# The Capacity of Equivolumetric Gradients in Zonal Rotors in the Separation of Ribosomes[1]

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We recently described equivolumetric gradients (POLLACK and PRICE, 1971) in which the volumetric distance through which a particle zone migrates is proportional to the sedimentation coefficient of the particle, and to the $\omega^2 dt$. Equivolumetric gradients can be thought of as the sectorial or cylindrical analogs of isokinetic gradients (NOLL, 1967). The general equation is,

$$\frac{r^2(\rho_p - \rho_m(r))}{\eta_m(r)} = \text{constant}, \tag{1}$$

where $r$ is the distance from the axis of rotation,
$\rho_p$ is the density of the particle,
$\rho_m(r)$ is the density of the medium as a function of radius,
$\eta_m(r)$ is the viscosity of the medium as a function of radius.

Because the widths of particle zones remain narrow in these gradients, the resolution obtained appears superior to that with other gradients and approaches the resolution of swinging bucket rotors. In a continuing effort to explore the limits of density gradient centrifugation, we have examined several factors affecting the capacity of these gradients.

Capacity. We speak of the capacity of a gradient as the maximum mass $m$ of particles which can be loaded onto a gradient without having any region becoming more dense than the underlying gradient. BERMAN (1966) pointed out that capacity may be exceeded as a particle zone, initially at less than capacity, is narrowed by migration into a steep gradient (Fig. 1). Since the equivolumetric model relies on rather drastic gradient-induced narrowing to balance the effects of sectorial dilution (Figs. 2 and 3), we can expect that capacity will decrease progressively with increasing volume.

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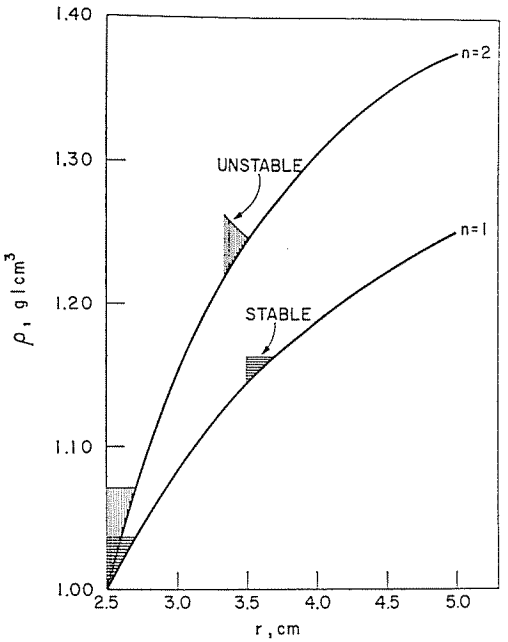


Fig. 1. Gradient-induced zone narrowing. If a gradient is sufficiently steep, the leading edge of a particle zone will be decelerated with respect to the trailing edge. This diagram (from BERMAN, 1966) illustrated how this phenomenon could cause a zone, initially stable, to become overloaded.

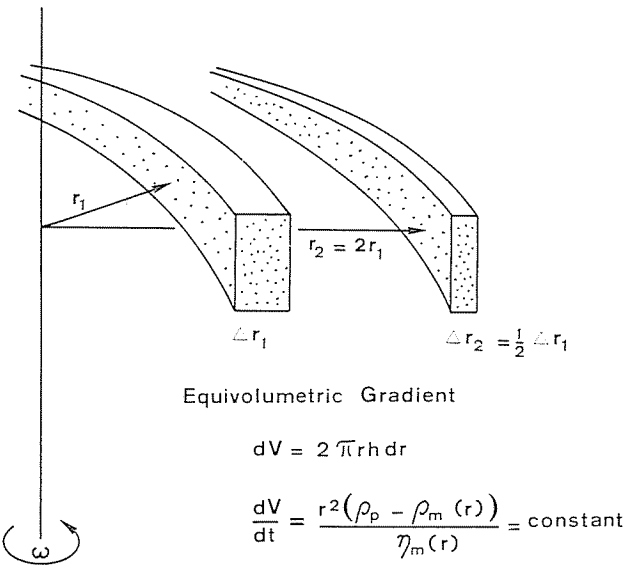


Fig. 2. Equivolumetric gradients. In an equivolumetric gradient the effect of gradient-induced narrowing is made exactly to counterbalance sectorial dilution; so that zone volumes remain constant.

For narrow, wedge-shaped zones, we can approximate the capacity as (EIKENBERRY et al., 1970) (Fig. 2)

$$m \cong \left(\frac{\bar{v}}{1-\bar{v}}\right)\left(\frac{d\rho}{dV}\right) V_s^2, \qquad (2)$$

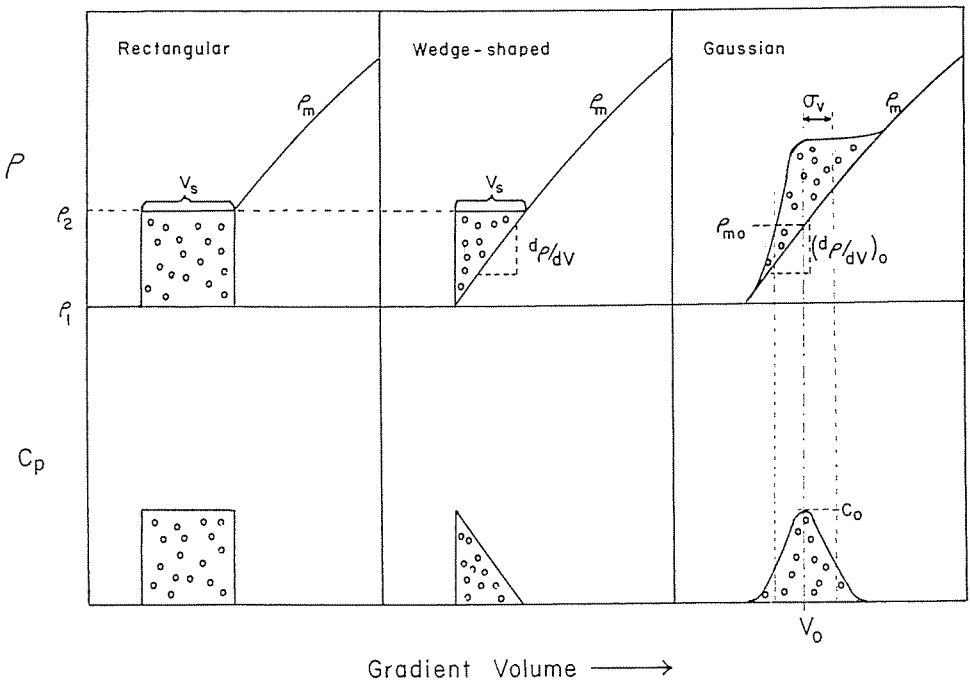


Fig. 3. Three shapes of particle zones on gradients. In the rectangular shape (left) the particle zone is homogeneous. In the wedge-shaped zone (center) the solute gradient extends through the sample zone. In the righthand figure, a Gaussian distribution of particles is superimposed on the gradient.

where $\bar{v}$ is the partial specific volume of the particles,
$\frac{d\rho_m}{dV}$ is the density gradient with respect to the volume,
$V_s$ is the volume of the particle zone.

We normally recover particles from equivolumetric gradients in Gaussian distributions with respect to volume, independently of the initial shape of the sample zone. The corresponding maximum capacity for Gaussian distributions is (VINOGRAD and BRUNER, 1966) (Fig. 3)

$$m = \frac{\sqrt{2\pi\epsilon}\,\sigma^2 \rho_p \frac{d\rho_m}{dV}}{\rho_p - \rho_0 - 2\sigma \frac{d\rho_m}{dV}}, \tag{3}$$

where $\sigma$ is the standard deviation of the particle zone,
$\rho_0$ is the density of the gradient (without particles) at the center of the particle zone.

In both Eqs. (2) and (3) we note that the density gradient $\frac{d\rho_m}{dV}$ is a factor.

Our object in testing the capacity of equivolumetric gradients was to inquire

whether the decreasing $\frac{d\rho_m}{dV}$ in fact limits the capacity of these gradients. It seems at first sight that this must be inevitable, except for two phenomena: 1. the width of particle zones gradually increases with time independently of the centrifugal field through the unexplained phenomenon of "anomalous zone broadening" (SPRAGG et al., 1969). 2. As we noted above, particles that are initially in a thin zone, whether rectangular or wedge-shaped, are recovered in zones that are Gaussian with respect to volume, for which the static capacity should be governed by Eq. (3). Thus the decreasing capacity due to decreasing $\frac{d\rho_m}{dV}$ may be offset by the spreading of the particle zone and its transformation to a Gaussian distribution.

Experimental Plan. Our object was to determine 1. if the capacity of an equivolumetric gradient was limited by the initial static capacity, i. e., the $\frac{d\rho_m}{dV}$ value at the initial sample zone, or 2. by the shallower density gradient in the middle of the rotor.

All of these tests were conducted with ribosomes from *Escherichia coli.*

## Methods and Materials

The general and specific methods of zonal centrifugation were as described previously (POLLACK and PRICE, 1971; PRICE, 1971), except that 70 S ribosomes from *E. coli* were employed. The ribosomes were obtained and purified on a Berman gradient as described by EIKENBERRY et al. (1970). The gradients contained 10 mM TRIS pH 7.4, 100 mM KCl, and, except in the initial experiments on resolution, 10 mM $MgCl_2$ throughout.

The gradients were generated in a Spinco 131 gradient pump. The rotor used was a B-30 A (International Equipment Company) in a B-60 drive unit. All experiments were conducted at a speed of 50,000 rpm for about 4 hours; reproducibility was achieved by measuring the $\int \omega^2 \, dt$ rather than time.

## Results

When a small amount of a mixture of *E. coli* ribosomes and subunits were centrifuged for $4.8 \times 10^{11}$ radians$^2 \cdot$ seconds$^{-1}$ in an equivolumetric gradient, a sedimentation profile was obtained as shown in Fig. 4. The particle zones were Gaussian with respect to volume and had $\sigma$-values of about 12 cc, as reported earlier for cytoplasmic ribosomes and subunits of *Euglena* (POLLACK and PRICE, 1971).

Capacity. In order to test capacity we first loaded increasing amounts of 70 S ribosomes in a 5-cc rectangular zone. The $\sigma$-value of the recovered particle zone increased progressively to 23 cc with 40 mg (Table I). The sedimentation profile of the 40-mg load is shown in Fig. 5.

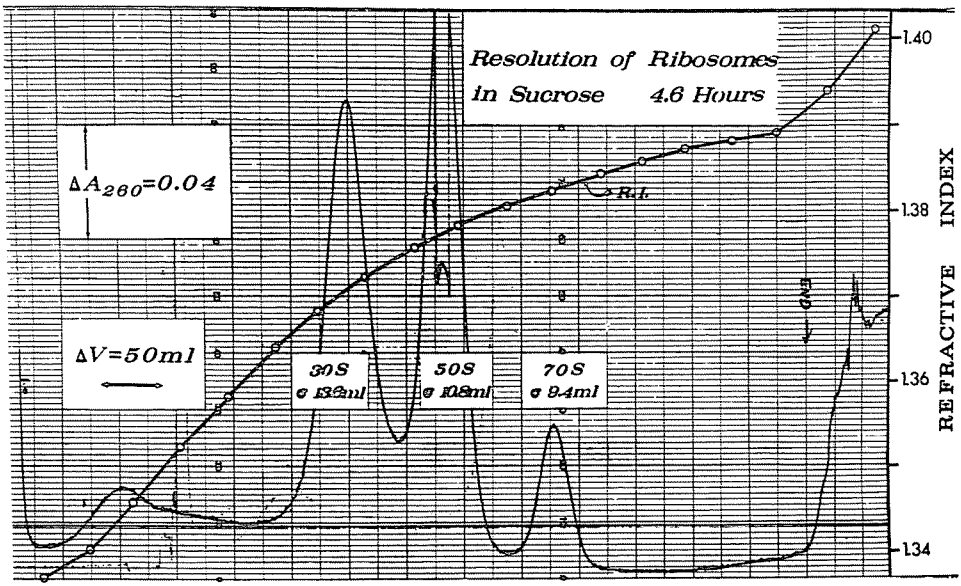


Fig. 4. Resolution of *E. coli* ribosomes and subunits in an equivolumetric gradient. Particles were centrifuged for $4.8 \times 10^{11}$ radians$^2$·seconds$^{-1}$ in a B-30 A rotor. Sedimentation is right to left.

We knew that significantly larger concentrations of ribosomes in a rectangular sample zone would inevitably show distortions due to droplet sedimentation; so we then changed to a 10-cc wedged-shape sample zone. The specific arrangement is shown in Fig. 6. A negligible quantity (5 mg) of ribosomes in this configuration sedimented into a zone of $\sigma = 22$ cc (Fig. 7). Comparing these results with those of 5-cc zones (Table I), we see that doubling the volume of the sample zone has increased the width of the recovered zone by only 50%.

*Table I.* Zone widths of 70 S ribosomes from 5-cc rectangular sample zones

The widths of the recovered zones were measured as the dispersions ($\sigma$ values), taken as the half width at 0.606 of the maximum height.

| Quantity of ribosomes and experiment number | $\sigma$-value (in cc) |
|---|---|
| 0.25 mg (68/48) | 16.9 |
| 1.2 mg (68/44) | 14.8 |
| 3.1 mg (68/46) | 16.4 |
| 12 mg (68/49) | 19.9 |
| 40 mg (68/55) | 23.5 |

We then proceeded directly to larger quantities of ribosomes, and to quantities that would certainly overload the gradient. The results (Table II and Fig. 8) quickly showed us that even with 100 mg of ribosomes, the zone of 70 S had increased to a $\sigma = 57$ cc, was distorted, and showed signs of overloading on the leading edge.

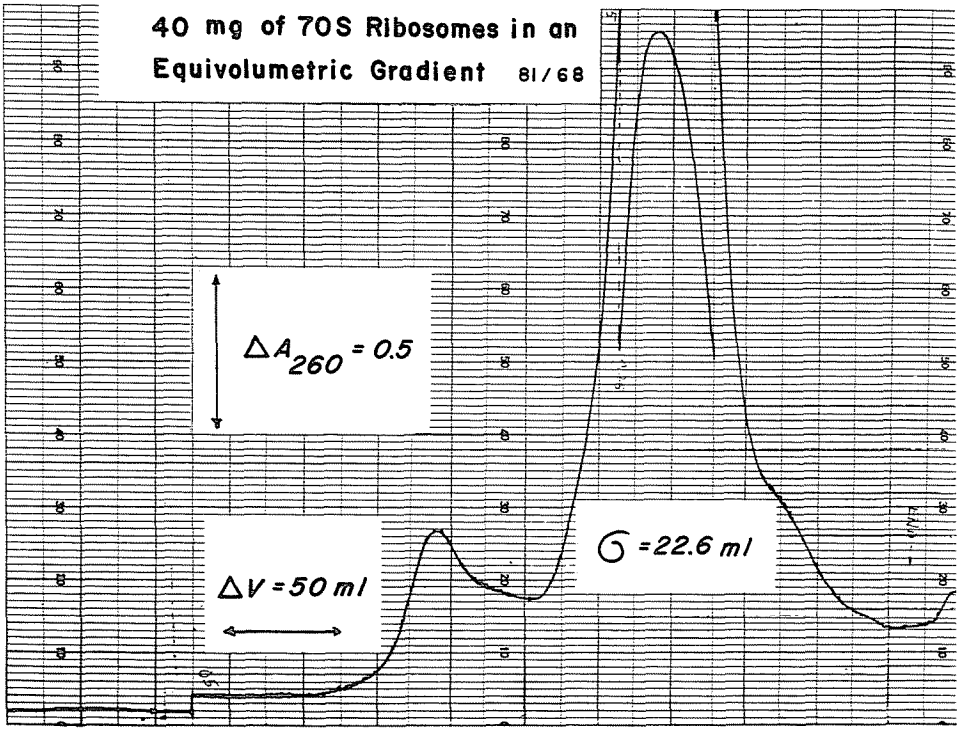


Fig. 5. Sedimentation of 40 mg of ribosomes from a rectangular sample zone. Particles were initially loaded in a 5-cc rectangular zone.

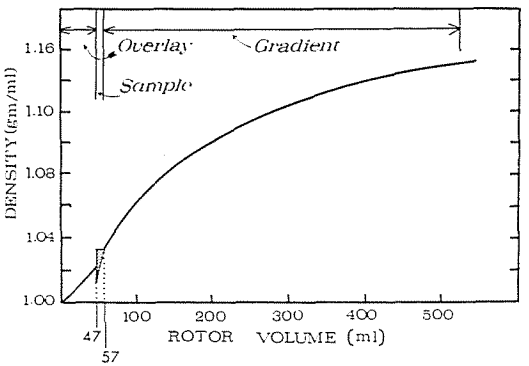


Fig. 6. Loading pattern with a 10-cc wedge-shaped sample zone.

We calculated from Eq. (2) that the capacity of the initial sample zone in these experiments should be 167 mg. If 100 mg of ribosomes were taxing the capacity of this initial sample zone, we could expect that the widths of the zones, which appear as minor bands in these experiments, should be the same as the 70 S zone. Instead we find that the subunits are recovered in zones of $\sigma$ equal to about 20 cc (Table II).

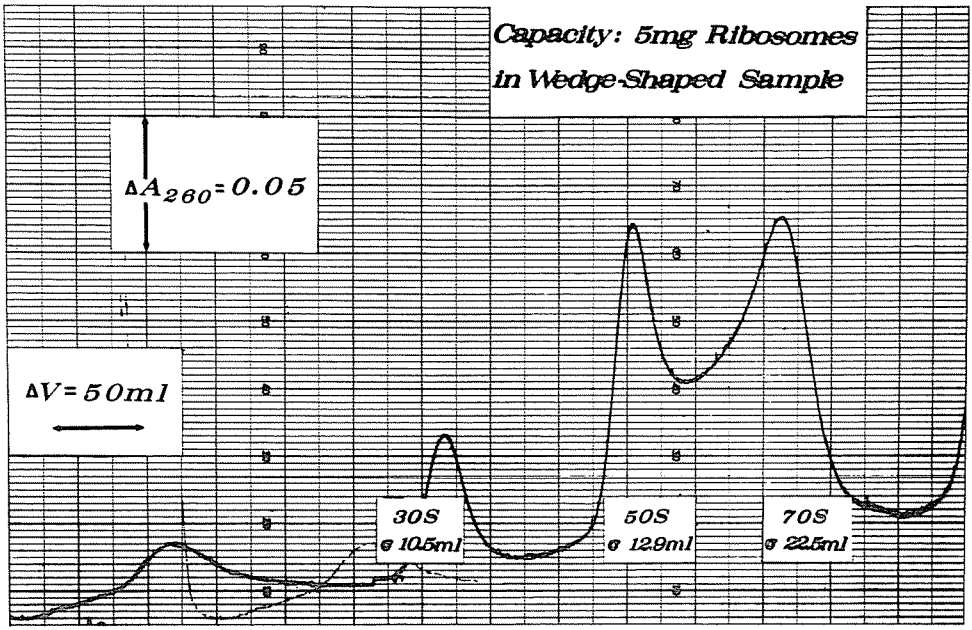


Fig. 7. Sedimentation of 5 mg of ribosomes from a 10-cc wedge-shaped sample zone. The configuration of the sample and gradient is shown in Fig. 6.

*Table II.* Zone widths of ribosomes from 10-cc wedge-shaped sample zones

The zone widths were measured as the dispersions ($\sigma$-values), taken as the half width at 0.609 of the maximum height. In addition to the 70 S ribosomes, small amounts of 30 S and 50 S subunits were also recovered in some cases and the zone widths recorded. The two experiments with 100 mg of ribosomes were conducted with different solute gradients within the sample zones. The zone shapes marked by * or ** deviated somewhat or substantially from Gaussian.

| Quantity of ribosomes and experiment number | $\sigma$-values in cc | | |
|---|---|---|---|
| | 30 S | 50 S | 70 S |
| 5 mg (85/8) | 10 | 13 | 22 |
| 100 mg (85/18)[1] | 24 | 21 | 58 |
| 100 mg (85/17)[2] | 23 | 20 | 57 |
| 250 mg (85/12) | 24 | 24 | 64 |
| 300 mg (85/9) | 28 | 26 | 64 |
| 400 mg (85/10) | 33 | 26 | 65 |

[1] 2–8% w/w sucrose gradient in sample zone.
[2] 4.5–8% w/w sucrose gradient in sample zone.

We take this as evidence that the failure of the gradient occurred after the particles had migrated sufficiently for some separation of the particle zones to occur. We asked, where did this occur?

We compute from Eq. (3) that the capacity of a Gaussian zone of $\sigma$=20 cc at

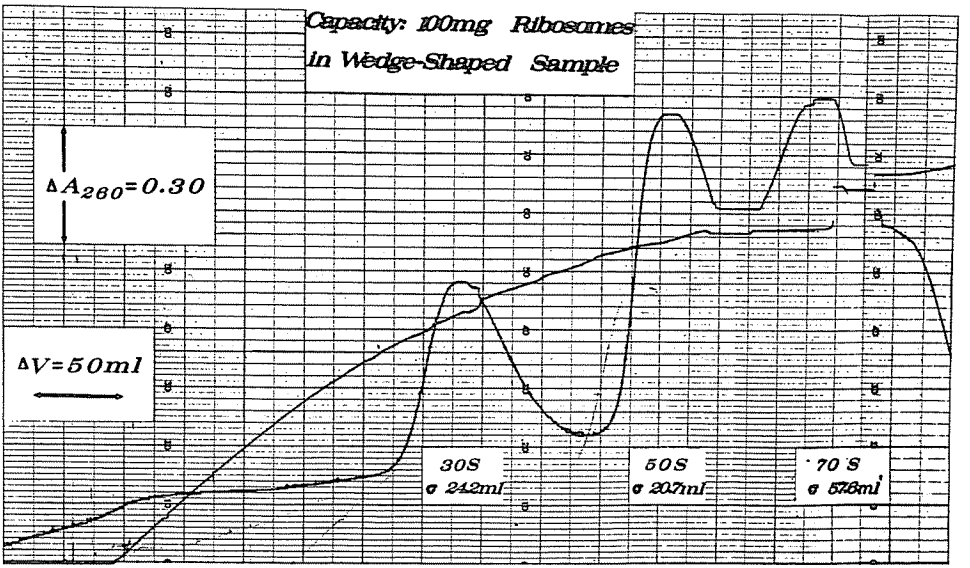


Fig. 8. Sedimentation of 100 mg of ribosomes from a 10-cc wedge-shaped sample zone. The configuration is shown in Fig. 6. The gradient within the sample zone was 4.5 to 8% w/w sucrose; results obtained with a 2–8% w/w gradient were indistinguishable.

400 cc into the gradient should be 1040 mg. Yet sedimenting particle zones containing only 10% of that value have doubled in size and shown clear indications of incipient collapse.

## Discussion

The capacity of particle zones has been examined theoretically or empirically by SVENSSON et al. (1957); BERMAN (1966); SARTORY (1969); SPRAGG and RANKIN (1967); and EIKENBERRY et al. (1970). It appears generally true from earlier work and from the study reported here that wedge-shaped sample zones may be loaded to somewhat more than half of their theoretical capacity (Eq. (2)) before substantial failure of the gradient occurs. Nonetheless large quantities of sedimenting particles come to occupy much larger volumes of the gradient than one would expect either from the behavior of smaller quantities or from the theoretical capacity of Gaussian distributions. Obviously, the available theory is inadequate.

From a practical standpoint we can conclude that 1. the limiting factor controlling the capacity of equivolumetric gradients is the relatively shallow gradient that occurs deep in the rotor, 2. for small quantities of ribosomes (under 50 mg in the B-30 A or B-XIV rotor), a rectangular sample zone gives zone widths no wider than wedge-shaped zones, and 3. that the limit for ribosomes under these conditions is about 100 mg, but that large quantities could presumably be accommodated at the cost of further dilution and poorer resolution.

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