zonal centrifugation should be directed to an improvement in loading and unloading methods to reduce the dispersion from these operations. Therefore, the separated resolution can be adequately maintained during the unloading.

(3) An optimal design for rotor configuration is required for a par-

ticular separation. Transport phenomena of particles in a rotor with respect to various configurations has to be studied in detail so that various factors affecting separation in a rotor can be evaluated quantitatively.

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REFERENCES

1. N. G. Anderson, "Studies on Isolated Cell Components VIII, High Resolution Gradient Differential Centrifugation," *Exp. Cell. Res.*, *9*, 446 (1955).

2. N. G. Anderson, "Zonal Centrifuges and Other Separation Systems," *Science*, *154*, 103 (1966).

3. N. G. Anderson, ed., *National Cancer Institute Monograph 21*, U.S. Government Printing Office, Washington, D.C., 1966.

4. N. G. Anderson, "An Introduction to Particle Separations in Zonal Centrifuges," in *National Cancer Institute Monograph 21*, U.S. Government Printing Office, Washington, D.C., 1966, p. 9

5. N. G. Anderson, "Preparative Particle Separation in Density Gradients," *Quart. Rev. Biophys.*, *1*(3), 217–263 (1968).

6. N. G. Anderson, H. P. Barringer, J. M. Amburgey, Jr., G. B. Cline, C. E. Nunley, and A. S. Berman, "Continuous-Flow Centrifugation Combined with Isopycnic Banding: Rotors B–VIII and B–XI," in *National Cancer Institute Monograph 21*, U.S. Government Printing Office, Washington, D.C., 1966, p. 199.

7. H. P. Barringer, N. G. Anderson, and C. E. Nunley, "Design of the B–V Continuous-Flow Centrifuge System," in *National Cancer Institute Monograph 21*, U.S. Government Printing Office, Washington, D.C., 1966, p. 191.

8. M. K. Brakke, "Zone Electrophoresis of Dyes, Proteins, and Viruses in Density Gradient Columns of Sucrose Solutions," *Arch. Biochem. Biophys.*, *55*, 175 (1955).

9. M. K. Brakke, "Nonideal Sedimentation and the Capacity of Sucrose Gradient Columns for Virus in Density-Gradient Centrifugation," *Ibid.*, *107*, 188–403 (1964).

10. M. K. Brakke, "Density-Gradient Centrifugation: Nonideal Sedimentation and the Interaction of Major and Minor Components," *Science*, *148*, 387–389 (1965).

11. V. N. Schumaker, "Zonal Centrifugation," in *Advances in Biological and Medical Physics* (C A. Tobias and J. H. Lawrence, eds.), Academic, New York, 1967, pp. 245–339.

12. T. Svedberg, *Z. Phys. Chem.*, *127*, 51 (1927).

13. T. Svedberg and K. O. Pederson, *The Ultracentrifuge*, Clarendon Press, Oxford, 1940.

14. J. W. Williams, *Ultracentrifugal Analysis in Theory and Experiment*, Academic, New York, 1963.

15. S. R. de Groot and P. Mazur, *Non-Equilibrium Thermodynamics*, 2nd ed., North-Holland, New York, 1962.

16. W. K. Sartory, "Instability in Diffusing Fluid Layers," *Biopolymers*, *7*, 251–263 (1969).

17. S. P. Spragg and C. T. Rankin, Jr., *Biophys. Acta, 141*, 164 (1967).

18. N. G. Anderson, *Semiannual Report of the U.S. AEC*, ORNL–1953, August 15, 1954, p. 17.

19. H. Elrod, N. G. Anderson, L. C. Patrick, and J. C. Shinpaugh, *J. Tenn. Acad. Sci.*, (1968).

20. H. W. Hsu and N. G. Anderson, *Biophys. J.*, *9*, 173–188 (1969).

21. H. W. Hsu, *Separ. Sci.*, *8*, 537 (1973).

22. H. D. Pham and H. W. Hsu, *Ind. Eng. Chem.*, *Process Des. Develop.*, *11*, 556 (1972).

23. H. W. Hsu, *Separ. Sci.*, *6*, 699–714 (1971).

24. M. K. Brakke, *Adv. Virus Res.*, *7*, 193–224 (1960).

25. N. G. Anderson, "Techniques for the Mass Isolation of Cellular Components," in *Physical Techniques in Biological Research*, Vol. 3, *Cells and Tissues*, (G. Oster and A. W. Polister eds.), Academic, New York, 1956, pp. 299–352.

26. H. Svensson, L. Hagdahl, and K. D. Lerner, *Sci. Tools*, *4*, 1–10 (1957).

27. A. S. Berman, "Theory of Centrifugation: Miscellaneous Studies," in *National Cancer Institute Monograph 21*, U.S. Government Printing Office, Washington, D.C., 1966, p. 41.

28. M. Gehetia and E. Katchalski, *J. Chem. Phys.*, *30*, 1334–1339 (1959).

29. M. K. Brakke, *Archs. Biochem. Biophys.*, *107*, 388–403 (1964).

30. M. K. Brakke, *Science*, *148*, 387–389 (1965).

31. S. P. Spragg and C. T. Rankin, Jr., *Biochem. Biophys. Acta*, *141*, 164–173 (1967).

32. H. B. Halsall and V. N. Schumaker, *Biochem. Biophys. Res. Commun.*, *43*, 601–606 (1971).

33. J. A. T. P. Meuwissen and K. P. N. Heirwegh, *Ibid.*, *41*, 675 (1970).

34. H. W. Hsu, *Math. Biosci.*, *13*, 361 (1972).

35. H. Fujita and L. J. Gostings, *J. Phys. Chem.*, *64*, 1256 (1960).

36. R. K. Genung and H. W. Hsu, *Separ. Sci.*, *7*, 249 (1972).

37. H. W. Hsu, *Math. Biosci.*, *23*, 179 (1975).

38. N. G. Anderson, C. A. Price, W. D. Fisher, R. E. Canning, and C. L. Burger, *Ann. Biochem.*, *7*, 1–9 (1964).

39. N. G. Anderson, H. P. Barringer, E. F. Babelay, and W. D. Fisher, *Life Sci.*, *3*, 667–671 (1964).

40. G. Dallner and R. Nilsson, *J. Cell. Biol.*, *31*, 181–193 (1960).

41. J. F. Albright and N. G. Anderson, *Exp. Cell Res.*, *15*, 271–281 (1958).

42. N. G. Anderson and K. M. Wilbur, *J. Gen. Physiol.*, *35*, 781–797 (1952).

43. F. Hoyle, B. Jolles, and R. G. Mitchell, *J. Hyg.*, *52*, 119–127 (1954).

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