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Transport Phenomena in Zonal Centrifuge Rotors. V. Dispersion Coefficient of Sample in Gradient Solutions*

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Summary

Dispersion coefficients of a polystyrene latex bead (diameter: 0.091 ± 0.0058 μ) and bovine serum albumin in sucrose and Ficoll (polysucrose by Pharmacia Fine Chemicals, Sweden) gradient solutions were measured in an Oak Ridge B-XV zonal centrifuge rotor. Measurements were analyzed by the moment method. Dispersion coefficients were then correlated in a reduced form in terms of Schmidt number as a function of reduced centrifugal force field strength, Taylor number, reduced sedimentation coefficient,

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‡ H. W. Hsu is with the Molecular Anatomy Program, Oak Ridge National Laboratory, as a consultant.

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density ratio between particle and gradient medium, and reduced time. The correlation is also given in a formula

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

which fits all the experimental data points with an average deviation of 2.5% and a maximum deviation of 6.3%. Some information on the eddy and molecular components of the dispersion coefficients is also presented.

INTRODUCTION

The zonal centrifuges have been successfully developed in a wide variety of separation processes, including separation of cell organelles by Elrod and Anderson (*1*), viruses by Anderson and Cline (*2*), and mammalian cells by Baggiolini et al. (*3*). The use of zonal centrifuges has two primary objectives: (a) separation and purification of biomaterial from sample mixtures, and (b) concentration of separated biomaterial 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 biomaterial in a band increases, likewise to achieve a high resolution in a separated zone.

The improvement of resolution in zonal centrifugations has been studied analytically by Svensson (*4*), Berman (*5*), Rubin and Katchalsky (*6*), and Sartory (*7*) and experimentally by Anderson (*8*), Brakke (*9*), Halsall and Schumaker (*10*), Nason et al. (*11*), Schumaker (*12*), and Spragg et al. (*13*, *14*).

A band broadening effect in zonal centrifugation is due to a complicated phenomena of interaction of diffusion and sedimentation. In this investigation we lumped all the contributing factors together and called it dispersion coefficients.

THEORY

The equation describing sedimentation of the ith chemical species in a centrifugal force field is given by Lamm (*15*) as

$$\frac{\partial c_i}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[r\sum_{j=1}^{\nu-1} D_{ij}\frac{\partial c_j}{\partial r} - s_i\omega^2 r^2 c_i\right] \qquad (i = 1, 2, \ldots) \qquad (1)$$

with the relation

$$\sum_{i=0}^{\nu-1} \bar{v}_i c_i = 1 \qquad (0 = \text{solvent}) \tag{2}$$

where c_i is the concentration of the ith component in gram per cubic centimeter of solution; $\bar{v}_i$, the partial specific volume of the ith component; D_{ij}, the multicomponent diffusivity between i and j components; s_i, the sedimentation coefficient of the ith component; ω, the angular velocity of rotation of a rotor; and r, the radial distance from the axis of rotation. In the use of the Lamm equation, partial specific volumes were assumed to be constants.

The Lamm equations describe a material balance for the ith component in a radial sector of a cylindrical rotor; a redistributing of component i has been caused by diffusion and sedimentation within the sector. The process involves diffusion coupling with sedimentation which is so complex that we have called the term D_{ij}, dispersion coefficient instead of diffusion coefficient, which is generally used in a sense of molecular diffusion.

The analysis of moments can be used in the determination of dispersion coefficients from experimental measurements. The method is summarized below following Schumaker's derivation (*12*) for the Lamm equation. If sedimentation and diffusion coefficients are constants, the nth moment of concentration i about the center of rotation is

$$\langle r^n \rangle = \int_{r_1}^{r_2} r^n c_i \, r dr \Big/ \int_{r_1}^{r_2} c_i r dr \tag{3}$$

in which the quantities r_2 and r_1 are related to the *bandwidth*, which is a significant measure of resolution in a separation process. A bandwidth w is defined from an experimental stripchart distribution diagram in such a way that

$$w = \int_{r_1}^{r_2} f(c) \, dr/h$$

where $f(c)$ is the shape and h is the peak of the distribution diagram. The quantity

$$\int_{r_1}^{r_2} f(c) \, dr$$

is always equal to the calculated theoretical area without dispersion.

Differentiation of Eq. (3) with respect to time gives

$$\left[\frac{\partial\langle r^n\rangle}{\partial t}\right]_r = \int_{r1}^{r2} r^n \left[\frac{\partial c_i}{\partial t}\right]_r r\,dr \Big/ \int_{r1}^{r2} c_i r^n\,dr \tag{4}$$

Through substitution of Eq. (4) into the Lamm equation, Eq. (1), and integration by parts, a recursion formula between the various moments of the *i*th component is obtained.

$$\frac{\partial\langle r^n\rangle}{\partial t} = n^2 D\langle r^{n-2}\rangle + ns\omega^2\langle r^n\rangle \tag{5}$$

Integration for the second moment, $n = 2$, yields

$$\frac{\langle r^2\rangle + 2D/s\omega^2}{\langle r^2\rangle_0 + 2D/s\omega^2} = \exp\left[2\omega^2 s(t - t_0)\right] \tag{6}$$

in which subscript 0 refers to the reference band without dispersion at time t_0. The reference bandwidth can be calculated from the amount of sample and the geometry of the rotor.

If the sedimentation coefficient s and the angular velocity ω are given, the dispersion coefficient can be obtained from Eq. (6). If the sedimenta-

TABLE 1

Relevant Physical Properties of Gradient Materials and Samples[a]

Gradient (20°C)	Density (g/cc)	Viscosity[a] (cp)
Sucrose		
9%	1.0339	1.2947
10%	1.0380	1.3371
Ficoll		
9%	1.030	4.8
10%	1.033	5.0

Particle	Diameter (cm)	Sedimentation coefficient (sec)	Density (g/cc)
Polystyrene bead	9.1×10^{-6}	0.230×10^{-10}	1.04
BSA	7.2×10^{-7}	0.430×10^{-12}	1.36

[a] C. T. Rankin, Jr., and L. H. Elrod, "Useful Data for Zonal Centrifugation," The Molecular Anatomy Program, Oak Ridge National Laboratory, January, 1970.

tion coefficient is unknown, Eq. (5) can be integrated for the fourth moment, $n = 4$. Thus it gives

$$\frac{\langle r^4\rangle - 2\langle r^2\rangle^2}{\langle r^4\rangle_0 - 2\langle r^2\rangle_0^2} = \exp\left[4\omega^2 s(t - t_0)\right] \tag{7}$$

Then the sedimentation and dispersion coefficients may be obtained by simultaneous solution of Eqs. (6) and (7).

MATERIALS

Sucrose solutions were prepared from distilled water and sucrose supplied by the Mallinckrodt Chemical Works, St. Louis, Missouri, in reagent grade. Ficoll (polysucrose) solutions were also prepared from distilled water and Ficoll supplied by the Pharmacia Fine Chemicals, Uppsala, Sweden, in reagent grade. The polystyrene latex beads used as sedimenting particles were manufactured by the Dow Chemical Co.,

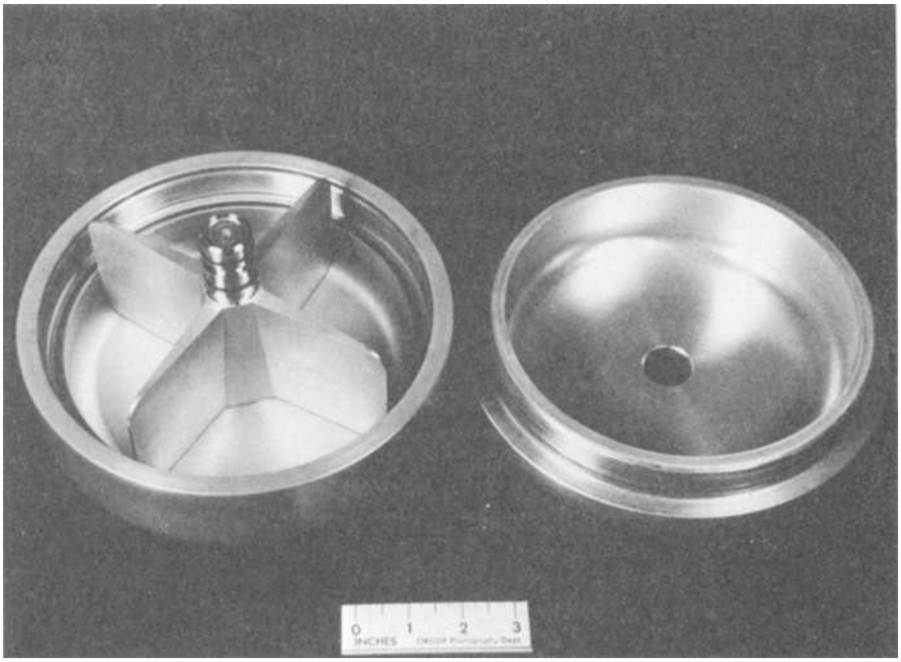

FIG. 1. Partial assembled B-XV rotor.

Midland, Michigan. The beads were $0.091 \pm 0.0058\,\mu$ in diameter. The beads were in 0.0025% w/v aqueous solution. Bovine serum albumin (BSA) was supplied by the Armour Pharmaceutical Co., Chicago, Illinois, in reagent grade. When used as a sample in sucrose gradient, BSA was in 5% w/v aqueous solution, while in Ficoll gradient the concentration was in 0.5% w/v aqueous solution. Both gradient solutions were buffered with 0.1 M NaCl solution when BSA was used as the sample material. Relevant physical properties of gradient materials and sedimenting samples are summarized in Table 1.

EXPERIMENTAL METHOD

An Oak Ridge B-XV rotor and a Spinco Model L preparative ultracentrifuge were used. A partially assembled B-XV rotor is shown in Fig. 1 and the completely assembled rotor with the removable upper seal in place is shown in Fig. 2. In Fig. 3 the rotor and seal in place in the

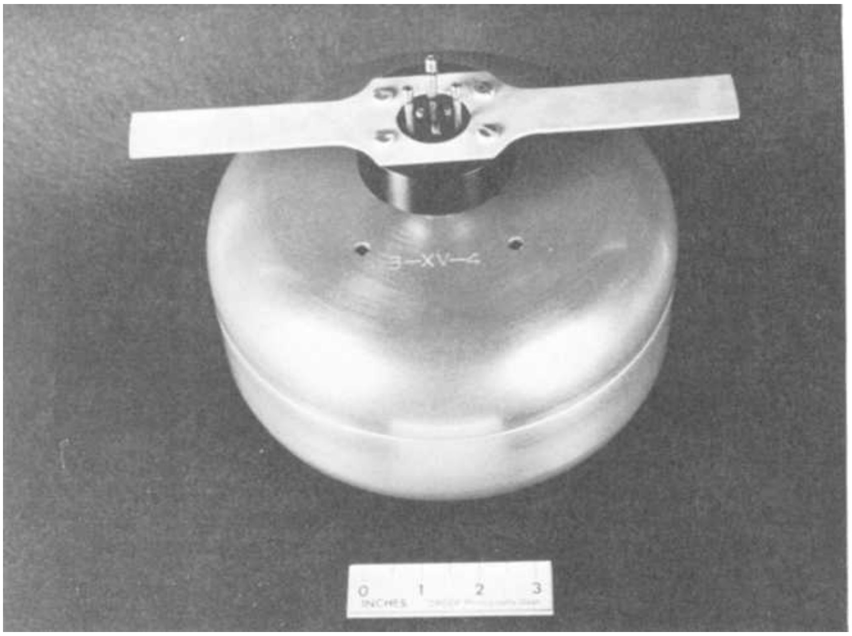

FIG. 2. Completely assembled B-XV rotor with removable upper seal in place.

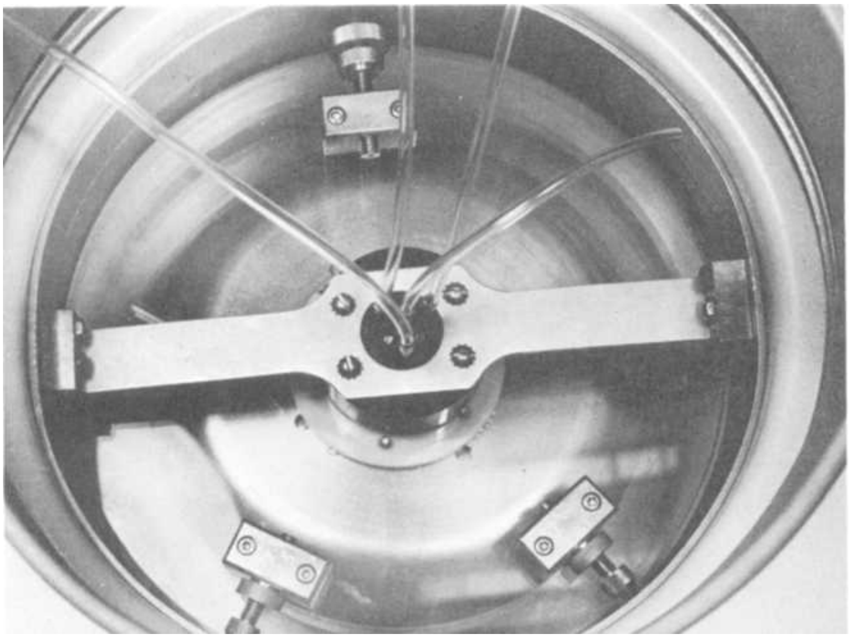

FIG. 3. Rotor and seal in place in Spinco Model L preparative ultracentrifuge.

ultracentrifuge are shown. A side view of the assembled rotor in position in the centrifuge is presented in Fig. 4. Several schematic diagrams of operations that apply to B-XV zonal centrifuge rotors are shown in Fig. 5.

Step-Gradient

In step-gradient runs the rotor was dynamically loaded while the rotor was spinning at 2500 rpm with 10% w/v sucrose until the sucrose overflowed through the exit line to Point A in Fig. 6. The exit line from the peristatic pump to Point A had been previously filled with an overlay material (9% w/v sucrose). A 5-ml sample solution was then injected into the rotor by a syringe at Point A at an approximate rate of 10 ml/min (see Fig. 5c). The sample was prepared in sucrose solution so that the resulting mixture would have 9.5% w/v sucrose density. The overlay material was then pumped into the rotor behind the sample at the rate of 20 ml/min (see Fig. 5d). The rate of pumping was controlled

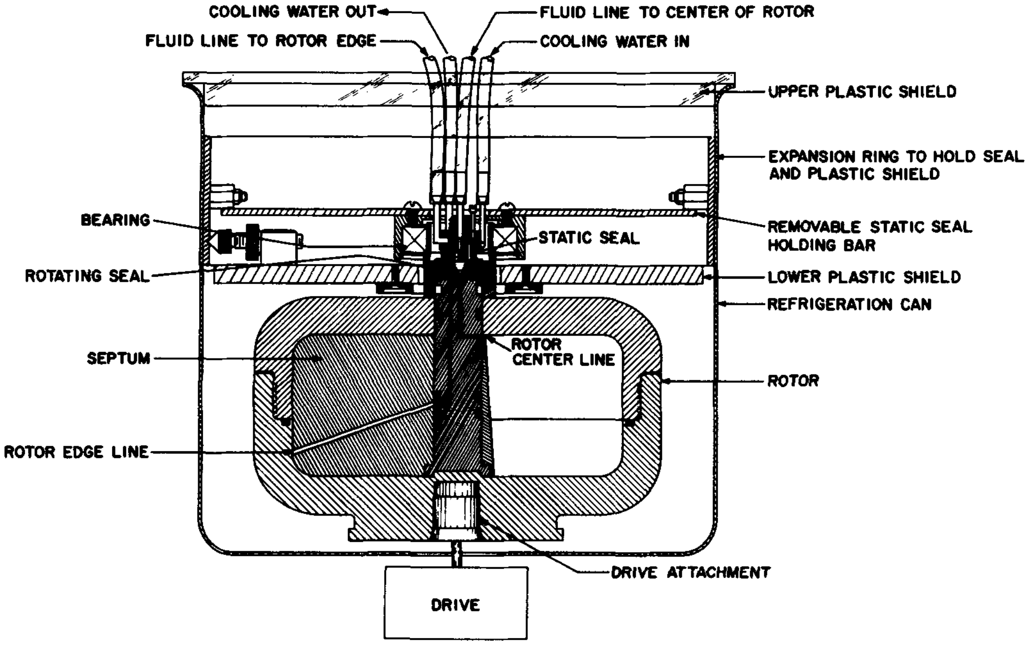

FIG. 4. Side view of assembled rotor in position in centrifuge during loading.

carefully so that uniform loading conditions were assured each time. The amount of overlay material was determined by the desired initial position of the sample zone in the rotor. In this investigation, 190 ml overlay was used in each run. A schematic diagram for a sample position in step-gradient runs is shown in Fig. 7a.

All centrifugation runs against step-gradients were made at 20°C at 2500 rpms. The count of centrifugation time began at the completion of placing the sample into the rotor and ended when unloading started.

Unloading was accomplished by pumping 10% w/v sucrose solution to the edge of the rotor, thus displacing overlay and gradient material from the center exit (see Fig. 5f). The exiting material passed through a flow cell and was scanned for optical density by a Model 270 Guilford Optical Density converter (see Fig. 5g). Optical density as a function of unloaded volume was obtained in a stripchart by a Guilford converter. The output of the converter was also monitored by a Honeywell Electronik Model 19 recorder which was used to amplify the optical density obtained.

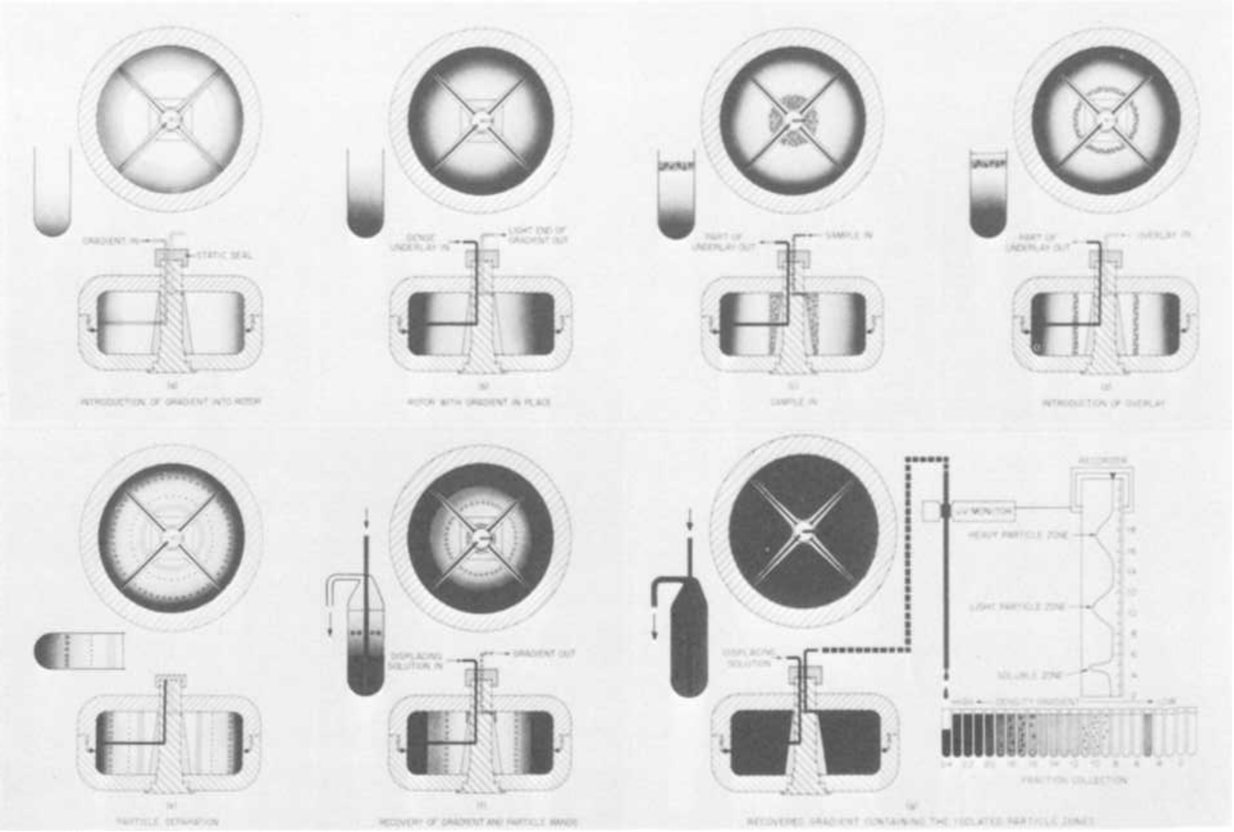

FIG. 5. Schematic diagrams of operation that apply to B-XV zonal centrifuge rotors.

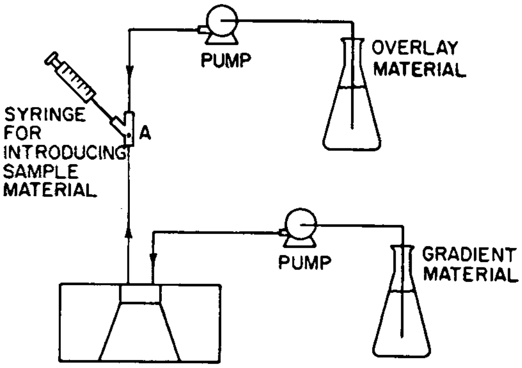

FIG. 6. Schematic diagram of rotor loading system.

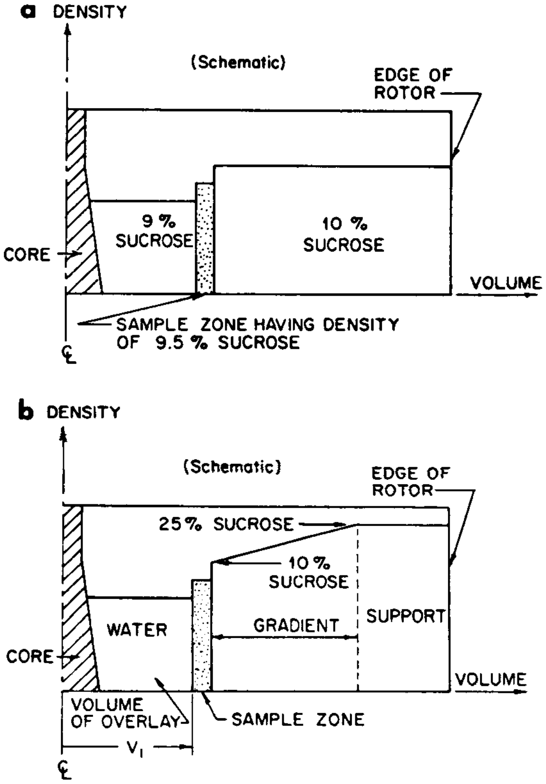

FIG. 7. Schematic diagrams of gradient solutions shapes: (a) a step-gradient solution, (b) a linear-with-volume gradient solution.

The optical density data obtained from the charts were converted to concentration. Polystyrene latex beads scatter light as well as absorb light, therefore empirical correlations were used in the conversions. For BSA, Beer's law was used in the conversion. The information on fraction cuts (volume) was converted to the radius for indication of position in the rotor.

All foregoing procedures were repeated for Ficoll gradient solution runs.

Linear-with-Volume Gradient

Linear-with-volume gradient solutions were dynamically loaded into the rotor by a Beckman gradient pump while the rotor was spinning at 2500 rpm, which allows the centrifugal force to stabilize the gradient solution. The gradient pump loaded the increased density gradient solution from the outer edge to the rotor until the desired gradient solution has been established. A cushion solution, a higher density gradient, was then introduced into the rotor from the outer edge of the rotor to force the stabilized gradient solution for sedimentation to the position of banding until the light gradient solution had overflown through the exit line to A in Fig. 6.

A 5-ml sample was then introduced into the rotor and followed with 190 ml of overlay as in the case for step-gradient solutions.

All the gradient solutions for the runs were constructed of sucrose which varied linearly with volume from 10 to 25% w/v. In common practice a 500-ml gradient solution was usually loaded with 30% w/v sucrose solution or cushion solution. The remaining procedures were the same as the case for step-gradient runs, except unloading was accomplished by pumping additional 30% w/v cushion solution from the outer edge of the septa instead of 10% w/v sucrose solution.

All foregoing procedures were repeated for Ficoll gradient solutions runs. A position of sample in linear-with-volume runs is depicted in Fig. 7b.

RESULTS AND DISCUSSION

The volume-concentration distribution data obtained from the chart were converted into the radius-concentration distribution forms for further processing of the data for both step and linear-with-volume gradient solutions. The converted results are presented graphically in

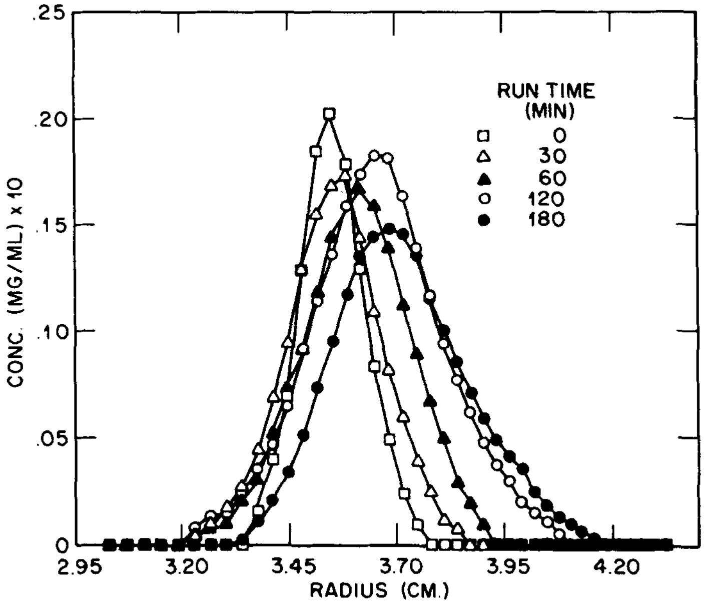

FIG. 8. Polystyrene beads banding in a step-gradient sucrose solution at various run times.

Figs. 8–11 for step gradient solutions, and the results for linear-with-volume gradient runs in Figs. 12 and 13.

Figure 8 shows the effect of sedimentation of polystyrene beads in sucrose with the leading edge of the sample zone, being at a greater radius, experiencing a larger centrifugal force and the width of the zone base continually expanding. The same beads in Ficoll gradient solution are shown in Fig. 9. The distribution curves retain a more symmetric shape and may be attributed to (a) a larger centrifugal force at the leading edge, which is offset by a fourfold increase in viscosity of Ficoll over sucrose, and (b) the viscosity gradient through the sample zone is steeper in Ficoll than in sucrose, thus offsetting the larger centrifugal force acting on the leading edge. Figures 10 and 11 show a BAS zone in sucrose and Ficoll gradient solutions. They show the same characteristic distributions as beads in the respective gradient solutions. It is interesting to note that for less than a 1-hr run the center of concentration distributions has shifted back to the axis instead of moving forward to the rotor edge. This may be attributed to the mixing of loaded sample with the gradient solution and an early rapid dispersion of gradient and overlay and of sample and overlay. This is probably an indication of the great instability that exists during this initial period of centrifugation.

Linear-with-volume gradient solution runs are shown in Figs. 12 and 13 for beads and a BSA in a sucrose solution, for less than 1 hr runs and for 1–3 hr runs, respectively. Instabilities during the initial period of centrifugation were also observed for both cases. After a certain period of centrifugation, the center of concentration distributions moved consistently toward the rotor edge. The zone base broadened with time as the leading edge advanced, while the trailing edge remained in a relatively constant position.

The second moments of each concentration distribution for step-gradient solutions were determined from Eq. (3), based on Figs. 8–13. The integration technique used was Simpson's method with 40 subintervals (5 ml increments through the 120–320 ml region of the rotor) for step-gradient solutions, and with eight subintervals (10 ml increments through the 165–245 ml region of the rotor) for linear-with-volume gradient solutions. Dispersion coefficients for each centrifugation

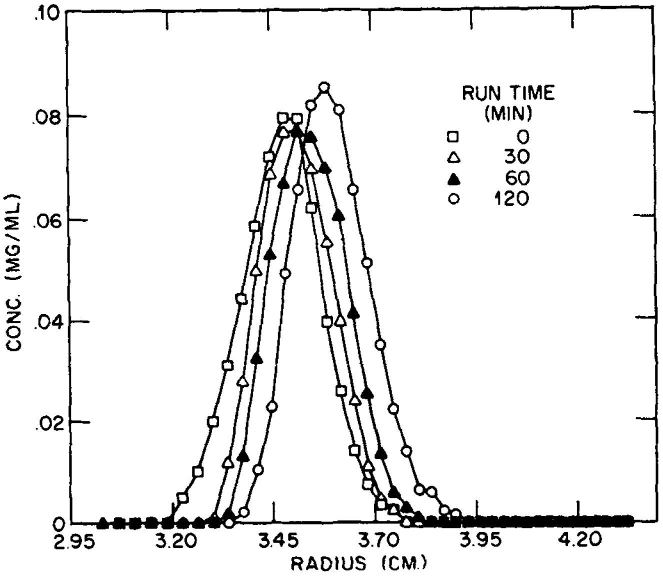

FIG. 9. Polystyrene beads banding in a step-gradient Ficoll solution at various run times.

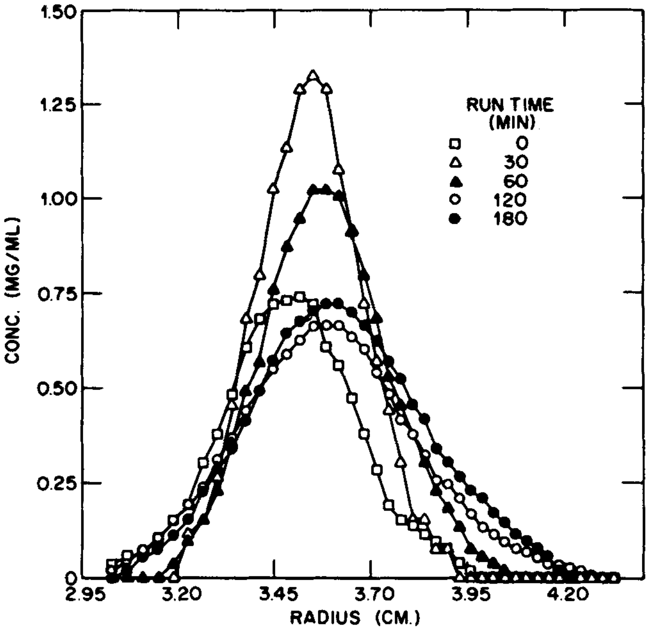

FIG. 10. BSA banding in a step-gradient sucrose solution at various run times.

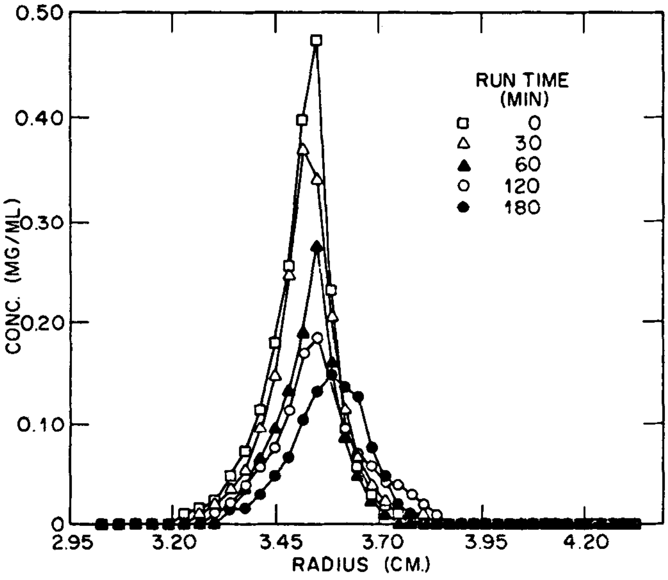

FIG. 11. BSA banding in a step-gradient Ficoll solution at various run time.

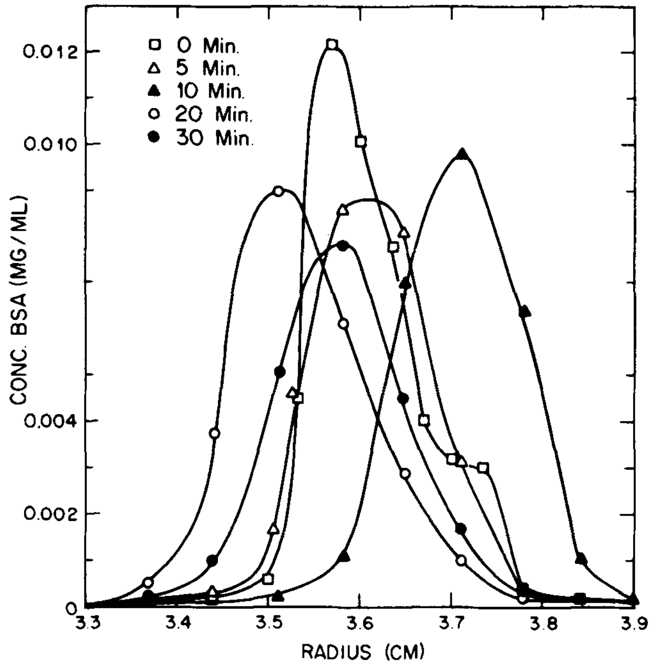

FIG. 12. Polystyrene beads banding in a linear-with-volume sucrose gradient solution at various run times.

run were also calculated from Eq. (6). Both results calculated are presented in Tables 2 and 3. Second moments ranged from 0.072 to 0.082 and dispersion coefficients ranged from 0.1×10^{-5} to 1.0×10^{-5} cm^2/sec, and both consistently decreased with centrifugation time. Dispersion coefficients as a function of centrifugation time are also presented in Fig. 14. The dispersion coefficient accounts for both eddy and molecular diffusions. From Fig. 15 we would like to suggest that the eddy contribution to the dispersion process decreases exponentially until a centrifugal work was approximately $\omega^2 t = 8.4 \times 10^8$ sec^{-1}, and after $\omega^2 t > 8.4 \times 10^8$ sec^{-1} the dispersion coefficient approaches to a constant value, a molecular diffusivity. Therefore the eddy diffusion coefficient may be estimated by subtracting the asymptotic constant from the observed dispersion coefficients.

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

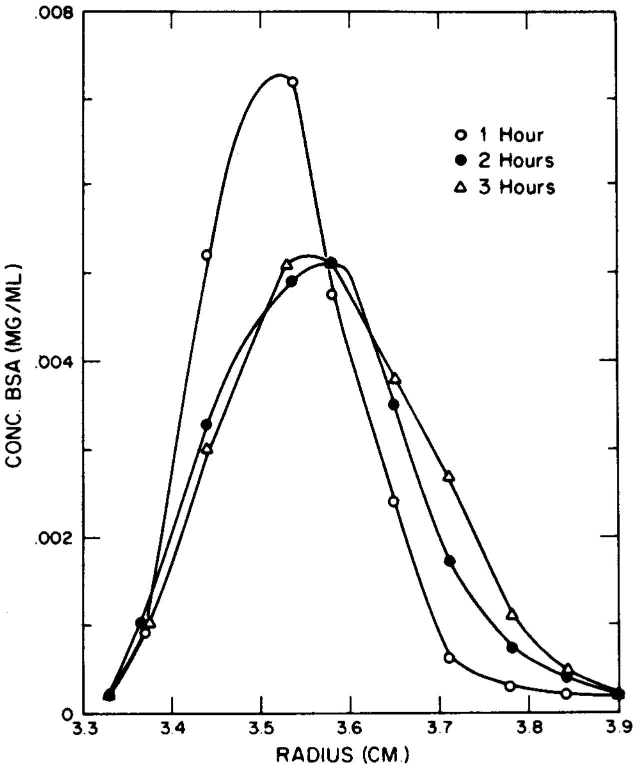

Fig. 13. BSA banding in a linear-with-volume sucrose gradient solution at various run times.

and-error method, it is found that

$$\mathrm{Sc} = 105[30F^{1.03}(\mathrm{Ta}\cdot Q)^{-1} + 19Se^{0.19}\tau]^{1.02} \tag{8}$$

fits all the data points within an average deviation of 2.5% and a maximum deviation of 6.3%. The quantities μ_0 and ρ_0 are the viscosity and density of the gradient solution evaluated over the bandwidth values, respectively. D is the dispersion coefficient between sample and a gradient solution, instead of the customary term of the diffusion coefficient. $F = \omega^2 t\rho_0 d_p/\mu_0$ is the reduced centrifugal force field strength and d_p is the size of sample dimension, and $\mathrm{Ta} = \omega^2 d_p R/\mu_0$ is the Taylor number which is equivalent to the Reynolds number in a rotating system. If $Q < 1$, a sedimentation takes place until $Q = 1$ (isopycnic

TABLE 2

Second Moments and Dispersion Coefficients from Step-Gradient Runs

Gradient	Sample	Run time (min.)	Second moment	Dispersion coefficients (cm^2/sec) × 10^5
Sucrose	Bead	0	0.07913	1.097
		30	0.07897	0.546
		60	0.07668	0.353
		120	0.07456	0.205
		180	0.07227	0.141
		720	0.07250	0.038
Ficoll	Bead	0	0.08275	1.115
		30	0.08106	0.562
		60	0.07946	0.367
		120	0.07709	0.214
Sucrose	BSA	0	0.08145	1.113
		30	0.07933	0.551
		60	0.07744	0.359
		120	0.07671	0.213
		180	0.07557	0.150
Ficoll	BSA	0	0.08093	1.124
		30	0.08042	0.558
		60	0.08022	0.371
		120	0.07885	0.219
		180	0.07799	0.155

TABLE 3

Second Moments and Dispersion Coefficients from Linear-with-Volume Sucrose Gradient Runs

Run time (min.)	Second moment	Dispersion coefficients (cm^2/sec) × 10^5
0	0.0767	1.065
5	0.0760	0.905
10	0.0727	0.758
20	0.0790	0.658
30	0.0773	0.537
60	0.0799	0.370
120	0.0781	0.217
180	0.0777	0.154

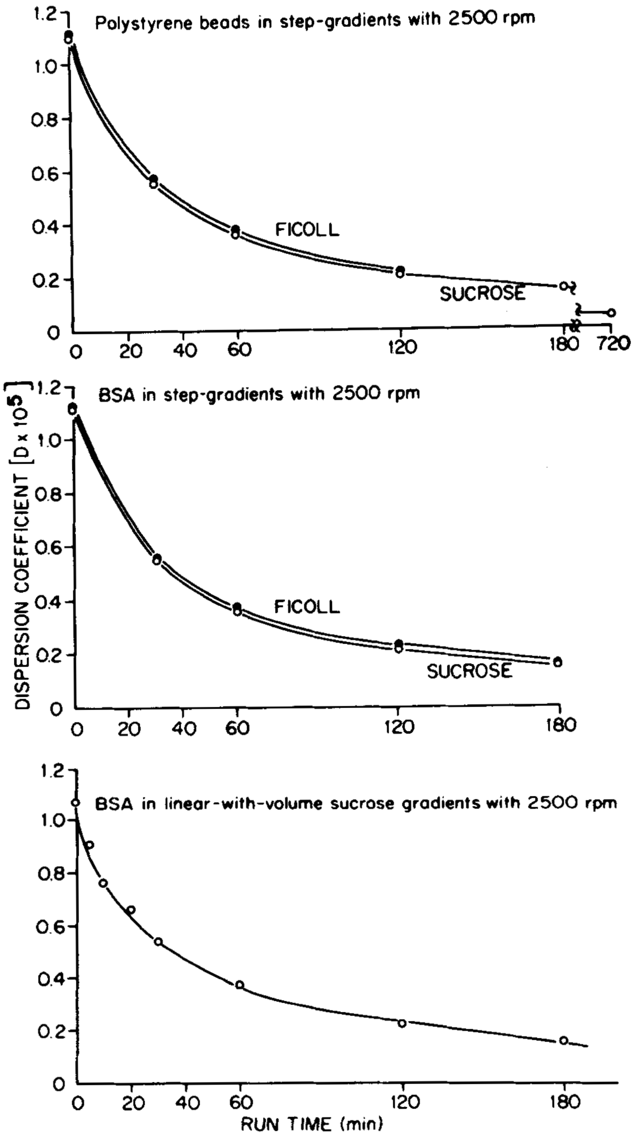

Fig. 14. Dispersion coefficients in gradient solutions at various run times.

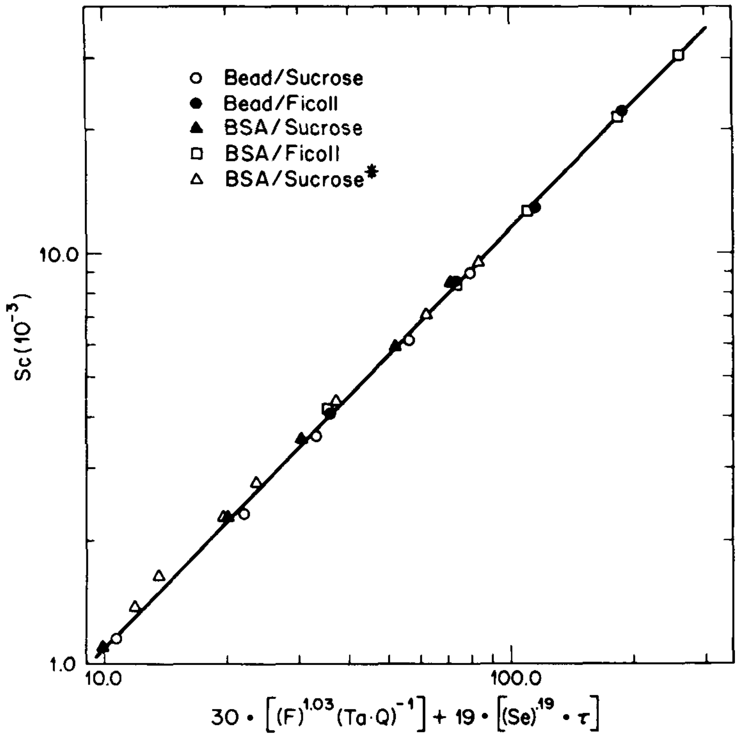

FIG. 15. A plot of Eq. (8), $\mathrm{Sc} = 105[30F^{1.03}(\mathrm{Ta}\cdot Q)^{-1} + 19Se^{0.19}\tau]^{1.02}$, and general correlation of experimental data points. (* Runs against linear-with-volume gradients which were not used in the correlation.)

point). $Se = s\omega^2\rho_0 R^2/\mu_0$ is the reduced sedimentation coefficient as defined by Hsu (*16*), and s is the local sedimentation coefficient in a gradient solution as defined by Svedberg (*17*).

The results obtained from the linear-with-volume gradient solution runs were checked against Eq. (8). 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 (8) and both correlations from a step-gradient solution and a linear-with-volume gradient solution are presented in Fig. 15.

Equation (8) and Fig. 15 may be used to estimate a dispersion coefficient on new biomaterials for which sizes of particle and their sedimentation coefficients are available.

SYMBOLS

c_i	concentration of chemical species i
d_p	size of particle
D_{ij}	dispersion coefficient
D	multicomponent diffusivity
F	reduced centrifugal force field $[= \omega^2 t \rho_0 \, d_p/\mu_0]$
n	index for moment
Q	density ratio $[= \rho/\rho_p]$
r	radial variable
R	radius of centrifugal rotor
s_i	Svedberg's sedimentation coefficient $[= dr/dt/\omega^2 r]$
Sc	Schmidt number $[= \mu_0/\rho_0 D]$
Se	reduced sedimentation coefficient $[= s\omega^2\rho_0 R^2/\mu_0]$
t	time variable
Ta	Taylor number $[= \omega^2 \, d_p R/\mu_0]$
$\bar{v}_i$	partial specific volume of chemical species i
ρ	density
μ	viscosity
τ	reduced time $[= \mu_0 t/\rho_0 R^2]$
ν	index for number of components
ω	angular velocity

Subscripts

0	average property of gradient fluid property
0	zero time as measured reference
p	particle

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