Three-Component Theory for the Approach to Sedimentation Equilibrium in a Density Gradient

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Synopsis

A three-component theory for the approach to sedimentation equilibrium in a density gradient is developed starting from the phenomenological equations of irreversible thermodynamics. It is concluded that in the limit of infinitely dilute macromolecule, the properties of the solvated molecule are being measured as might be expected.

Introduction

Meselson and Nazarian¹ have presented the equations for the approach to sedimentation equilibrium in a density gradient neglecting the selective solvation of the macromolecule. The purpose of this work is to generalize their result to the three-component case starting with the phenomenological equations of irreversible thermodynamics.

The approximations required for these calculations are similar to those used by Hearst and Vinograd in their equilibrium theory.² A Taylor expansion about the equilibrium position of the band is made for all cell variables and only first-order terms in the displacement parameter retained. Such an assumption limits the use of the theory to short liquid columns but simultaneously eliminates the computational problems associated with a radial field and a sector-shaped cell. The salt gradient is assumed to be at equilibrium at zero time.

The simplicity of the equations is a testament to the ease with which this particular three-component system can be understood in the limit of infinitely dilute macromolecule. The introduction of ionic components, which will be considered in a future publication, does not significantly complicate the basic equations derived here.

Theory

Neglecting Coriolis forces, the phenomenological equations for a three-component system in a centrifugal field are:^{3,4}

$$J_1 = L_{11}M_1(1 - \bar{v}_1\rho)\omega^2r + L_{13}M_3(1 - \bar{v}_3\rho)\omega^2r - D_{11}(dc_1/dr) - D_{13}(dc_3/dr) \quad (1)$$

$$J_3 = L_{33}M_3(1 - \bar{v}_3\rho)\omega^2r + L_{31}M_1(1 - \bar{v}_1\rho)\omega^2r - D_{33}(dc_3/dr) - D_{31}(dc_1/dr) \quad (2)$$

The M_i are molecular weights and the $\bar{v}_i$ are partial specific volumes, where the subscript 1 refers to water and the subscript 3 refers to the macromolecule. This convention is consistent with past publications of the author. The symbols ω , r , and ρ are angular velocity, distance from the center of rotation, and solution density, respectively. The J_i represent the molar flux of component i relative to the centrifuge cell. The c_i are concentrations in moles per unit volume. The phenomenological coefficients, L_{ij} , are related to the diffusion coefficients, D_{ij} , by eq. (3)

$$D_{ij} = \sum_k L_{ik}\mu_{kj} \quad (k = 1, 3) \quad (3)$$

where

$$\mu_{kj} = (\partial\mu_k/\partial c_j)_{T,P,c_k}$$

Multiplying eq. (1) by $-(D_{31}/D_{11})$ and adding to eq. (2) results in eq. (4).

$$J_3 - \frac{D_{31}}{D_{11}} J_1 = \left(D_{33} - \frac{D_{13}D_{31}}{D_{11}} \right) \left[M_3(1 + \Gamma') \left(\frac{\partial\mu_3}{\partial c_3} \right)_{\mu_1}^{-1} \times \left(1 - \frac{\bar{v}_3 + \Gamma'\bar{v}_1}{1 + \Gamma'} \rho \right) \omega^2r - \frac{dc_3}{dr} \right] \quad (4)$$

Equation (3) was used to eliminate the phenomenological coefficients from eq. (4). The solvation parameter, Γ' , is equal to $-[(M_1/M_3) - (\partial\mu_3/\partial c_1)_{c_3}]/(\partial\mu_1/\partial c_1)_{c_3}$ which is equivalent to the similar definition of Hearst and Vinograd² based on molalities in the limit of infinitely dilute component 3.

In the limit of infinitely dilute macromolecule, $D_{31} \rightarrow 0$ while D_{33} , D_{11} , and D_{13} remain finite.⁴ This is necessary in order that the flux of component 3 go to zero when the concentration of component 3 is zero. In addition J_1 is of order c_3 . Therefore to first order in c_3 we may write:

$$J_3 = D_{33}M_s(\partial\mu_3/\partial c_3)\mu_1^{-1}(1 - \bar{v}_s\rho)\omega^2r - D_{33}(dc_3/dr) \quad (5)$$

where

$$M_s = M_3(1 + \Gamma')$$

and

$$\bar{v}_s = (\bar{v}_3 + \Gamma'\bar{v}_1)/(1 + \Gamma')$$

At equilibrium $J_3 = 0$, and band position is defined by the point where $(dc_3/dr) = 0$. This position we denote r_0 , and the deviation from this position, $r - r_0$, is called δ . If all cell variables are expanded about r_0 and then substituted into eq. (5) with the retention of first-order terms in δ , eq. (6) is obtained. The subscript zero refers to the value of the variable at

r_0 . D_0 is D_{33} at r_0 . The gradient in polymer concentration, dc_3/dr , is of order δ . This fact eliminates the higher order term in the expansion of D_{33} .

$$J_3 = (D_0 c_3 / RT) M_{s,0} \delta [\bar{v}_{s,0} (d\rho/dr) + \rho_0 (d\bar{v}_{s,0}/dr)] \omega^2 r_0 - D_0 (dc_3/dr) \quad (6)$$

The substitution

$$(\partial \mu_3 / \partial c_3) \mu_1 = RT / c_3 \quad (7)$$

has been made in eq. (6). The validity of this ideality assumption has been discussed previously.² Because the effective gradient originally introduced² is defined by the relation

$$(d\rho/dr)_{\text{eff}} = (d\rho/dr) + (\rho_0 / \bar{v}_{s,0}) (d\bar{v}_{s,0}/dr)$$

eq. (6) may be further simplified by writing

$$J_3 = -(D_0 / \sigma^2) c_3 \delta - D_0 (dc_3/d\delta) \quad (8)$$

where

$$\sigma^2 = RT / M_{s,0} \bar{v}_{s,0} (d\rho/dr)_{\text{eff}} \omega^2 r_0$$

It is shown in the appendix by using the continuity equation that to first order in δ/r_0 we may write

$$\frac{dm_n}{dt} = n \int_a^b J_3 \delta^{n-1} d\delta \quad (9)$$

where a and b are the air-solution meniscus and the bottom of the cell. The moment m_n is defined by eq. (10).

$$m_n = \int_a^b c_3 \delta^n d\delta \quad (10)$$

By employing the method used by Baldwin and Shooter⁵ and Meselson and Nazarian¹ a recursion relation is obtained from eqs. (8), (9), and (10) which enables one to calculate the time dependence of various moments of the distribution.

$$dm_n/dt = -n(D_0/\sigma^2)m_n + D_0 n(n-1)m_{n-2} \quad (11)$$

Equation (11) is integrated to yield:

$$m_0 \equiv 1 \quad n = 0$$

$$m_1 = [m_1(0)] e^{-D_0 t / \sigma^2} \quad n = 1 \quad (12)$$

$$[m_2 - m_2(\infty)] = \{ [m_2(0) - m_2(\infty)] e^{-2D_0 t / \sigma^2} \} \quad n = 2 \quad (13)$$

where

$$m_2(\infty) = \sigma^2 \quad (14)$$

The symbols $m_1(0)$ and $m_2(0)$ are the first and second moments of the distribution at zero time.

These solutions are identical in form to those presented by Meselson and Nazarian.¹ It is clear from this treatment that the properties of the solvated species are being measured.

Appendix

The continuity equation for a force field with cylindrical symmetry is

$$dc/dt = -(1/r)d(Jr)/dr \quad (15)$$

Multiplying eq. (15) by δ^n and integrating from a to b we find

$$\frac{dm_n}{dt} = - \int_a^b \frac{1}{r_0 + \delta} \frac{d(Jr)}{d\delta} \delta^n d\delta \quad (16)$$

If we now expand

$$\frac{1}{r_0 + \delta} = \frac{1}{r_0} \left[1 - \frac{\delta}{r_0} + \left(\frac{\delta}{r_0} \right)^2 - \dots \right]$$

and integrate by parts, remembering that J is zero at a and at b ,

$$dm_n/dt = n \int_a^b J \delta^{n-1} d\delta - (1/r_0) \int_a^b J \delta^n d\delta + (1/r_0^2) \int_a^b J \delta^{n+1} d\delta \dots \quad (17)$$

Equation (17) is equivalent to eq. (9) to first-order in δ/r_0 . There is no justification in keeping the higher order terms here since they were not retained in any of the previous discussion.

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