Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Schumaker, Verne N.(1966) 'Limiting Law for Boundary Spreading in Zone Centrifugation', Separation Science and Technology, 1: 4, 409 — 411
To link to this Article: DOI: 10.1080/01496396608049456
URL: http://dx.doi.org/10.1080/01496396608049456

SEPARATION SCIENCE, 1(4), 409–411 (1966)

Limiting Law for Boundary Spreading in Zone Centrifugation*

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Summary

The superimposed density and viscosity gradient required in zone centrifuge experiments will create a gradient of sedimentation velocity which in many cases may offset the spreading of the zone due to diffusion. Equations are developed which show that, as a first approximation, the limiting shape of the sedimenting zones of macromolecules will be gaussian.

Precise determination of sedimentation coefficients by zone centrifugation has been demonstrated for virus particles (*1*), proteins (*2*), and nucleic acids (*3,4*). Zone techniques have been successfully employed in analytical as well as preparatory centrifugation (*4–6*). With the development of the zonal centrifuge (*7*), the separation as well as the characterization of relatively large quantities of macromolecular components appears feasible (*8*). Because resolution of the various components during separation procedures depends upon the width as well as the location of the migrating zones, we have become interested in the factors which govern the spread of these zones.

As a first approximation to this problem, we have decided to neglect any convective disturbance which would cause additional spreading in systems in which it occurred. Factors which might create convection, such as droplet sedimentation (*9*), the turn-over effect (*10*), or nonradial tube shape, are assumed to be absent, and

* Contribution No. 1947. This work was supported by U.S. Public Health Service Grant GM-13914.

the migration of the zones is calculated from the sedimentation and diffusion of the individual macromolecules.

In calculating band width, it becomes important to consider the gradient in viscosity and density which will always accompany a gradient in the concentration of the supporting solute, and which will cause a variation in the sedimentation coefficient of the macromolecules throughout the length of the centrifuge tube. In this paper we are interested in the state of affairs which will exist when a sizable variation in the velocity of the sedimenting particles is involved. In this case there will be a change in the sedimentation velocity of the macromolecules across the width of the migrating zone. If this change is large and negative it will balance the effects of diffusion and limit the width of the zone. It is readily shown that the sharpening effects of a small variation in sedimentation coefficient are quite great and can override even a rather large diffusive tendency. To a first approximation the resulting zones will be gaussian in shape, and the limiting width will be a function of the molecular weight of the macromolecules.

To develop equations for this effect we shall first write the expression for the sedimentation velocity, V:

$$V = s_0 \omega^2 r \frac{[1 - \bar{v}\rho]}{[1 - \bar{v}\rho_0]} \frac{\eta_0}{\eta} \tag{1}$$

In Eq. (1), the symbol s_0 is the sedimentation coefficient of the macromolecules in water at 20°C, ω is the angular velocity of the centrifuge, r is the distance from the axis of rotation, $\bar{v}$ is the partial specific volume of the macromolecules, ρ is the density of the solution and of ρ_0 that of water at 20°, and η and η_0 are the viscosities of the solution and of water at 20°C.

The migrating zone will spread due to diffusion. At the same time it will tend to sharpen, because of the sedimentation gradient. As the zone broadens, the rate of boundary spreading due to diffusion decreases, while the sharpening effect to the sedimentation velocity gradient increases. Eventually the latter will equal the former, and an equilibrium distribution will be reached. When this occurs, we may equate at any point within the spreading boundary the mass flow due to diffusion and due to the sedimentation gradient:

$$\frac{[\partial V]}{[\partial r]} xcA = D \frac{[\partial c]}{[\partial x]} A \tag{2}$$

where we have used a new symbol, x, to denote the distance from the center of the migrating zone to any position within the migrating zone.

Solving Eq. (2) for the concentration at any point within the migrating zone, c, with respect to the concentration at the center of the migrating zone, c_0 yields the following expression:

$$\frac{c}{c_0} = \exp\left(\frac{[1\ \partial V]}{[D\ \partial r]}\, x^2\right) \tag{3}$$

where, as a first approximation, $\partial V/\partial r$ is assumed to be a negative constant.

The exponent in Eq. (3) can be seen from Eq. (1) to be a function of s/D, and hence a function of the molecular weight of the macromolecules. Therefore, the limiting shape of the migrating zone is that of a gaussian curve, and the standard deviation will be inversely proportional to the square root of the molecular weight of the macromolecules.

The sharpening caused by the sedimentation gradient may be very great if the latter is deliberately chosen to maximize this effect. We suggest that the resolving power of zone centrifugation might be considerably enhanced by this means in order to fractionate materials which are reasonably close in sedimentation coefficient.

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Received by editor May 17, 1966
Submitted for publication June 8, 1966