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Transport Phenomena in Zonal Centrifuge Rotors. III. Particle Sedimentation in Gradient Solutions

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Summary

An analysis is presented for the prediction of (a) a particle position as a function of rotation time, (b) an instantaneous particle sedimentation velocity, and (c) an instantaneous observed particle sedimentation coefficient in a gradient solution characterized by viscosity and density profiles which are expressed in polynomials as functions of radial distance of the zonal rotor. Effect on variation of those predicted quantities with (a) particle size and rotor speed, (b) the density ratio between a particle and the light end of the gradient solution, and (c) the profiles of a gradient solution are also discussed from the computed numerical results.

INTRODUCTION

The recent theoretical and experimental advances in zonal centrifugation have led to more efficient methods of separating biologically-

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active material ranging in size from whole cells to protein molecules resolvable with the electron microscope (*1–10*). In exploring cell substructure and the isolation of cell components or viruses, the Molecular Anatomy (MAN) Program at Oak Ridge National Laboratory has been engaged in developing high-resolution and high-capacity centrifugation techniques for a wide variety of biomaterials (*1–7*).

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 like concentration of gradient and sedimentation velocity bands, which are usually measured by an optical method in an ultraanalytical centrifugation, are not easily made during the zonal centrifugation. It is thus necessary to rely on a refined theory of mathematical modeling for prediction of such quantities.

The problem has been investigated by Thomson and Mikuta (*11*), Rosenbloom and Cox (*12*), and Duve et al. (*13*) by modifying density and viscosity in a sustaining gradient solution. Recently Pretlow et al. (*14*) used linear density and exponential viscosity for the Ficoll gradient to analyze their particle position data.

The objective of this paper is to evaluate quantitatively the location of a particle, its instaneous sedimentation velocity, and its sedimentation coefficient of a particle during a zonal centrifugation run in an arbitrary gradient solution, which is characterized by the density and viscosity profiles given as functions of radial distance in polynomials. Thus, the analysis will permit the rational selection of centrifuge conditions for mass separation of new biomaterials in a specified gradient solution.

FORMULATION OF PROBLEM

A cylindrical rotor system of radius R filled with a gradient solution whose viscosity and density increase with distance r from the axis is expressed by the polynomials

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

$$\rho = \rho_o(1 + \epsilon_1'r + \epsilon_2'r^2 + \epsilon_3'r^3 + \cdots) \tag{1b}$$

where μ_o and ρ_o 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 cylindrical rotor is to rotate about its own axis at an angular velocity ω. The biomaterials or particles are suspended in the light-end of a gradient solution. Before the rotor reaches a given constant angular velocity ω, we assume that the particles ride in the gradient solution and do not exercise their sedimenting action under the centrifugal force field. The sedimenting motion will begin after a pseudo-steady-state has been reached *(15)*. The particles are all assumed to be spherical, and drag force on the particles during the sedimentation can be approximated by Stokes' resistance law

$$F_d = -3\mu\pi D_p \mathbf{v} \tag{2}$$

where μ is the viscosity of the sedimenting medium, D_p is the particle diameter, and $\mathbf{v}$ the particle velocity measured relative to the rotating axes. The sedimentation equation of the particle in an incompressible gradient solution relative to the rotating coordinate system, following the derivation of Berman *(16)*, can be given by

$$\beta \mathbf{v} = \kappa(\partial \mathbf{v}/\partial t) + 2\kappa \boldsymbol{\omega} \times \mathbf{v} + \boldsymbol{\omega} \times (\boldsymbol{\omega} \times \mathbf{r}) \tag{3}$$

with

$$\beta = \frac{18\mu_o(1 + \lambda_1'r + \lambda_2'r^2 + \lambda_3'r^3 \cdots)}{[\rho_p - \rho_o(1 + \epsilon_1'r + \epsilon_2'r + \epsilon_3'r^3 + \cdots)]D_p^2} \tag{4}$$

and

$$\kappa = \frac{\rho_p}{\rho_p - \rho_o(1 + \epsilon_1'r + \epsilon_2'r^2 + \epsilon_3'r^3 + \cdots)} \tag{5}$$

where $\mathbf{r}$ is the position vector of a particle in the rotating coordinate system and ρ_p the particle density. Since we are considering the particle's sedimentation after a rotor reaches a pseudo-steady-state, a change in the axial direction can be neglected. Thus, the particle sedimentation equation, Eq. (3), for the radial velocity component, v_r, and the tangential velocity component, v_θ, are:

$$-\beta v_r = \kappa(dv_r/dt) - r\omega^2[1 + 2(\kappa\Omega/\omega) + (\kappa\Omega^2/\omega^2)] \tag{6}$$

$$-\beta v_\theta = \kappa(dv_\theta/dt) + 2\kappa v_r \omega[1 + (\Omega/2\omega)] \tag{7}$$

where r is the radial distance of a particle from the axis of rotation, and Ω, the angular velocity of a particle in the rotating coordinate system, is defined as $\Omega = v_\theta/r$. It is obvious that $\Omega/\omega \ll 1$; therefore, one may approximate Eqs. (6) and (7) into the following equations without error to obtain

$$\frac{dv_r}{dt} + \frac{18\mu_o(1 + \lambda_1'r + \lambda_2'r^2 + \lambda_3'r^3 + \cdots)}{\rho_p D_p^2} v_r - \frac{r\omega^2}{\rho_p}[\rho_p - \rho_o(1 + \epsilon_1'r + \epsilon_2'r^2 + \epsilon_3'r^3 + \cdots)] = 0 \quad (8)$$

$$\frac{dv_\theta}{dt} + \frac{18\mu_o(1 + \lambda_1'r + \lambda_2'r^2 + \lambda_3'r^3 + \cdots)}{\rho_p D_p^2} v_\theta + 2\omega v_r = 0 \quad (9)$$

Since the radial velocity component, v_r, can be written as $v_r = dr/dt$, Eq. (8) is a second-order differential equation for the radial coordinate r. We have assumed that particle sedimentation takes place after the rotor reaches a pseudo-steady-state; hence, Eq. (9) does not directly relate to the present investigation.

Now, Eq. (8) can be rewritten with the reduced variables for the radial coordinate as

$$P(d^2\zeta/d\tau^2) + 18Q(1 + \lambda_1\zeta + \lambda_2\zeta^2 + \lambda_3\zeta^3 + \cdots)(d\zeta/d\tau) + [Q(1 + \epsilon_1\zeta + \epsilon_2\zeta^2 + \epsilon_3\zeta^3 + \cdots) - 1]N\zeta = 0 \quad (10)$$

in which

$$P = (D_p/R)^2 \quad (11a)$$

$$Q = \rho_o/\rho_p \quad (11b)$$

$$N = (\rho_o\omega R D_p/\mu_o)^2 \quad (11c)$$

$$\zeta = r/R \quad (11d)$$

$$\tau = \mu_o t/\rho_o R^2 \quad (11e)$$

$$\lambda_i = \lambda_i' R^i \quad (11f)$$

and

$$\epsilon_i = \epsilon_i' R^i \quad (11g)$$

For systems of interest in the present study, the largest particle diameter, D_p, will be in the order of 1 to 3 μ; the radius of the rotor, R, approximately 6 to 8 cm (for B- and K-series rotors); the rotor speed, approximately 30,000 to 36,000 rpm; and the density and viscosity of the light-end of the gradient solution, approximately 1.1 to 1.3 g/cc and 1.2 to 20 cP, respectively. Therefore, the order of magnitude for the dimensionless coefficients P, Q, and N in Eq. (10) is roughly in the order of 10^{-10}, 10^{-1}, and 10^{-2}, respectively. Hence, Eq. (10) can be approximated to the first-order equation by neglecting the second-order term with P as a coefficient without error. Thus Eq. (10) can be approximated to

$$(1 + \lambda_1\zeta + \lambda_2\zeta^2 + \lambda_3\zeta^3 + \cdots)(d\zeta/d\tau) - A\left[1 - \frac{Q}{1-Q}(\epsilon_1\zeta + \epsilon_2\zeta^2 + \epsilon_3\zeta^3 + \cdots)\right]\zeta = 0 \quad (12)$$

where

$$A = [(1/Q) - 1](N/18) \quad (13)$$

The boundary condition for the case is

$$\tau = 0, \qquad \zeta = \xi_i \quad (14)$$

where $\xi_i = r_i/R$ is the reduced initial position of the particle, which can be obtained from the loading condition of the sample into the rotor (*15*). Usually the length of the outer edge of the rotor core can be used as r_i.

SOLUTION OF RADIAL EQUATION

Equation (12) is to be solved by a perturbation method with the boundary condition given in Eq. (14). One assumes that the solution is to be of the following form:

$$\zeta = \zeta_0 + A\zeta_1 + A^2\zeta_2 + A^3\zeta_3 + \cdots \quad (15)$$

By substituting Eq. (15) into Eq. (12) and setting the coefficients of each power of A equal to zero, one obtains a set of differential equations which are to be solved for ζ_n ($n = 0, 1, 2, 3, \ldots$). The quantity A is used as a perturbation parameter. With the boundary condition, Eq. (14), the solutions for ζ_n are obtained to be as follows:

$$\zeta_0 = \xi_i \quad (16)$$

$$\zeta_1 = C_1\tau \quad (17)$$

where

$$C_1 = \epsilon_{10}/\lambda_{10} \quad (18)$$

in which

$$\epsilon_{10} = \left[1 - \frac{Q}{1-Q}(\epsilon_1\xi_i + \epsilon_2\xi_i^2 + \epsilon_3\xi_i^3 + \cdots + \epsilon_n\xi_i^n + \cdots)\right]\xi_i \quad (19)$$

$$\lambda_{10} = (1 + \lambda_1\zeta_i + \lambda_2\xi_i^2 + \lambda_3\zeta_i^3 + \cdots + \lambda_n\xi_i^n + \cdots) \quad (20)$$

$$\zeta_2 = (C_2/2)\tau^2 \quad (21)$$

where

$$C_2 = (C_1/\lambda_{10})[\epsilon_{21} - \lambda_{21}C_1] \tag{22}$$

in which

$$\epsilon_{21} = 1 - \frac{Q}{1-Q}[2\epsilon_1\xi_i + 3\epsilon_2\xi^2 + 4\epsilon_3\xi^3 + \cdots + (n+1)\epsilon_n\xi_i^n + \cdots] \tag{23}$$

$$\lambda_{21} = \lambda_1 + 2\lambda_2\xi_i + 3\lambda_3\epsilon_i^2 + \cdots + n\lambda_n\xi_i^{n-1} + \cdots \tag{24}$$

$$\zeta_3 = (C_3/3)\tau^3 \tag{25}$$

where

$$C_3 = (1/\lambda_{10})[(\epsilon_{21} - C_1\lambda_{21})(C_2/2) - (\epsilon_{31} + C_1\lambda_{31})C_1^2 - \lambda_{21}C_1C_2] \tag{26}$$

in which

$$\epsilon_{31} = \frac{Q}{1-Q}\left[\epsilon_1 + 3\epsilon_2\xi_i + 6\epsilon_3\xi_i^2 + \cdots \binom{n}{2}\epsilon_n\xi_i^{n-1} + \cdots\right] \tag{27}$$

$$\lambda_{31} = \lambda_2 + 3\lambda_3\xi_i + \cdots + \binom{n}{2}\lambda_{n-1}\xi_i^{n-2} + \cdots \tag{28}$$

$$\zeta_4 = (C_4/4)\tau^4 \tag{29}$$

where

$$C_4 = (1/\lambda_{11})[(\epsilon_{21} - 4\lambda_{21}C_1)(C_3/3) - (\epsilon_{31} + 2\lambda_{31}C_1)C_1C_2 - (\epsilon_{41} + \lambda_{41}C_1)C_1^3 - \lambda_{21}(C_2^2/2)] \tag{30}$$

in which

$$\epsilon_{41} = \frac{Q}{1-Q}\left[\epsilon_2 + 4\epsilon_3\xi_i + \cdots + \binom{n}{3}\epsilon_{n-1}\xi_i^{n-2} + \cdots\right] \tag{31}$$

$$\lambda_{41} = \lambda_3 + 4\lambda_4\xi_i + \cdots + \binom{n}{3}\lambda_n\xi_i^{n-3} + \cdots \tag{32}$$

By substituting Eqs. (9) and (16)–(32) into Eq. (15), one obtains

$$\zeta = \xi_i\left(1 + C_1'A\tau + \frac{C_2'}{2!}A^2\tau^2 + \frac{C_3'}{3!}A^3\tau^3 + \frac{C_4'}{4!}A^4\tau^4 + \cdots\right) \tag{33}$$

where

$$C_1' = C_1/\xi_i, \qquad C_2' = C_2/\xi_i,$$
$$C_3' = C_3/\xi_i 2!, \qquad \text{and} \qquad C_4' = C_4/\xi_i 3! \tag{34}$$

It is interesting to note that, if the viscosity and density of the sedimenting medium are constants, i.e., λ_i and ϵ_i are all zero, Eq. (12) can be easily integrated with the boundary condition, Eq. (14), to give

$$\zeta = \xi_i e^{A\tau}$$
$$= \xi_i\left(1 + A\tau + \frac{A^2\tau^2}{2!} + \frac{A^3\tau^3}{3!} + \frac{A^4\tau^4}{4!} + \cdots\right) \quad (35)$$

Comparison of Eqs. (35) and (33) shows that the coefficients, C_i's, in Eqs. (34) represent the correction factors due to the viscosity and density variations in a rotor.

SEDIMENTATION VELOCITY OF PARTICLES

The sedimentation velocity of particles in a gradient solution now can be easily obtained from the expression of the position of particles as a function of time. The sedimentation velocity of particles in a reduced form is obtained by differentiating Eq. 15 with respect to the reduced rotation time, τ, which gives

$$V_r = d\zeta/d\tau = A(C_1 + AC_2\tau + A^2C_3\tau^2 + A^3C_4\tau^3 + \cdots) \quad (36)$$

in which the quantity A and all the C_i's were were defined previously.

OBSERVED SEDIMENTATION COEFFICIENT OF PARTICLES

The sedimentation coefficient of a particle, s, is defined by Svedberg (*17, 18*) as

$$s = \frac{dr/dt}{\omega^2 r} \quad (37)$$

If the sedimenting medium is a gradient solution characterized by Eqs. (1a) and (1b), the observed sedimentation coefficient of a particle, or a macromolecule, can be expressed in a reduced form by

$$S = s\omega^2 R^2 \rho_o/\mu_o = V_r/\zeta \quad (38)$$

It is emphasized here that Eq. (37) gives an expression for an instantaneous observed particle sedimentation coefficient which is not, in a general sense, the sedimentation coefficient customarily given.

In order to make the characteristics of biomaterials unique, the observed sedimentation coefficient for the bioparticle is customarily converted to the sedimentating medium of water at 20°C. The conversion formula is

$$S_{H_2O,20°C} = S_{obsv}\left(\frac{\mu_{G,T}}{\mu_{H_2O,20°C}}\right)\left(\frac{1 - \bar{v}\rho_{H_2O,20°C}}{1 - \bar{v}\rho_{G,T}}\right) \tag{39}$$

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

RADIAL SHEAR-STRESS OF PARTICLES

Knowledge of shear-stress exerted by the particles during the sedimentation may be informative to prevent damage to biomaterials in the separation, which can be obtained from the radial velocity component (*19*). The shear-stress exerted by a particle is the same as that of the sustaining liquid, but opposite in sign.

$$\tau_{rr} = \mu\left\{2\frac{\partial v_r}{\partial r} - \frac{2}{3}\left[\frac{1}{r}\frac{\partial}{\partial r}(rv_r)\right]\right\} \tag{40}$$

In a reduced form Eq. (40) can be rewritten

$$T_{rr} = \frac{\tau_{rr}}{2s\omega^2\mu_0} = \tfrac{1}{3}(1 + \lambda_1\zeta + \lambda_2\zeta^2 + \lambda_3\zeta^3 + \cdots)\left[\frac{2}{S}\frac{d\ln V_r}{d\tau} - 1\right] \tag{41}$$

NUMERICAL RESULTS

In order to simplify the numerical calculation, the following constants for a hypothetical gradient solution were used:

$\lambda_1 = a,\quad \lambda_2 = \lambda_1^2/2,\quad$ and $\quad \lambda_3 = \lambda_1^3/6;\quad a = -0.5, 1.0(0.5),^* 3.0$

$\epsilon_1 = b,\quad \epsilon_2 = \epsilon_1^2/2,\quad$ and $\quad \epsilon_3 = \epsilon_1^3/6;\quad b = 0.1(0.1),^* 1.0$

The profiles of the gradient solution were parts of the natural exponential curves, $e^{a\zeta}$ or $e^{b\zeta}$. Figure 1 shows the viscosity and density profiles of a gradient solution in rotors for $\lambda_1 = 1.5$, 2.0, 2.5, and 3.0, and $\epsilon_1 = 0.10$, 0.15, 0.20, and 0.25.

Figures 2 and 3 show the reduced particle position in a rotor as a

* The numbers in parentheses indicate the interval for the increment.

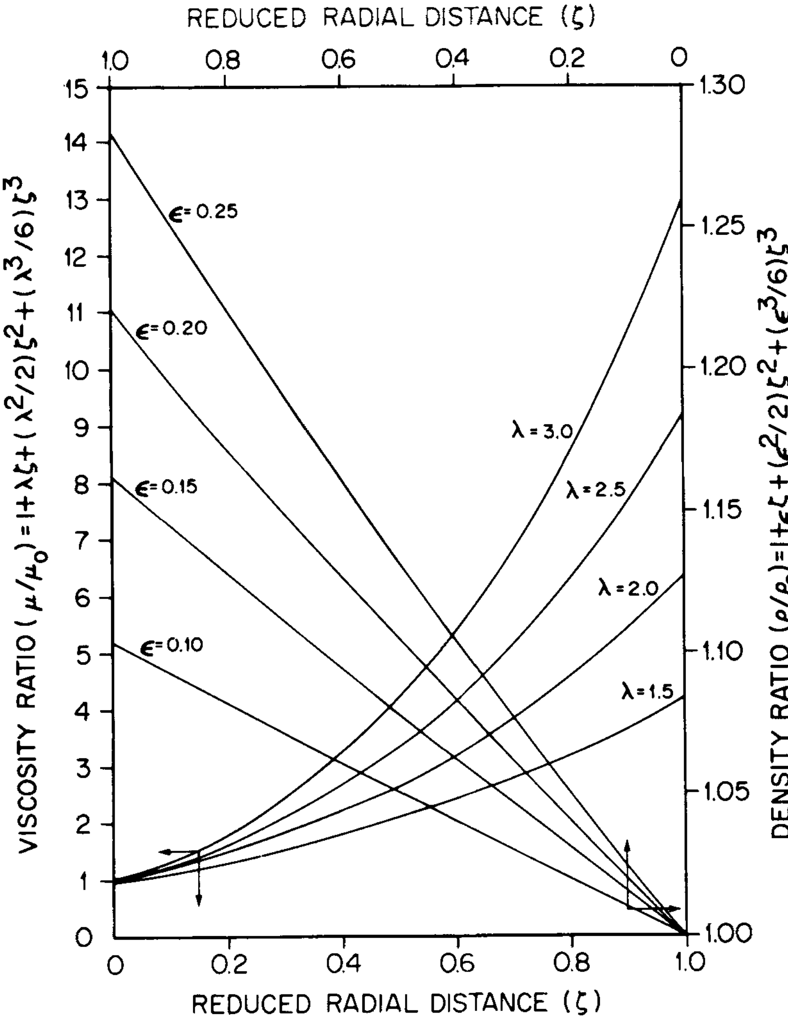

FIG. 1. Profiles of gradient solutions in a rotor.

function of reduced rotation time ($A\tau$). If the viscosity and density of a sedimenting medium are constants, a particle's position and the rotation time have a commonly known linear relationship, as can be seen from Eq. (35).

$$\ln \zeta = \ln \xi_i + A\tau$$

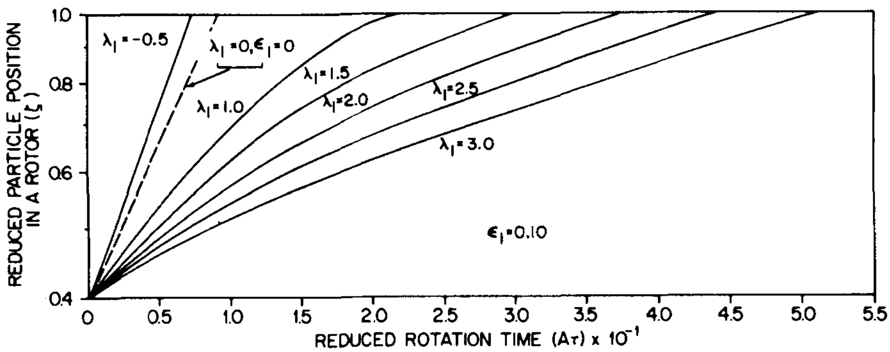

FIG. 2. Particle's position in a rotor as a function of rotation time with variations in λ_1 for $\epsilon_1 = 0.10$.

In a semilog plot, the above equation gives a straight line which is shown as a broken line in Figs. 2 and 3. The curves in the figures show the correction for the gradient solution. The variations of λ_1 from -0.5 to 3.0 for $\epsilon_1 = 0.1$ are shown in Fig. 2, and the variations for ϵ_1 from 0.1 to 0.9 for $\lambda_1 = 1.5$ are in Fig. 3.

It is interesting to note that, for the gradient solution with $\lambda_1 = -0.5$ and $\epsilon_1 = 0.1$, in which the viscosity of a gradient solution decreases while the density of a gradient solution increases, a particle sediments much faster than in the medium of constant density and viscosity. Therefore, we may conclude that the viscosity of a gradient solution is

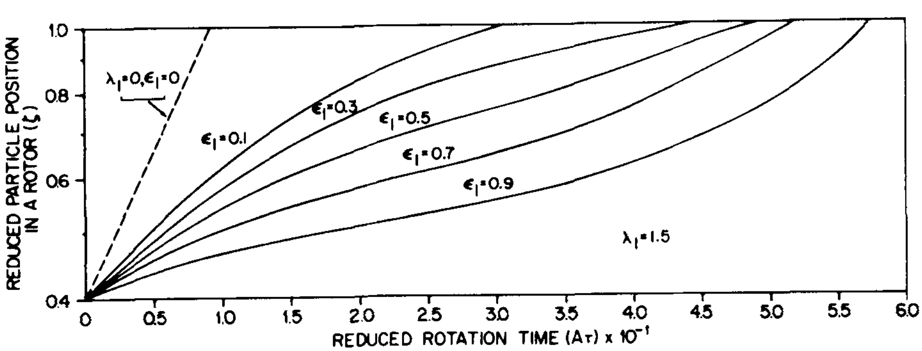

FIG. 3. Particle's position in a rotor as a function of rotation time with variations in ϵ_1 for $\lambda_1 = 1.5$.

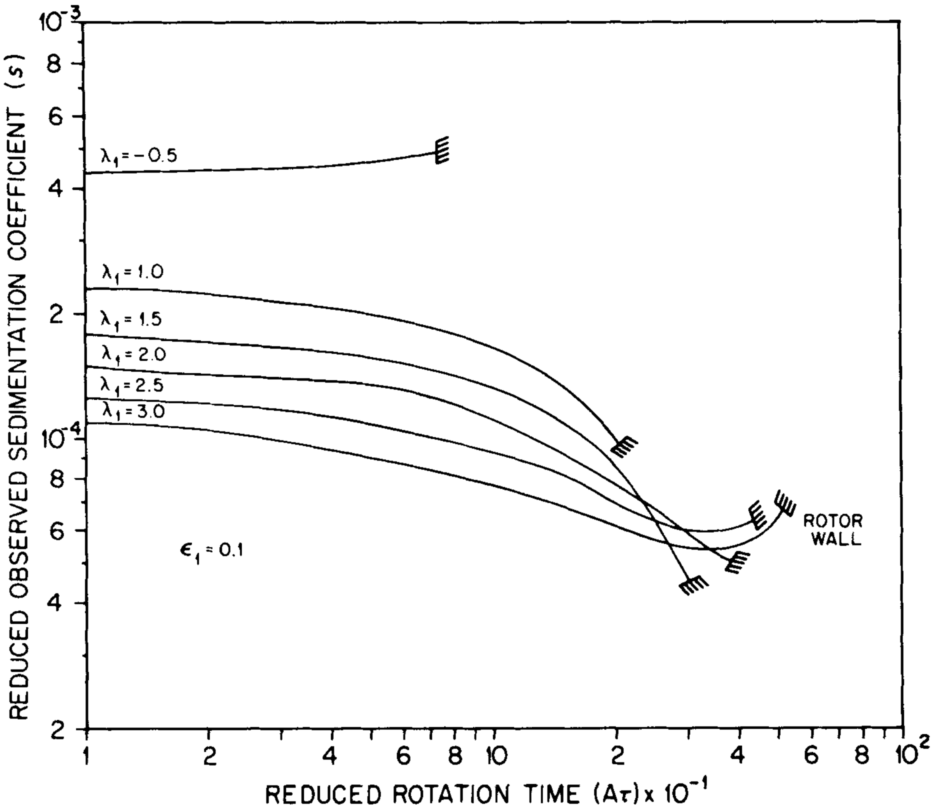

FIG. 4. Observed sedimentation coefficient as a function of rotation time with variations in λ_1 for $\epsilon_1 = 0.10$.

the controlling factor in the design of length of time required for a zonal centrifugation run.

The observed sedimentation coefficients as a function of reduced rotation time are shown in Figs. 4 and 5. The variations of λ_1 from -0.5 to 3.0 for $\epsilon_1 = 0.1$ on the observed sedimentation coefficients are shown in Fig. 4 and the variations of ϵ_1 from 0.1 to 0.9 for $\lambda_1 = 1.5$ are in Fig. 5. It is interesting to note that there is a minimum point in Figs. 4 and 5 which suggests the importance of the profiles of viscosity and density in a gradient solution for a zonal run. With a properly designed gradient profile, one can conduct zonal velocity separation

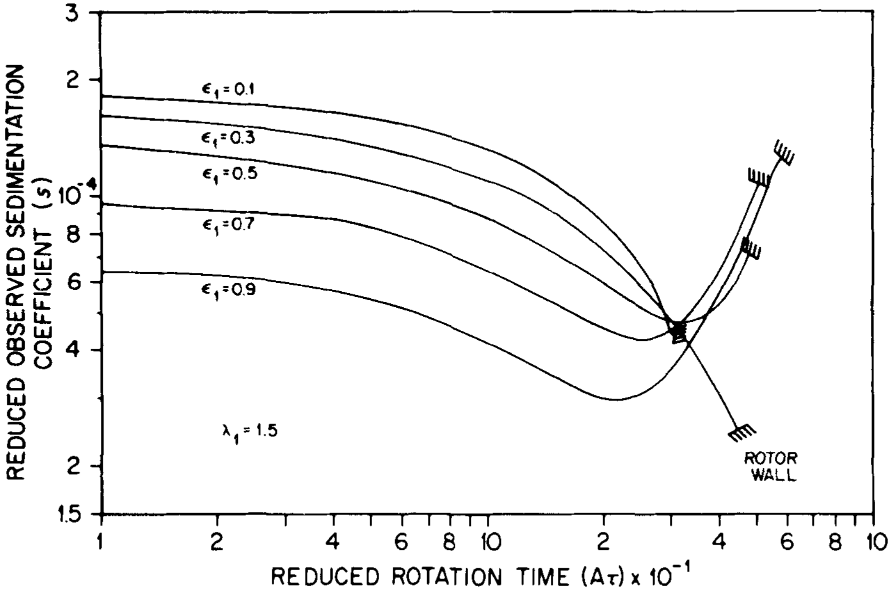

FIG. 5. Observed sedimentation coefficient as a function of rotation time with variations in ϵ_1 for λ_1 = 1.5.

according to the specifications. This suggests that investigation on mixed gradient medium solutions is warrantable.

An example of shear-stress distribution of particles is presented in Fig. 6. The shear-stress reaches a maximum and disappears immediately before the particle reaches the wall.

DISCUSSION

The results presented here are based on the assumptions that the particles ride in the gradient solution and do not sediment before the rotor reaches its given, full angular velocity, ω. Usually, the dynamic loading for B- and K-rotors was performed at rotor speeds of 2000 to 3000 rpm. For zonal centrifuge separations of the particle sizes in consideration, the sedimentation velocity is negligible around the dynamic loading rotation speed. Therefore, this assumption seems to be reasonable and without error. It is also assumed that all the particles are spherical with D_p as their particle diameters. The method of handling the nonspherical particles can be modified to the usual fashion by the introduction of appropriate shape factors. During the sedimentation period the particles will generally orient themselves to the minimum drag position. Therefore, the shortest characteristic length of the particle will be recommended for use as D_p. For osmometric particles, the equivalent particle diameter can be obtained from the information on partial specific volume of particles and partial specific quantity of a gradient solution. The diffusion effect in a gradient solution during the sedimentation period and the Brownian motion of ultrasmall particles in a gradient solution are also neglected. Experimental investigations on these effects are warrantable.

For the application of these results, one must first find viscosity and density profiles of a gradient solution in a rotor by curve-fitting to determine λ_i' and ϵ_i'. The diameter and density of particles have to be known to use these mathematical results. By using Eq. (15) together with Eqs. (18)–(34) one can determine where and when the particles

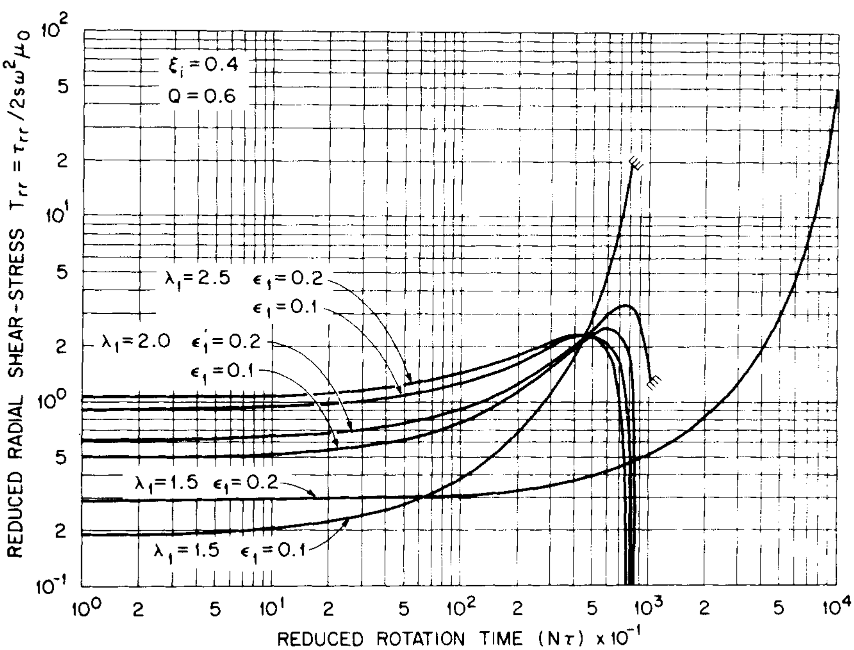

FIG. 6. Shear-stress distribution of a particle during sedimentation.

will be in a given gradient solution in a rotor. If one is interested in a velocity sedimentation, Eq. (37), together with information on the gradient solution, can be used to determine when and where the centrifugal separation can be optimum. With a gradient solution where $\lambda_1 = 1.5$ and $\epsilon_1 = 0.1$, one will find that the maximum separation between $N = 0.1$ and 0.01, when $Q = 0.6$ and $\xi_i = 0.4$ (approximately for B-XV rotor), will take place at $\tau = 2.5 \times 10^2$. Then, using the definition of τ, the best velocity sedimentation centrifugation time can be found. Furthermore, the location for optimum velocity sedimentation can also be found.

If information on the gradient solution and on experimental runs for new biomaterials is available, one can use the mathematical results obtained to analyze the separated biomaterial's characteristics, such as the diameter, density, and sedimentation coefficient. Thus, the analysis will serve as a method of analytical zonal centrifugation. In subsequent investigations the optimum profile of a gradient solution with respect to the resolution and to the loading capacity, etc., will be considered.

LIST OF SYMBOLS

A	parameter defined in Eq. (13)
C_i, C_i'	constants defined in Eqs. (18), (22), (26), (30), and (34)
D_p	diameter of particle
F_d	drag force on the particle
N	parameter defined in Eq. (11c)
P	parameter defined in Eq. (11a)
Q	parameter defined in Eq. (11b)
r	radial length variable
R	radius of rotor
s	Svedberg's sedimentation coefficient
S	dimensionless observed sedimentation coefficient given in Eq. (38)
t	time variable
T_{rr}	reduced shear-stress given in Eq. (41)
$\mathbf{v}$	velocity vector
v_r	radial velocity component
v_θ	tangential velocity component
V_r	reduced sedimentation velocity defined in Eq. (36)

β	parameter defined in Eq. (4)
ϵ_i, ϵ_i'	characteristic coefficients for gradient density variations
ζ	reduced radial variable defined in Eq. (11d)
κ	parameter defined in Eq. (5)
λ_i, λ_i'	characteristic coefficients for gradient viscosity variations
μ	dynamic viscosity
ρ	density
ξ_i	reduced initial position of particles
τ	reduced time variable defined in Eq. (11e)
τ_{rr}	radial shear-stress
ω	angular velocity

Subscripts

d	drag
i	initial condition or indices of the coefficients
o	quantity evaluated at light-end of the gradient solution
0, 1, 2, 3, 4	indices for the degree of approximation
p	particle
r	radial direction
θ	tangential direction

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