Mass Transport Equations for Diffusion during Zone Sedimentation*

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ABSTRACT: Equations are developed to determine the diffusion coefficient during zone sedimentation. The effect on the measured diffusion coefficient of the supporting density and viscosity gradient is considered.

In the first paper in this series (Schumaker and Rosenbloom, 1965) we have derived exact equations by which sedimentation coefficients may be determined for macromolecules sedimenting as a zone through a stabilizing density gradient. For a rectangular cell in either a uniform or a centrifugal field, the migration of the center of gravity of the distribution was shown to be simply related to the sedimentation velocity of the macromolecules. For a radially shaped cell in a centrifugal field, the migration of the center of sedimentation, defined as the mass average value of the logarithm of the radius, was shown to be a function of the sedimentation coefficient. The effects of concentration dependence, polydispersity, and the presence of a superimposed viscosity and density gradient were treated in detail. Vinograd and Bruner (1966), using the method of moments and neglecting small terms, have described alternative derivations for the fundamental equations. Gehatia and Katchalski (1959) have presented a solution to the continuity equation for a zone of infinitesimal width in a sector-shaped cell, while Rubin and Katchalski (1966) have presented a solution to the continuity equation assuming an initial band of finite width containing a uniform concentration of macromolecules.

In the present paper we develop equations which allow the evaluation of diffusion coefficients from an examination of the change in shape of the sedimenting zone. Initially the zones may be of arbitrary shape and width. In the case of a rectangular cell in a uniform field, the time rate of change of the variance of the zone is shown to be directly proportional to the diffusion coefficient of the macromolecules. In the case of a rectangular or sector-shaped cell in a radial field the time rate of change of similar moment functions are shown to be proportional to the diffusion coefficient. The effect on the measured diffusion coefficient of the viscosity and density gradient and of concentration dependence of the sedimentation coefficient is considered.

In the following derivations we assume there is no convection parallel to the field. In the first three sections below, s and D are assumed to be constants.

A Cell of Uniform Cross-Sectional Area in a Homogeneous Field. Let J be the total flux across a stationary plane at position x. The relation between J and mass transport owing to sedimentation and diffusion is given by eq 1.

$$AJ + AD\left(\frac{\partial c}{\partial x}\right)_t = Avc \qquad (1)$$

The symbols D, v, c, and A represent the diffusion coefficient, the sedimentation velocity, the concentration, and the cross-sectional area. If eq 1 is multiplied by $(x - \langle x\rangle)\mathrm{d}x$ and integrated from the meniscus, a, to the cell bottom, b, the following expression is obtained

$$\int_{\mathrm{a}}^{\mathrm{b}} AJ(x - \langle x\rangle)\mathrm{d}x + \int_{\mathrm{a}}^{\mathrm{b}} AD\frac{\partial c}{\partial x}(x - \langle x\rangle)\mathrm{d}x = \int_{\mathrm{a}}^{\mathrm{b}} Avcx\mathrm{d}x - \int_{\mathrm{a}}^{\mathrm{b}} Avc\langle x\rangle\mathrm{d}x \qquad (2)$$

where

$$\langle x\rangle \equiv \int_{\mathrm{a}}^{\mathrm{b}} x\mathrm{d}m \Big/ \int_{\mathrm{a}}^{\mathrm{b}} \mathrm{d}m$$

and

$$\mathrm{d}m = Ac\mathrm{d}x$$

The quantity $\langle x\rangle$ may be called the "x coordinate of the center of gravity." If v is constant, then using the definitions of $\langle x\rangle$ and $\mathrm{d}m$ it follows immediately that the two terms on the right of eq 2 are equal but of opposite sign and therefore cancel. By this simple

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expedient, the sedimentation velocity has been removed from further consideration.

The first term on the left may be evaluated by integrating by parts. Thus the first term may be written

$$\int_{\mathrm{a}}^{\mathrm{b}} AJ(x - \langle x\rangle)\mathrm{d}x = \int_{\mathrm{a}}^{\mathrm{b}} \frac{\partial}{\partial x}\left[AJ\left(\frac{x^2}{2} - x\langle x\rangle\right)\right]_t \mathrm{d}x - \int_{\mathrm{a}}^{\mathrm{b}}\left(\frac{x^2}{2} - x\langle x\rangle\right)\left(\frac{\partial AJ}{\partial x}\right)_t \mathrm{d}x$$

For zones which have pulled away from the meniscus and not yet reached the bottom, the concentrations and mass flows vanish at the meniscus and bottom. Hence, $c_{\mathrm{a}} = c_{\mathrm{b}} = J_{\mathrm{a}} = J_{\mathrm{b}} = 0$. The perfect differential vanishes at the limits, and the last expression becomes

$$\int_{\mathrm{a}}^{\mathrm{b}} AJ(x - \langle x\rangle)\mathrm{d}x = \langle x\rangle \int_{\mathrm{a}}^{\mathrm{b}} x\left(\frac{\partial AJ}{\partial x}\right)_t \mathrm{d}x - \int_{\mathrm{a}}^{\mathrm{b}} \frac{x^2}{2}\left(\frac{\partial AJ}{\partial x}\right)_t \mathrm{d}x \qquad (3)$$

The equation of continuity may be written (Tanford, 1961)

$$A\left(\frac{\partial c}{\partial t}\right)_x = -\left(\frac{\partial AJ}{\partial x}\right)_t \qquad (4)$$

Combining eq 3 and 4, and using the definitions of $\langle x\rangle$ and dm yields

$$\int_{\mathrm{a}}^{\mathrm{b}} AJ(x - \langle x\rangle)\mathrm{d}x = \frac{\mathrm{d}}{\mathrm{d}t}\int_{\mathrm{a}}^{\mathrm{b}} \frac{1}{2}(x^2 - \langle x\rangle^2)\mathrm{d}m \qquad (5\mathrm{a})$$

where the total time derivative may be used since the definite integral is not a function of x.

Returning to eq 2, the second term on the left may be integrated by parts and evaluated at the limits to yield

$$\int_{\mathrm{a}}^{\mathrm{b}} AD\left(\frac{\partial c}{\partial x}\right)_t (x - \langle x\rangle)\mathrm{d}x + D\int_{\mathrm{a}}^{\mathrm{b}} Ac\mathrm{d}x = 0 \qquad (5\mathrm{b})$$

Substituting eq 5a and b back into eq 2 gives

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_{\mathrm{a}}^{\mathrm{b}} (x^2 - \langle x\rangle^2)\mathrm{d}m = 2D\int_{\mathrm{a}}^{\mathrm{b}} \mathrm{d}m \qquad (5\mathrm{c})$$

By expansion it follows directly that

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_{\mathrm{a}}^{\mathrm{b}} (x - \langle x\rangle)^2\mathrm{d}m = \frac{\mathrm{d}}{\mathrm{d}t}\int_{\mathrm{a}}^{\mathrm{b}} (x^2 - \langle x\rangle^2)\mathrm{d}m$$

Substituting this expression into eq 5c, and using the fences $\langle\rangle$ to denote mass-averaged quantities (Schumaker and Rosenbloom, 1965), yields

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle(x - \langle x\rangle)^2\rangle = 2D \qquad (5\mathrm{d})$$

Thus, the diffusion coefficient is equal to the time rate of change of the variance of the diffusing zone. In deriving this expression it has been necessary to assume that v and D are constants independent of x, t, and c. The initial shape of the diffusing zone may be arbitrary, however, and of finite width. Equation 5d is identical with Vinograd and Bruner's Eq. (29).

A Uniform Cell in a Centrifugal Field. The relationship between mass flow, sedimentation, and diffusion may be written as

$$AJ + AD\left(\frac{\partial c}{\partial r}\right)_t = As\omega^2 rc \qquad (6)$$

The treatment of this equation is almost completely analogous to the preceding case. To make the right-hand side, which involves the sedimentation coefficient, vanish, it is necessary to multiply by $((r - \langle r\rangle)/r)\mathrm{d}r$ and to integrate from the top to the bottom.

The left-hand side is expanded by parts, and the boundary conditions $c_{\mathrm{a}} = c_{\mathrm{b}} = J_{\mathrm{a}} = J_{\mathrm{b}} = 0$ are used. Collecting terms yields the following expression

$$-\int_{\mathrm{a}}^{\mathrm{b}} r\left(\frac{\partial AJ}{\partial r}\right)_t \mathrm{d}r + \langle r\rangle\int_{\mathrm{a}}^{\mathrm{b}} \ln r\left(\frac{\partial AJ}{\partial r}\right)_t \mathrm{d}r - \int_{\mathrm{a}}^{\mathrm{b}} ADc\frac{\langle r\rangle}{r^2}\mathrm{d}r = 0$$

The equation of continuity (eq 4) is then employed, and the final expression may be written in the form

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle\ln\langle r\rangle - \ln(r)\rangle = D\langle 1/r^2\rangle \qquad (7)$$

where

$$\langle 1/r^2\rangle \equiv \int_{\mathrm{a}}^{\mathrm{b}} (1/r^2)\mathrm{d}m \Big/ \int_{\mathrm{a}}^{\mathrm{b}} \mathrm{d}m$$

Here again, eq 7 is valid for zones of finite width and arbitrary shape, but D and s are assumed constant.

A Radially Shaped Cell in a Centrifugal Field. The relationship between J, s, and D is now

$$b\theta rJ + b\theta rD\left(\frac{\partial c}{\partial r}\right)_t = b\theta rs\omega^2 rc \qquad (8)$$

where $b\theta r$ is the cross-sectional area of the radially shaped cell. Multiplying eq 8 by $((\ln r - \langle\ln r\rangle)/r)\mathrm{d}r$ and integrating from a to b will cause the right-hand side, and, hence, the terms containing s, to vanish. Collecting terms yields the following expression

$$\langle \ln r\rangle \int_{\mathrm{a}}^{\mathrm{b}} \ln r\left(\frac{\partial(b\theta rJ)}{\partial r}\right)_t \mathrm{d}r - \int_{\mathrm{a}}^{\mathrm{b}} \frac{(\ln r)^2}{2}\left(\frac{\partial(b\theta rJ)}{\partial r}\right)_t \mathrm{d}r - D\int_{\mathrm{a}}^{\mathrm{b}} b\theta c\frac{\mathrm{d}r}{r} = 0$$

Integrating the left-hand side by parts, using the boundary conditions $c_{\mathrm{a}} = c_{\mathrm{b}} = J_{\mathrm{a}} = J_{\mathrm{b}} = 0$, and the equation of continuity yields

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle(\ln r - \langle \ln r\rangle)^2\rangle = 2D\langle 1/r^2\rangle \qquad (9)$$

Equation 9 is valid for a zone of finite width and arbitrary shape. Both s and D have been assumed to be constant.

Effects of Superimposed Density and Viscosity Gradients Due to the Presence of the Supporting Solute. If the supporting gradient can be assumed to cause a linear variation in s and D over the width of the migrating zone, then eq 8 becomes

$$b\theta rJ + b\theta r\left(D^* + \left(\frac{\partial D}{\partial r}\right)_t x\right)\left(\frac{\partial c}{\partial r}\right)_t = b\theta r^2\omega^2\left(s^* + \left(\frac{\partial s}{\partial r}\right)_t x\right)c \qquad (10)$$

where $(\partial D/\partial r)_t$ and $(\partial s/\partial r)_t$ describe the variations in D and s due to the supporting gradient across the width of the migrating boundary. These quantities may vary with time but at a particular time are assumed to be constant in r. The s^* and D^* are the values of s and D at the center of sedimentation. The x is the distance from the center of sedimentation to any point, r, and c, as usual, represents the concentration of macromolecules. If eq 10 is multiplied by $((\ln r - \langle \ln r\rangle)/r)\mathrm{d}r$ and integrated from a to b, etc., as before, and the equation of continuity employed, then an expression analogous to eq 9 results.

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle(\ln r - \langle \ln r\rangle)^2\rangle - 2D^*\langle 1/r^2\rangle = 2\omega^2\left(\frac{\partial s}{\partial r}\right)_t \langle x(\ln r - \langle \ln r\rangle)\rangle - \left(2\left(\frac{\partial D}{\partial r}\right)_t \int_{\mathrm{a}}^{\mathrm{b}} b\theta x(\ln r - \langle \ln r\rangle)\mathrm{d}c\right)\Big/\int_{\mathrm{a}}^{\mathrm{b}} \mathrm{d}m \qquad (11)$$

Equation 11 could be employed directly with experimental data, using a computer. The term involving $\partial D/\partial r$ will usually be small, however, since it involves both positive and negative values. We will assume it can be neglected. The sedimentation term may be greatly simplified. If we define r^* as the coordinate of the center of sedimentation, such that $\ln r^* \equiv \langle \ln r\rangle$ and $x = r - r^*$, then $\ln r - \langle \ln r\rangle \overset{\vee}{=} (x/r^*)$. Equation 11 becomes

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle(\ln r - \langle \ln r\rangle)^2\rangle - 2D^*\langle 1/r^2\rangle \overset{\vee}{=} 2\omega^2\left(\frac{\partial s}{\partial r}\right)_t \frac{\langle x^2\rangle}{r^*} \qquad (12)$$

The quantity $\langle x^2\rangle$ is the variance of the zone computed about the center of sedimentation.

We note that $(\partial s/\partial r)_t$ is negative and thus the effect of the gradient is to reduce the measured value of the diffusion coefficient. The term can be quite large, and may even cause sharpening. Moreover, when $D\langle 1/r^2\rangle = -(\omega^2\partial s/\partial r\langle x^2\rangle)/r^*$, then the zone will neither spread nor sharpen but continue to migrate down the centrifuge cell with constant width (Schumaker, 1966).

Effect of Self-Concentration Dependence of the Sedimentation Coefficient. If $s = s_0(1 - kc)$, then the expression analogous to eq 9 is

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle(\ln r - \langle \ln r\rangle)^2\rangle - 2D\langle 1/r^2\rangle = -2\omega^2 s_0 k\langle xc\rangle/r^* \qquad (13)$$

In deriving this expression, the effects of the supporting viscosity and density gradient, treated in the previous section, are ignored. The complexities resulting from simultaneous consideration of the dependence of s and D upon concentration as well as upon the viscosity and density gradients of the supporting solute have so far prevented a solution of the equation for the most general case.

Discussion

From the equations presented diffusion coefficients may be calculated from the time rate of change of various moment functions. An examination of the broadening of the zone with time is necessary under any approach which makes no assumptions concerning the initial distribution of the macromolecules. While this, of course, precludes calculation of diffusion coefficients from examination of the distribution at a single time point, it does have the advantage that zones of various initial shapes can be studied. Thus, experimental difficulties, such as slight mixing on layering, can be accommodated.

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