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Width of Zonal Centrifugation Bands

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NOTE

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Summary

A method is presented to obtain a true band width in zonal centrifugation runs from an experimental measurement either by a photographic method or by a light-scanning method.

In a zonal centrifugation technique, a "layer" of particles is loaded at the low-density end of a density gradient that has previously been built up in a rotor. The density gradient, which is stabilized by the centrifugal force field, is usually obtained by either sucrose or various salts dissolved in an appropriately buffered water solution. The particles settle through the density gradient at a rate depending on (a) the particle size and shape, (b) the local viscosity of the medium, (c) the difference in density between the particles and the local density of the medium, and (d) the speed of rotation of the rotor.

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Thus, if the original particle layer consisted of a mixture of particles with different properties, these different particles will, after a given period of centrifugation, appear at various points along the density gradient as bands.

As the sedimenting macromolecules move outward from the core, before reaching their respective zone of isodensity in the gradient, diffusion can cause band broadening. Consequently, the resolution on separation is greatly reduced by this effect, unless there is a measure to calibrate the design of a specific density gradient.

In this paper, we would like to point out that the true width of a zonal centrifugation band is narrower than the observed band width or the band width obtained from light scanning or photography (*1*, *2*). This is particularly true for heavy macromolecules which do not require a high centrifugal force field for separation. It is hoped that the following information will be of help to those who are working in the area of resolution broadening effects.

METHOD OF CALIBRATION

During sedimentation, the surface of each band becomes part of a paraboloid of revolution, as shown schematically in Fig. 1. The equation describing the parabolic surface of revolution is well-known and is given by Bird et al. (*3*) as

$$Z = \frac{\omega^2 r^2}{2g} + Z_0 \tag{1}$$

where Z is a vertical axial coordinate; r, a radial coordinate, as shown in Fig. 1; ω, an angular velocity; g, gravitational force; and Z_0, the minimum of Z in the paraboloid, which depends on the angular velocity and the loading location of sample. For the case of band sedimentation, the paraboloid configuration is classified as type 4 (see Ref. 4). Thus, one has

$$Z_0 < 0,\ r_t < R,\ h_p = H, \qquad (r_b < r_t \text{ always})$$

where r_t is the radius of the paraboloid at the top of rotor wall, h_p is the height at the intersection of the paraboloid with the rotor wall, and r_b is the radius of the paraboloid at the bottom of rotor wall. R and H are the radius and the height of the rotor, respectively. The true width of a sedimentation band (BW_t), referring to Fig. 1, should be

$$BW_t = 1/2[(r_{to} - r_{ti}) + (r_{bo} - r_{bi})] \tag{2}$$

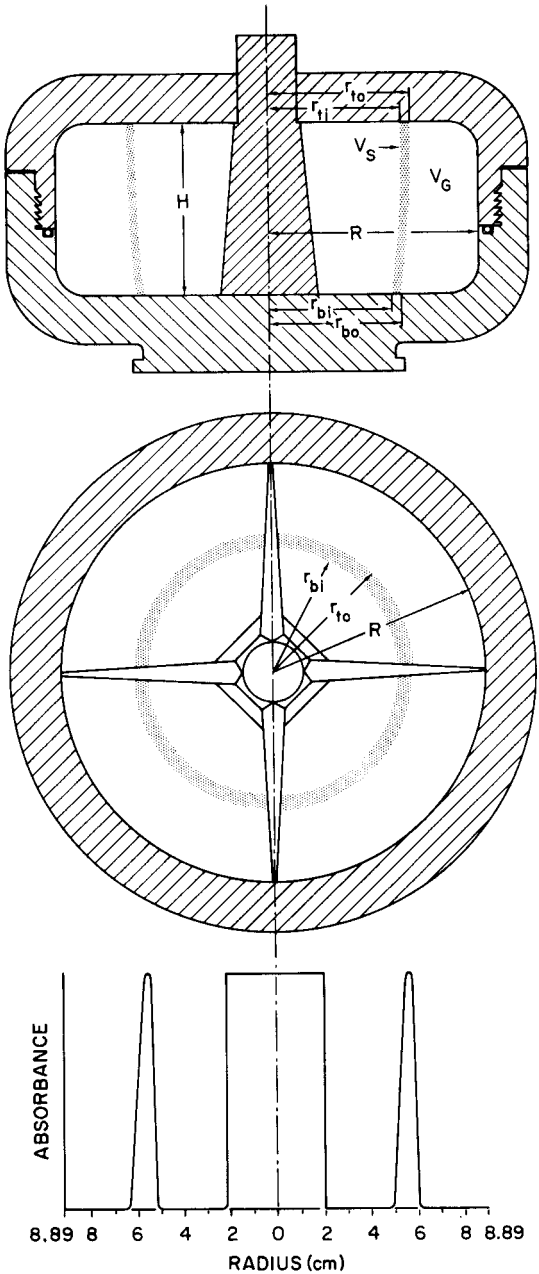

FIG. 1. Schematic diagram of a zonal centrifugation band showing an apparent band width, a true band width, and a typical scanning absorbance graph.

However, the apparent observed band width (BW_a), which is the top view of the rotor, is

$$BW_a = r_{to} - r_{bi} \tag{3}$$

The quantities, r_{to} and r_{bi}, are the measured or observed radii from experiments (see Fig. 1 for notation used). Therefore, it is necessary to know the radii r_{bi} and r_{bo} for obtaining the true width of the sedimenting band. Usually in a zonal run, volumes of gradient, sample, and overlay as indicated in Fig. 1, are known. Then, the quantities r_{ti} and r_{bo} required for the calibration can be obtained from Eq. (18) in Ref. *4*

$$r_{ti} = \left[R^2(1 - \alpha_i) + \frac{Hg}{\omega^2}\right]^{1/2} \tag{4}$$

where

$$\alpha_i = \frac{V_G + Vs}{\pi R^2 H} \tag{5}$$

in which V_G and Vs are volumes of a gradient solution on the cushion side and of a sample, respectively. The quantity of r_{bo} can be obtained from Eq. (1) by setting $Z = 0$ and using an expression, Z_0, given in Ref. *4*, Eq. (17). Thus one obtains

$$r_{bo} = \left[R^2(1 - \alpha_0) - \frac{Hg}{\omega^2}\right]^{1/2} \tag{6}$$

in which

$$\alpha_0 = \frac{V_G}{\pi R^2 H} \tag{7}$$

In the above equations, the quantity g is the acceleration of gravity [980 cm sec^{-2}], and ω, angular velocity [sec^{-1}], is equal to 2π(rpm)/60. The values of r_{ti} and r_{bo} needed for the calibration for the B-XV rotor (R = 8.89 cm, H = 7.62 cm, and total volume excluding core = 1665 cm^3) are calculated from Eqs. (4)–(6), with α varying from 0.04 to 0.70 in 0.01 increments and with ω varying from 1000 to 3000 rpm in 500 rpm increments. The numerical values are presented in Table 1.

We have made a zonal run with a B-XV rotor in which a 40 cm^3 sample of polystyrene latex beads (diameter 0.3 μ) sedimented through a 1225 cm^3 5 $\sim$ 15% sucrose gradient at 1000 rpm. From the volume-

TABLE 1

Values of r_{ti} and r_{bo} Calculated from Eqs. (4) and (6) for B-XV Rotor

	rpm									
	1000		1500		2000		2500		3000	
α	r_{ti}	r_{bo}	r_{ti}	r_{bo}	r_{ti}	r_{bo}	r_{ti}	r_{bo}	r_{ti}	r_{bo}
0.40	6.94	6.84	6.91	6.86	6.90	6.87	6.89	6.88	6.89	6.88
0.41	6.88	6.78	6.85	6.81	6.84	6.82	6.84	6.82	6.83	6.82
0.42	6.82	6.72	6.79	6.75	6.78	6.76	6.78	6.76	6.78	6.76
0.43	6.76	6.66	6.73	6.64	6.72	6.70	6.72	6.70	6.72	6.71
0.44	6.70	6.60	6.68	6.63	6.67	6.64	6.66	6.64	6.66	6.65
0.45	6.64	6.54	6.62	6.57	6.61	6.58	6.60	6.58	6.60	6.59
0.46	6.58	6.48	6.56	6.51	6.55	6.52	6.54	6.52	6.54	6.53
0.47	6.52	6.42	6.50	6.45	6.49	6.46	6.48	6.46	6.48	6.47
0.48	6.46	6.36	6.43	6.39	6.42	6.40	6.42	6.40	6.42	6.40
0.49	6.40	6.29	6.37	6.32	6.36	6.34	6.36	6.34	6.35	6.34
0.50	6.34	6.23	6.31	6.26	6.30	6.27	6.29	6.28	6.29	6.28
0.51	6.28	6.17	6.25	6.20	6.24	6.21	6.23	6.21	6.23	6.22
0.52	6.21	6.10	6.18	6.13	6.17	6.14	6.17	6.15	6.17	6.15
0.53	6.15	6.04	6.12	6.07	6.11	6.08	6.10	6.09	6.10	6.09
0.54	6.09	5.97	6.05	6.00	6.04	6.01	6.04	6.02	6.04	6.02
0.55	6.02	5.91	5.99	5.94	5.98	5.95	5.97	5.95	5.97	5.96
0.56	5.95	5.84	5.92	5.87	5.91	5.88	5.91	5.89	5.90	5.89
0.57	5.89	5.77	5.86	5.80	5.84	5.81	5.84	5.82	5.84	5.82
0.58	5.82	5.70	5.79	5.74	5.78	5.75	5.77	5.75	5.77	5.75
0.59	5.75	5.63	5.72	5.67	5.71	5.68	5.70	5.68	5.70	5.69
0.60	5.68	5.56	5.65	5.60	5.64	5.61	5.63	5.61	5.63	5.62
0.61	5.61	5.49	5.58	5.52	5.57	5.54	5.56	5.54	5.56	5.54
0.62	5.54	5.42	5.51	5.45	5.50	5.46	5.49	5.47	5.49	5.47
0.63	5.47	5.34	5.44	5.38	5.42	5.39	5.42	5.40	5.41	5.40
0.64	5.40	5.27	5.36	5.31	5.35	5.32	5.34	5.32	5.34	5.33
0.65	5.32	5.19	5.29	5.23	5.28	5.24	5.27	5.25	5.27	5.25
0.66	5.25	5.12	5.21	5.15	5.20	5.17	5.19	5.17	5.19	5.18
0.67	5.17	5.04	5.14	5.08	5.12	5.09	5.12	5.10	5.11	5.10
0.68	5.10	4.96	5.06	5.00	5.05	5.01	5.04	5.02	5.04	5.02
0.69	5.02	4.88	4.98	4.92	4.97	4.93	4.96	4.94	4.96	4.94
0.70	4.94	4.80	4.90	4.84	4.89	4.85	4.88	4.86	4.88	4.86

radius relationship for the rotor, the band width in the rotor should be 0.18 cm. However, a Ne–He laser (6328 Å) mounted on a moving bench scanning vertically through a plastic B-XV size rotor with a photocell as a receiver attached to the recorder right after the completion of loading at 1000 rpm gives a 0.32 cm band width. With the

procedure outlined above, the correction factor is 0.133 cm, which demonstrates good agreement with the predicted width. After the band broadening effect takes place, the above correction is important for studying the dispersion between the band and the gradient solution.

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