

THE THEORY OF SEDIMENTATION ANALYSIS^{1, 2}

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INTRODUCTION

Ten years ago a small conference on the ultracentrifuge was held. From a perusal of the brief account of this meeting (1) it will be apparent that much of the discussion was organized around each of the several terms that appear in the familiar Svedberg molecular weight equation; further, centrifuge design and operation received much attention. It is perhaps just as well that descriptions of more than just the basic principles underlying the procedures in the laboratory and with the computing machine were not elaborate, because in the intervening years the whole theory of sedimentation analysis has undergone a profound change. The approaches to the essential working equations through descriptions

of kinetic theory and of flow in binary systems have been or are being replaced by more general thermodynamic treatments. It is in order to attempt to consolidate some of the significant changes and gains in these directions that the authors have prepared this report.

The laws of sedimentation and diffusion have been the object of much mathematical study. Outstanding for their significance in connection with later developments in ultracentrifugal analysis are the articles of Mason and Weaver bearing the titles "The Settling of Small Particles in a Fluid" (2) and "The Duration of the Transient State in the Settling of Small Particles" (4). While the teachings of these researches were restricted to sedimentation in a uniform gravitational field it was not long before the counterparts of their equations for centrifugal fields began to appear in the literature. Coincident with the contributions of Mason and Weaver came the remarkable conception and development of the ultracentrifuge by Svedberg and his associates (3). Great as were the abilities of these pioneers, one may pause to reflect whether they could have foreseen the manifold and complicated ways in which the theory (and practice) of sedimentation analysis would unfold.

Except in a comprehensive monograph, a complete treatment of the subject can no longer be presented; hence this review is not a balanced, objective account, but rather the topics described are ones with which the authors have a relatively greater acquaintance. In the treatment of the transport cases, flow equations are derived by means of the thermodynamics of irreversible processes; for the equilibrium problem the Gibbs system of thermodynamics provides the essential working equations. In these ways there are obtained mathematical relationships which are more accurate than the experiments of the present day require. An important point is that in the derivations of the expressions used for the calculation of molecular weights it is not at all necessary to assume ideal behavior (and in transport, two-component systems), although this has been quite common practice in the past.

It is a well-known fact that the sedimentation methods have enjoyed a spectacular success in protein chemistry. It is now apparent that because of his enthusiasm for the transport method the protein chemist has on occasion allowed himself to be carried to some excesses. For instance, ideal equations descriptive of behavior in two-component systems with no volume change on mixing have been used to describe the experimental observations in multicomponent and not entirely ideal systems. Apparent single translational friction coefficients have been combined with other data and assumptions to provide information about the shape and volume of protein and polysaccharide molecules, when several such coefficients must have been involved. Other experiments have been performed to give amounts of water bound per gram of protein, but the theoretical interpretation of them has been inadequate.

One of the main reasons why the experiments to determine the molecular weights of proteins have been so generally successful is because thermodynamic nonideality terms are relatively small with these globular homogeneous molecules. Again, Svedberg, Tiselius, Pedersen, and Lamm early realized that special

mathematical problems were involved in the interpretation of sedimentation experiments in which charged molecules or ions are present. The several devices designed to repress the effects of charged macro-ions, such as operation near the isoelectric point with a supporting electrolyte, have been largely effective because the ratios of charge to weight in proteins were low.

The polymer chemist, working with solutions of long flexible molecules, is often skeptical of the value of sedimentation measurements. The common optical systems, based upon ray optics, have made it necessary to work at too high concentrations of solute, with the result that serious theoretical complications are introduced in the evaluation of the experiments. In transport the pronounced dependence of the sedimentation coefficient in the now accessible ranges of solution concentration produces an abnormal sharpening of boundary gradient curves; in equilibrium it appears that the weight-average and other average molecular weights of this type of solute can be directly obtained only in ideal solutions, making almost imperative the use of the theta solvent. However, the ever-growing commercial importance of the synthetic high polymeric substances has accentuated the necessity of studying these systems in detail, and the sedimentation methods may yet come into significant use with them.

It shall be the purpose of this review then to attempt to describe what may and what may not be done in sedimentation analysis—always from the theoretical point of view—and to indicate the directions in which, in the opinion of the authors, immediate progress is most likely to be made. Each of the three sections which follow forms an entity, with its own set of equations, figures, and references.

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PART I. THE SEDIMENTATION-DIFFUSION EQUILIBRIUM

I. INTRODUCTORY REMARKS

There are many observable properties which are characteristic of systems in a state of internal balance or equilibrium. Of them, it is sedimentation equilibrium which is here involved. The effects of nonideality in any real system are so significant that the thermodynamic approach should be always employed in its study. Such an analysis possesses the advantage that the solution nonideality observed in sedimentation equilibrium may be related to that found in other kinds of measurement, notably osmotic pressure and light scattering. The purpose of the sedimentation equilibrium method is commonly supposed to be that of finding the molecular weights of macromolecular substances; actually, from the measurement of the concentration distribution in sedimentation equilibrium much valuable information regarding the thermodynamic properties of the system is made available.

The basic assumption is the validity of the first and second laws of thermodynamics. As other convenient assumptions are used they may be evaluated for the derivation of the more rigorous equations. The real point is that the desired mathematical relationship descriptive of the behavior can be derived from a single equation which expresses the criterion for equilibrium in the system: namely, that in the presence of the externally applied ultracentrifugal field the total potential of any constituent is constant in each phase, that is, at each radial distance in the cell, at equilibrium. The system may be a mixture of many components.

A. BASIC DEFINITIONS AND EQUATIONS

For a closed system of fixed composition and in a state of internal equilibrium the combination of the first and second laws of thermodynamics gives $dE = TdS - PdV$. When the body is not of fixed composition there must be introduced into the equation the variables which determine the amount of each component and hence the composition of the system. In a homogeneous phase in which there are k different substances the composition may be given in terms of the mole numbers, $n_1, n_2, n_3, \dots, n_k$ of the several substances in the whole of the phase. For variable composition the internal energy is

$$E = E(S, V, n_1, n_2, \dots, n_k)$$

and

$$dE = \left(\frac{\partial E}{\partial S}\right)_{V, n_i} dS + \left(\frac{\partial E}{\partial V}\right)_{S, n_i} dV + \sum_1^k \left(\frac{\partial E}{\partial n_i}\right)_{S, V, n_j} dn_i$$

The Gibbs chemical potential, μ_i , is defined as

$$\mu_i = \left(\frac{\partial E}{\partial n_i}\right)_{S, V, n_j}$$

thus

$$dE = TdS - PdV + \sum_i \mu_i dn_i$$

B. EQUILIBRIUM FOR TRANSFER BETWEEN PHASES

In an open system of several phases in thermal equilibrium there exists the possibility of free exchange of chemical substance between adjacent phases. It can be demonstrated that the condition of equilibrium with respect to these transfers requires that the chemical potential of each substance be the same in all of the phases.

For the sedimentation equilibrium experiment there is an externally applied centrifugal potential which must be taken into consideration. In addition, ions and charged molecules may be present in the system, giving rise to electrochemical potentials. In the presence of those additional fields of force, it is the total potential, $\bar{\mu}_i$, which is constant in all phases at equilibrium, thus:

$$\bar{\mu}_i = \mu_i - \frac{M_i \omega^2 r^2}{2} + z_i e \psi$$

In these equations, M_i is the molecular weight of the i^{th} ionic species or neutral component, ψ is the electrical potential of the phase, e is the charge per mole of protons, and z_i is the valence of the i^{th} component per mole in chemical units. In the case of systems which contain only neutral molecules the electrochemical potential term does not appear.

C. CONCENTRATION SCALES

It is often necessary to transform concentrations and activity coefficients from one concentration scale to another. Perhaps the most advantageous means for describing the composition of a solution is the use of the mole-fraction scales, but one often finds data in the literature expressed as a function of the molality of the solution. In the study of transport phenomena another description of the composition is required, one in which the concentration is specified in units of mass per volume of solution. The most common definition of ideality makes use of the mole-fraction scale. A solution ideal on this scale will not be *exactly* so on m or c scales. The transformation from one scale to another is achieved by algebraic methods.

The activity coefficients of an i^{th} component which correspond to the several concentration scales are defined by writing the corresponding expressions for μ_i , the chemical potential per mole of this component. Because their values differ it is necessary to assign different symbols to the activity coefficients (and reference potentials) of the several concentration scales.

$$\mu_i = (\mu_i)_c + RT \ln y_i c_i$$

$$\mu_i = (\mu_i)_N + RT \ln f_i N_i$$

$$\mu_i = (\mu_i)_m + RT \ln \gamma_i m_i$$

The molality, m_i , of the i^{th} component is the number of moles of i per 1000 g. of solvent. The volume concentration, c_i , may refer either to the number of grams or to the number of moles of the component per 100 ml. of solution. (Small c_i and capital C_i are often used to make this distinction, but the activity coefficient has the same value, γ_i , in both cases.) The molal scale finds frequent use in the derivation of equations, since it possesses the great advantage that m_i is independent of T and P . Volume concentration scales are convenient in experimental operations, especially where the solutes involved cannot be freed from solvent. The concentrations are then obtained by measurement of refractive index, light absorption per unit volume, etc. Equations derived in terms of molalities may be converted to expressions involving volume concentrations.

II. BINARY SYSTEMS

A. INCOMPRESSIBLE SOLUTIONS

For a solution containing two electrically neutral components, at equilibrium in a centrifugal field, component 1 will arbitrarily be termed the solvent and component 2 the solute.¹ The criteria for equilibrium in this system are (2, 15, 21):

$$\frac{dT}{dr} = 0 \quad (1)$$

$$\frac{dP}{dr} - \rho\omega^2 r = 0 \quad (2)$$

$$\frac{d\mu_1}{dr} - M_1\omega^2 r = 0 \quad (3)$$

$$\frac{d\mu_2}{dr} - M_2\omega^2 r = 0 \quad (4)$$

Here r is the distance from the center of rotation of a rotor revolving with an angular velocity ω . The quantities T , P , and ρ are the temperature, pressure, and solution density, respectively, at the point r . The chemical potentials and molecular weights of components 1 and 2 are denoted by μ_1 and μ_2 , and M_1 and M_2 , respectively, at the pressure and composition at r .

At constant temperature, the chemical potential of the solute is a function of the pressure and the molality of the solute. Then:

$$\frac{d\mu_2}{dr} = \left(\frac{\partial\mu_2}{\partial P}\right)_{m_2} \frac{dP}{dr} + \left(\frac{\partial\mu_2}{\partial m_2}\right)_P \frac{dm_2}{dr} \quad (5)$$

Furthermore,

$$\left(\frac{\partial\mu_2}{\partial P}\right)_{m_2} = \bar{V}_2 = M_2 \bar{v}_2 \quad (6)$$

¹ Use is made of the term "solution," implying as it does a distinction between solvent and solute. It is preferable to refer to a system which consists of two components.

Here \bar{V}_2 and \bar{v}_2 are the partial molal volume and partial specific volume, respectively, of the solute. Combination of equations 4, 5, and 6 then yields:

$$M_2(1 - \bar{v}_2 \rho) \omega^2 r = \left(\frac{\partial \mu_2}{\partial m_2} \right)_P \frac{dm_2}{dr} \quad (7)$$

This may be regarded as the fundamental equation for the sedimentation equilibrium of a binary system. For many purposes, it would be advantageous to replace the molality with the concentration in grams per deciliter. This can be done both simply and rigorously if it is assumed that the system is incompressible. Then,

$$M_2(1 - \bar{v}_2 \rho) \omega^2 r = \left(\frac{\partial \mu_2}{\partial c_2} \right)_P \frac{dc_2}{dr} \quad (8)$$

On this concentration scale, the chemical potential can be expressed as

$$\mu_2 = \mu_2^\circ + RT \ln c_2 y_2 \quad (9)$$

where y_2 is the appropriate activity coefficient.² Then:

$$\frac{M_2(1 - \bar{v}_2 \rho) \omega^2 r \cdot c_2}{RT} = \left\{ 1 + c_2 \left(\frac{\partial \ln y_2}{\partial c_2} \right)_P \right\} \frac{dc_2}{dr} \quad (10)$$

Equation 10 can be used for the calculation of the molecular weight from sedimentation equilibrium data, if values of the quantity $(\partial \ln y_2 / \partial c_2)_P$ are available. Strictly speaking, these values should correspond to the hydrostatic pressure in the ultracentrifuge cell (22, 32). For the time being, however, we shall neglect both the dependence of the activity coefficient on pressure and the variation of the partial specific volume with solute concentration. That this is consistent is indicated by the relation (32):

$$\frac{\partial}{\partial P} \left(\frac{\partial \ln \gamma}{\partial m} \right)_P = \frac{1}{RT} \left(\frac{\partial \bar{V}}{\partial m} \right)_P \quad (11)$$

where γ is the activity coefficient on the molality scale.

For many substances, particularly macromolecules of unknown molecular weight, the quantity $(\partial \ln y_2 / \partial c_2)_P$ is unknown, and so we require a method for extrapolating the data to infinite dilution. We shall assume that $\ln y_2$ may be represented by a series of the form

$$\ln y_2 = BM_2 c_2 + CM_2^2 c_2^2 + \dots \quad (12)$$

where B and C are unknown constants. Equation 10 then becomes:

$$\frac{M_2(1 - \bar{v}_2 \rho) \omega^2 r \cdot c_2}{RT} = (1 + BM_2 c_2 + 2CM_2^2 c_2^2 + \dots) \frac{dc_2}{dr} \quad (13)$$

² In an "ideal" system, $y_2 = 1$, and equation 10 becomes

$$\frac{M_2(1 - \bar{v}_2 \rho) \omega^2 r \cdot c_2}{RT} = \frac{dc_2}{dr}$$

This equation, or its equivalent, has been printed in many places. However, for organic high polymers in a θ -solvent there is the polydispersity to consider, and even in the simplest binary system the safe procedure is to treat data with the full equation 10.

It has been suggested by Mandelkern, Williams, and Weissberg (10) that equation 13 be used directly for the extrapolation to infinite dilution, since a fairly broad concentration range is covered in most sedimentation equilibrium experiments. With the assumption that $C = B^2/8$, equation 13 may be rearranged to yield:

$$\frac{(1 - \bar{v}_2 \rho) \omega^2 r \cdot c_2}{RT(dc_2/dr)} = \frac{1}{M_2} (1 + BMc)^2 \quad (14)$$

Thus the square root of the left side of equation 14 could be plotted versus the solute concentration. However, as has been pointed out, extreme precision in the data is required. A further objection is that small amounts of heterogeneity would markedly affect the results obtained. For these reasons it would seem preferable, at least at the present time, to integrate equation 13 over the ultracentrifuge cell in order to determine an "apparent" molecular weight, which can be then extrapolated to infinite dilution. In carrying out the integration, use is made of the relation

$$\int_a^b c_2 r dr = c_2^\circ \int_a^b r dr = c_2^\circ \frac{(b^2 - a^2)}{2} \quad (15)$$

which amounts to a statement of the conservation of mass during the experiment. The quantity c_2° is the initial concentration, and a and b are the ends of the solution column. Integration of equation 13 then yields:³

$$\frac{(1 - \bar{v}_2 \rho_1) \left(1 - \frac{\bar{v}_2 c_2^\circ}{100}\right) \omega^2 (b^2 - a^2)}{2RT} \left\{ \frac{c_2^\circ}{c_2(b) - c_2(a)} \right\} \\ = \frac{1}{M_2} + B \frac{c_2(b) + c_2(a)}{2} + \frac{2}{3} C [c_2^2(b) + c_2(b) \cdot c_2(a) + c_2^2(a)] \quad (16)$$

where ρ_1 is the density of pure solvent. The term $(1 - \bar{v}_2 c_2^\circ/100)$ arises from the fact that the solution density will be a linear function of the concentration if the partial specific volume is constant.⁴ Equation 16 represents a working equa-

³ A similar equation, in terms of differences in refractive index, has been developed by Van Holde and Baldwin (23).

⁴ Evaluation of this term involves expressing the density as

$$\rho = \rho_1 + \frac{(1 - \bar{v}_2 \rho_1)}{100} c_2$$

and the approximation

$$\int_a^b c_2^2 r dr \cong c_2^\circ \int_a^b c_2 r dr$$

Since in most cases

$$\frac{\bar{v}_2 c_2^\circ}{100} \ll 1$$

the approximation will not introduce a significant error.

tion for the extrapolation to infinite dilution. If we define the "apparent" molecular weight by

$$M_2^{\text{app}} = \frac{2RT}{(1 - \bar{v}_2 \rho_1) \left(1 - \frac{\bar{v}_2 c_2^0}{100}\right) \omega^2 (b^2 - a^2)} \left\{ \frac{c_2(b) - c_2(a)}{c_2^0} \right\} \quad (17)$$

and assume that the concentration is sufficiently low so that only the first two terms on the right of equation 16 need be retained, then there results:

$$\frac{1}{M_2^{\text{app}}} = \frac{1}{M_2} + B \left(\frac{c_2(b) + c_2(a)}{2} \right) \quad (18)$$

Both M_2^{app} and $(c_2(b) + c_2(a))/2$ are measurable quantities. Equation 18 should be compared with the expression previously proposed by Wales, Adler, and Van Holde (27) for the extrapolation of ultracentrifuge data:

$$\frac{1}{M_2^{\text{app}}} = \frac{1}{M_2} + Bc_2^0 \quad (19)$$

This equation is similar to equation 18, except that the initial concentration, c_2^0 , has been used instead of $(c(b) + c(a))/2$. These two concentrations are approximately the same, and become identical as the concentration gradients in the cell approach zero. However, the use of equation 19 in experimental work may lead to error, as shown by figure 1. A hypothetical solute with $M_2 = 2 \times 10^5$,

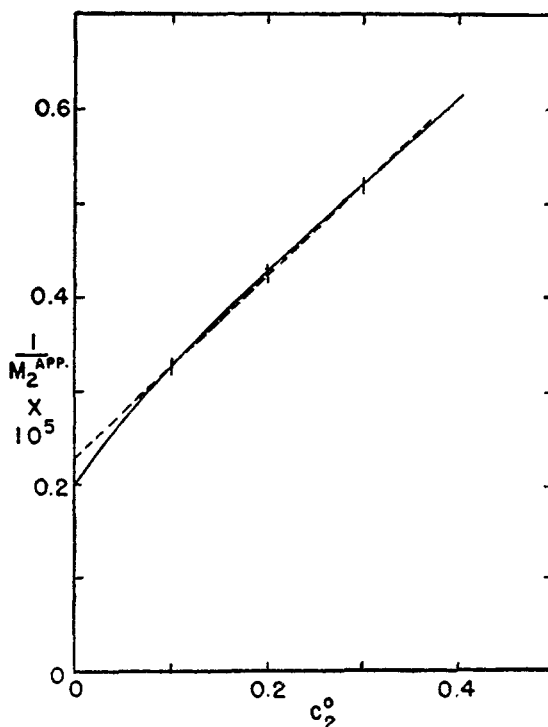


FIG. 1. Graphs of $1/M_2^{\text{app}}$ vs. c_2^0 , showing the limitation in use of equation 19

$(1 - \bar{v}_\rho) = 0.3$, $B = 1 \times 10^{-5}$, and $C = 0$ has been considered, and the quantities involved in equations 18 and 19 calculated. The solid line, which is a graph of $1/M_2^{\text{app}}$ vs. c_2^0 , shows a pronounced curvature. This curvature is such that if data obtained at concentrations above 0.1 g./100 ml. were used to extrapolate to $c_2^0 = 0$ an appreciable error in M_2 would result. The dotted line represents the results which would be attained from attempted linear extrapolation of data obtained at concentrations of 0.1, 0.2, and 0.3 g./100 ml. and a rotor velocity of 60 r.p.s. These results indicate that the use of equation 18 represents a substantial improvement over the earlier practice. At lower rotor speeds the effect is much less pronounced. Equation 18 is of the same form as the equations which are used to extrapolate light-scattering and osmotic pressure data to infinite dilution. The molecular weight obtained by such an extrapolation of the sedimentation equilibrium data is unaffected by solvation of the macromolecules, as has been shown by Goldberg (2). It must be emphasized, however, that this statement applies only to binary systems; it will be shown in the following sections that results obtained from experiments in ternary or more complex systems may be influenced by solvation.

B. PRESSURE CORRECTIONS

The theory as outlined above should be satisfactory for the analysis of sedimentation equilibrium data obtained with the precision available at the present time. However, a number of assumptions have been made which must be examined critically under special circumstances, and which will be no longer justified in general as experimental accuracy is improved. Two of these assumptions are: (1) the solution is incompressible; (2) variation of the partial specific volume with concentration, and variation of the activity coefficient with pressure are negligible. A very general integrated form of the sedimentation equilibrium equation has been obtained by Young, Kraus, and Johnson (32) for cases where the above assumptions cannot be made:

$$RT \ln \frac{m_2(r)}{m_2(a)} + RT \ln \frac{\gamma_2(m_2(r), P(a))}{\gamma_2(m_2(a), P(a))} \\ = M_2 \omega^2 \left(\frac{r^2 - a^2}{2} \right) - M_2 \omega^2 \int_a^r [\bar{v}_2(m_2(r), P(r')) \rho(m_2(r'), P(r'))] r' dr' \quad (20)$$

In this equation, the activity coefficients are referred to the pressure at the meniscus of the solution (e.g., 1 atm.). The partial specific volume is referred to the molality at the point r , but must be integrated over the range of pressure between a and r . The solution density must be integrated over the pressure and concentration between a and r . The symbol $P(r')$ represents the equilibrium pressure which exists at any point, r' , in the cell where the molality is $m_2(r')$, with r' being a variable with values from a to r .

If the proper data are available, equation 20 could be used in the form in which it is written. Since this would involve the numerical integration of the values of

\bar{v}_ρ , it is perhaps preferable to rewrite the equation in terms of a set of correction factors. The partial specific volume and density will be expressed in the form

$$\bar{v}_2(m(r), P(r')) = \bar{v}_2^\circ + g_m m(r) + g_P(P(r') - P(a)) \quad (21)$$

$$\rho(m(r'), P(r')) = \rho_1 + h_m m(r') + h_P(P(r') - P(a)) \quad (22)$$

where \bar{v}_2° and ρ_1 refer to the conditions of zero solute concentration and atmospheric pressure, and the coefficients g_m , g_P , h_m , and h_P are assumed to be independent of molality and pressure. Insertion of these equations into equation 20 and neglect of all cross-terms in small quantities yield:⁵

$$RT \ln \frac{m(r)}{m(a)} + RT \ln \frac{\gamma(m(r), P(a))}{\gamma(m(a), P(a))} = M_2 \omega^2 \left(\frac{r^2 - a^2}{2} \right) \cdot \left\{ 1 - \bar{v}_2^\circ \rho_1 \left(1 + \frac{h_m}{\rho_1} \int_a^r m(r') r' dr' + \frac{g_m}{\bar{v}_2^\circ} m(r) + \left(\frac{\rho_1 g_P}{\bar{v}_2^\circ} + h_P \right) \frac{\omega^2}{2} \left(\frac{r^2 - a^2}{2} \right) \right) \right\} \quad (23)$$

If $r = b$ we obtain, to a good approximation:

$$RT \ln \frac{m(b)}{m(a)} + RT \ln \frac{\gamma(m(b), P(a))}{\gamma(m(a), P(a))} = M_2 \omega^2 \left(\frac{b^2 - a^2}{2} \right) \cdot \left\{ 1 - \bar{v}_2^\circ \rho_1 \left(1 + \frac{h_m}{\rho_1} m_2^\circ + \frac{g_m}{\bar{v}_2^\circ} m(b) + \left(\frac{\rho_1 g_P}{\bar{v}_2^\circ} + h_P \right) \frac{\omega^2}{2} \left(\frac{r^2 - a^2}{2} \right) \right) \right\} \quad (24)$$

where m_2° is the initial molality of component 2. The equations have been left in terms of molalities, since these corrections are most likely to be employed in the determination of the activity coefficients of materials of known molecular weight. For the determination of unknown molecular weights, the concentrations should be changed to some mass-based scale (grams solute per 1000 g. solvent, for example). The coefficients in equations 21 and 22 then must be referred to this scale.

If refractometric methods are used for the determination of dm_2/dr , and hence m , other difficulties arise. Johnson, Kraus, and Young (6) have assumed that the refractive index gradient in the ultracentrifuge cell is given by

$$\frac{dn}{dr} = \left(\frac{\partial n}{\partial m} \right)_{P,T} \frac{dm}{dr} + \left(\frac{\partial n}{\partial P} \right)_{m,T} \frac{dP}{dr} + \epsilon \quad (25)$$

where the coefficients $(\partial n/\partial m)_{P,T}$ and $(\partial n/\partial P)_{m,T}$ are independent of molality and concentration. The refractive index gradient from a reference experiment is given by

$$\left(\frac{dn}{dr} \right)_0 = \left(\frac{\partial n_1}{\partial P} \right)_T \frac{dP}{dr} + \epsilon' \quad (26)$$

⁵ The approximation,

$$P(r') - P(a) \cong \int_a^{r'} \omega^2 \rho_1 r'' dr'' = \omega^2 \rho_1 \left(\frac{(r')^2 - a^2}{2} \right)$$

is also made.

where n_1 is the refractive index of the solvent. The quantities ϵ and ϵ' are corrections for the distortion of the windows of the ultracentrifuge cell and may be assumed to be the same in both experiments. If it may be further assumed that the coefficients $(\partial n/\partial P)_{m,T}$ and $(\partial n_1/\partial P)_T$ are identical, then the gradient in the molality may be calculated from:

$$\left(\frac{\partial n}{\partial m}\right)_{P,T} \frac{dm}{dr} = \frac{dn}{dr} - \left(\frac{dn}{dr}\right)_0 - \left(\frac{\partial n_1}{\partial P}\right)_T \omega^2 r (\rho - \rho_1) \quad (27)$$

In cases where the density of the solution and that of the solvent differ by only a small amount the application of the correction implied by the second term would seem doubtful, since the coefficients $(\partial n/\partial P)_{m,T}$ and $(\partial n_1/\partial P)_T$ might differ by comparable amounts.

III. TERNARY SYSTEMS

A. THE GENERAL EQUATIONS

The system solvent(1)–macromolecular solute(2)–solvent(3) is of especial interest, since it represents either the system in which a macromolecular solute is dissolved in a “mixed solvent,” or the case in which the sedimentation equilibrium of a macromolecular solute is studied in the presence of a third substance of low molecular weight. It will be shown that in such ternary systems solvation, which was of no importance for binary systems, markedly influences the result.

For simplicity, it will be assumed that the solution is incompressible, and that \bar{v}_2 , \bar{v}_3 , and ρ are independent of composition. Then the chemical potential of any component may be regarded as a function of the pressure and the molalities⁶ of any two of the components. Therefore, instead of the single equation 8, one obtains the pair of equations:

$$M_2(1 - \bar{v}_2 \rho) \omega^2 r = \left(\frac{\partial \mu_2}{\partial m_2}\right)_{m_3,P} \frac{dm_2}{dr} + \left(\frac{\partial \mu_2}{\partial m_3}\right)_{m_2,P} \frac{dm_3}{dr} \quad (28)$$

$$M_3(1 - \bar{v}_3 \rho) \omega^2 r = \left(\frac{\partial \mu_3}{\partial m_2}\right)_{m_3,P} \frac{dm_2}{dr} + \left(\frac{\partial \mu_3}{\partial m_3}\right)_{m_2,P} \frac{dm_3}{dr} \quad (29)$$

In order to simplify the results to follow, a more compact symbolism will be adopted:

$$A_i = \frac{M_i(1 - v_i \rho) \omega^2}{2RT} \quad (30)$$

$$a_{ij} = \frac{1}{RT} \left(\frac{\partial \mu_i}{\partial m_j}\right)_{m_k \neq j, P} \quad (31)$$

It can be shown (20) that:

$$a_{23} = a_{32} \quad (32)$$

⁶ The use of molalities, instead of concentrations per unit volume, simplifies the treatment.

The general sedimentation equations now become:

$$2A_2 \cdot r = a_{22} \frac{dm_2}{dr} + a_{23} \frac{dm_3}{dr} \quad (33)$$

$$2A_3 \cdot r = a_{32} \frac{dm_2}{dr} + a_{33} \frac{dm_3}{dr} \quad (34)$$

B. THE "BINDING" COEFFICIENT

To provide a thermodynamic measure of "solvation" or "binding" a number Γ will be defined as:

$$\Gamma = - \frac{(\partial \mu_3 / \partial m_2)_{m_3, P}}{(\partial \mu_3 / \partial m_3)_{m_2, P}} = - \frac{a_{32}}{a_{33}} = \left(\frac{\partial m_3}{\partial m_2} \right)_{\mu_3, P} \quad (35)$$

This quantity can be thought of as a measure of the relative solvation of the macromolecule by components 3 and 1. Thus, if it is assumed that none of component 1 is bound by the macromolecular component, then a positive value of Γ may be interpreted as the number of moles of component 3 bound by each mole of component 2. In general, it would seem wise to avoid such assumptions and regard Γ as a measure of *relative* "solvation" or "binding." Similar quantities have been used by Stockmayer (20) in interpreting the light scattering of ternary systems, and by Wales and Williams (29) in their study of the sedimentation equilibrium in systems of this kind.

Making use of the above definition, and solving equations 33 and 34 simultaneously, expressions are obtained for the molality gradients of components 2 and 3:

$$\frac{dm_2}{dr} = 2r \left\{ \frac{A_2 + \Gamma A_3}{a_{22} - \Gamma^2 a_{33}} \right\} \quad (36)$$

$$\frac{dm_3}{dr} = 2r \left\{ \frac{(a_{22}/a_{33})A_3 + \Gamma A_2}{a_{22} - \Gamma^2 a_{33}} \right\} \quad (37)$$

If these concentration gradients can be independently measured in the ultracentrifuge cell, it is possible to calculate Γ from sedimentation equilibrium data. It can be shown from equations 36 and 37 that at low concentrations of component 2,

$$\Gamma = \frac{\left(\frac{dm_3}{dr} \right) - \left(\frac{dm_3}{dr} \right)_0}{\left(\frac{dm_2}{dr} \right)} \quad (38)$$

Here the quantity $(dm_3/dr)_0$ represents the gradient of component 3 in a reference experiment, in which only components 1 and 3 are present in the same concentrations as before, i.e.,

$$\left(\frac{dm_3}{dr} \right)_0 = 2r \cdot \frac{A_3}{a_{33}} \quad (39)$$

According to equation 38, the quantity Γ determines the gradient in the molality of component 3 produced by the existence of a gradient in the molality of component 2.

Since it is sometimes advantageous, or even necessary, to use a "mixed solvent" in the determination of the molecular weight of a macromolecular substance, it is of interest to develop the equation for the extrapolation of the apparent molecular weight to $m_2 = 0$. It is assumed that the concentration gradient of component 2 can be measured independently. Then, by rearranging equation 36 and setting (20)

$$a_{22} = \frac{1}{m_2} + \beta_{22} \quad (40a)$$

and

$$a_{33} = \frac{1}{m_3} + \beta_{33} \quad (40b)$$

where

$$\beta_{22} = \left(\frac{\partial \ln \gamma_2}{\partial m_2} \right)_{P, m_3} \quad (41a)$$

and

$$\beta_{33} = \left(\frac{\partial \ln \gamma_3}{\partial m_3} \right)_{P, m_2} \quad (41b)$$

one obtains

$$\frac{1}{M_2^{\text{app}}} = \frac{(1 - \bar{v}_2 \rho) \omega^2 r \cdot m_2}{RT \left(\frac{dm_2}{dr} \right)} = \frac{\left\{ 1 + m_2 \left(\beta_{22} - \Gamma^2 \left(\frac{1}{m_3} + \beta_{33} \right) \right) \right\}}{M_2 \left\{ 1 + \Gamma \frac{M_3 (1 - \bar{v}_3 \rho)}{M_2 (1 - \bar{v}_2 \rho)} \right\}} \quad (42a)$$

For materials of unknown molecular weight, a weight-based rather than mole-based concentration scale is preferable. Molalities will be replaced by the concentrations $W_2 = M_2 m_2$, $W_3 = M_3 m_3$, in grams per 1000 g. of solvent. Similarly, the substitution $\Gamma(M_3/M_2) = \Gamma'$ will be made, where Γ' now has dimensions of grams per gram. Then equation 42a becomes

$$\frac{1}{M_2^{\text{app}}} = \frac{(1 - \bar{v}_2 \rho) \omega^2 r \cdot W_2}{RT \frac{dW_2}{dr}} = \frac{\left\{ 1 + W_2 \left(\frac{\beta_{22}}{M_2} - \frac{M_2}{M_3} \Gamma'^2 \left(\frac{1}{W_3} + \frac{\beta_{33}}{M_3} \right) \right) \right\}}{M_2 \left\{ 1 + \Gamma' \frac{(1 - \bar{v}_3 \rho)}{(1 - \bar{v}_2 \rho)} \right\}} \quad (42b)$$

and in the limit as $W_2 \rightarrow 0$:

$$M_2^{\text{app}} \rightarrow M_2 \left\{ 1 + \Gamma' \frac{(1 - \bar{v}_3 \rho)}{(1 - \bar{v}_2 \rho)} \right\} \quad (43)$$

Thus, if $\Gamma' \neq 0$ and $(1 - \bar{v}_3 \rho) \neq 0$, the presence of component 3 will have two effects on the determination of the molecular weight. In the first place, it will alter the slope of the $1/M_2^{\text{app}}$ vs. c_2 graph, and secondly, it will influence the limit approached as $c_2 \rightarrow 0$. Since the quantity $(1 - \bar{v}_3 \rho)$ may differ appreciably from

zero, the second effect may be quite serious. This difficulty was first pointed out by Lansing and Kraemer (9), who obtained equation 43 on the basis of a molecular kinetic picture of solvation. The problem has also been considered by Wales and Williams (29, 30); their treatment, while more general than that presented here in that it allows for heterogeneity of the solute, explicitly omits consideration of the nonideality of components 2 and 3.

If, as is often the case, it is not possible simultaneously to measure the concentration gradients of components 2 and 3, the problem does not seem to allow a general solution. The quantity usually measured in a sedimentation equilibrium experiment is the refractive index gradient, which for the ternary system considered will be given by

$$\frac{dn}{dr} = \phi_2 \frac{dW_2}{dr} + \phi_3 \frac{dW_3}{dr} \quad (44)$$

where ϕ_2 and ϕ_3 are differential refractive-index increments on the W scale. Equations 36 and 37 can be inserted into equation 44, and the result rearranged to yield the equation:

$$\frac{\phi_2(1 - \bar{v}_2\rho)\omega^2 r \cdot W_2}{RT \left\{ \frac{dn}{dr} - \left(\frac{dn}{dr} \right)_0 \right\}} = \frac{\left\{ 1 + W_2 \left(\frac{\beta_{22}}{M_2} - \frac{M_2}{M_3} \Gamma'^2 \left(\frac{1}{W_3} + \frac{\beta_{33}}{M_3} \right) \right) \right\}}{M_2 \left\{ 1 + \Gamma' \frac{(1 - \bar{v}_3\rho)}{(1 - \bar{v}_2\rho)} \right\} \left\{ 1 + \Gamma' \frac{\phi_3}{\phi_2} \right\}} \quad (45)$$

Here $(dn/dr)_0$ is the refractive index gradient from a "blank" experiment, where component 2 is absent. It is assumed that the concentration of component 3 is approximately the same, in both experiments, at the point in question. Since the quantity W_2 is not measurable under the conditions assumed, equation 45 cannot be generally used for extrapolation. If the sedimentation equilibrium experiments are carried out with a very short column of solution, it can be shown that at the center of the column W_2 may be replaced by the known initial concentration W_2^0 , with negligible error. In this case, equation 45 could be used, and the results extrapolated to infinite dilution. The apparent molecular weight would then approach the limit

$$M_2^{\text{app}} \rightarrow M_2 \left\{ 1 + \Gamma' \frac{(1 - \bar{v}_3\rho)}{(1 - \bar{v}_2\rho)} \right\} \left\{ 1 + \Gamma' \frac{\phi_3}{\phi_2} \right\} \quad (46)$$

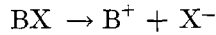
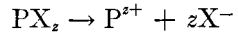
which differs from equation 43 only in the inclusion of a term in the differential refractive increments.

Perhaps it should be remarked at this point that the situation is quite different in the case of osmotic pressure measurements in mixed solvents. At high dilutions ($c_2 \rightarrow 0$), $\pi = (c_2/M)RT$, and c_2 is identical with the volume concentration of the solution with respect to the macromolecular component, even if the addition of macromolecule alters the activity coefficients of the components of the solvent. On the other hand, it has been demonstrated (20) that in light-scattering measurements both the slope and the intercept of the $1/M_2^{\text{app}}$ vs. m_2 curve may be altered by the presence of a third component.

IV. STRONG ELECTROLYTES

If the solute in a binary system ionizes, the sedimentation equilibrium will be markedly affected by this ionization. It has been shown by Tiselius (22) and by Pedersen (15) that a single solute which dissociates into $(z + 1)$ ions will yield an apparent molecular weight which is only $(1/(z + 1))$ of the true value. In the determination of the molecular weights of proteins, it has been the general practice to add an excess of a low-molecular-weight electrolyte, in order to "swamp out" the electrostatic effects which produce the above-mentioned result. The sedimentation equilibrium established in such ternary systems (macroelectrolyte, low-molecular-weight electrolyte, water) has been discussed in detail by Johnson, Kraus, and Scatchard (5).

Let us consider a system containing water, a macroelectrolyte PX_z , and a low-molecular-weight electrolyte BX . It is assumed that the solutes ionize according to the equations:



It will be further assumed that the system is incompressible and thermodynamically ideal, and that the partial specific volumes of all components and the density are independent of concentration. Then the sedimentation equilibrium equations, in terms of molalities, reduce to

$$\frac{d \ln m_{P^{z+}} \cdot m_{X^-}^z}{dr} = \frac{d \ln m_{P^{z+}}}{dr} + z \frac{d \ln m_{X^-}}{dr} = 2A_{PX_z} \cdot r \quad (47)$$

$$\frac{d \ln m_{B^+} m_{X^-}}{dr} = \frac{d \ln m_{B^+}}{dr} + \frac{d \ln m_{X^-}}{dr} = 2A_{BX} \cdot r \quad (48)$$

where

$$A_{PX_z} = \frac{M_{PX_z} (1 - \bar{v}_{PX_z} \rho) \omega^2}{2RT} \quad (49)$$

$$A_{BX} = \frac{M_{BX} (1 - \bar{v}_{BX} \rho) \omega^2}{2RT} \quad (50)$$

We also have the condition of electrical neutrality:

$$zm_{P^{z+}} + m_{B^+} = m_{X^-} \quad (51)$$

It should be noted that the molalities of the ions $m_{P^{z+}}$ and m_{B^+} are equal to the molalities of the neutral components m_{PX_z} and m_{BX} , respectively; for brevity the latter will henceforth be written as m_P and m_B . Thus, equations 47 and 48 become:

$$\frac{d \ln m_P}{dr} + z \frac{d \ln m_{X^-}}{dr} = 2A_P \cdot r \quad (52)$$

$$\frac{d \ln m_B}{dr} + \frac{d \ln m_{X^-}}{dr} = 2A_B \cdot r \quad (53)$$

If it is possible to measure simultaneously the concentrations of both neutral components, the problem of determining A_P (and hence M_P) is relatively simple. The quantity $d \ln m_x/dr$ can be eliminated between equations 52 and 53, to yield:

$$\frac{d \ln m_P}{dr} - z \frac{d \ln m_B}{dr} = 2(A_P - zA_B) \cdot r \quad (54)$$

When rearranged, and written in terms of the c scale,⁷ this becomes:

$$\frac{1}{r} \frac{d \ln c_P}{dr} = 2A_P + z \left\{ \frac{1}{r} \frac{d \ln c_B}{dr} - 2A_B \right\} \quad (55)$$

Since r , c_P , c_B , and A_B are known, the left side may be plotted versus the quantity in brackets on the right, to obtain the charge z . Even if this is only roughly known, A_P should be obtained with good precision, since the second term on the right of equation 55 will generally be small.

In many cases, however, the only information available is the refractive index gradient, dn/dr . As in Section III, this will be expressed as

$$\frac{dn}{dr} = \theta_P \frac{dc_P}{dr} + \theta_B \frac{dc_B}{dr} \quad (56)$$

where θ_P and θ_B are differential refractive-index increments on the c scale. Equations 52 and 53 may be solved simultaneously, with the aid of the condition of electrical neutrality, to yield expressions for the concentration gradients of the neutral components:

$$\frac{dc_P}{dr} = \frac{\left\{ 2A_P \left(\frac{z M_B c_P}{2 M_P c_B} + 1 \right) - A_B \cdot z \right\}}{\left\{ \frac{z^2 M_B c_P}{2 M_P c_B} + \frac{z M_B c_P}{2 M_P c_B} + 1 \right\}} \cdot c_P \cdot r \quad (57)$$

$$\frac{dc_B}{dr} = \frac{\left\{ A_B \left(\frac{M_P c_B}{M_B c_P} + z + z^2 \right) - A_P \cdot z \right\}}{\left\{ \frac{z^2 M_B c_P}{2 M_P c_B} + \frac{z M_B c_P}{2 M_P c_B} + 1 \right\}} \cdot \frac{M_B}{M_P} \cdot c_P \cdot r \quad (58)$$

It has been assumed here that the solution is sufficiently dilute for ratios of concentrations to be substituted for ratios of molalities. Equations 57 and 58 can be considerably simplified if it is permissible to neglect the term

$$\left(\frac{z M_B c_P}{2 M_P c_B} \right)$$

as compared to unity. For a solution containing 0.5 g./dl. of a macromolecule ($M = 50,000$ and $z = 20$) and 0.5 g./dl. (0.1 M) of a low-molecular-weight electrolyte ($M = 50$), we have

$$\left(\frac{z M_B c_P}{2 M_P c_B} \right) = 0.01$$

⁷ This will be permissible as long as both solutes are dilute and the solution is incompressible.

Neglecting terms of this order, equations 56, 57, and 58 are combined to obtain:

$$\frac{dn}{dr} \left\{ 1 + \frac{z^2 M_B c_P}{2 M_P c_B} \right\} = 2\theta_P c_P A^* \cdot r + \theta_B \cdot A_B \cdot c_B \cdot r \quad (59)$$

where

$$A^* = \left(1 - \frac{z M_B}{2 M_P} \cdot \frac{\theta_B}{\theta_P} \right) \left(1 - \frac{z M_B (1 - \bar{v}_B \rho)}{2 M_P (1 - \bar{v}_P \rho)} \right) \quad (60)$$

If from the above we subtract the "reference" gradient, which is given by

$$\left(\frac{dn}{dr} \right)_0 = \theta_B \cdot A_B c_B' \cdot r \quad (61)$$

the result, after neglecting the term

$$\theta_B \cdot A_B c_B \cdot r - \theta_B \cdot A_B c_B' \cdot r \left\{ 1 + \frac{z^2 M_B c_P}{2 M_P c_P} \right\}$$

and rearranging, is

$$\frac{1}{M^{app}} = \frac{(1 - \bar{v}_P \cdot \rho) \omega^2 r}{RT} \cdot \frac{c_P \cdot \theta_P}{\left\{ \frac{dn}{dr} - \left(\frac{dn}{dr} \right)_0 \right\}} = \frac{1}{M^*} \left\{ 1 + \frac{z^2 M_B}{2 M_P} \cdot \frac{c_P}{c_B} \right\} \quad (62)$$

where

$$M^* = M_P \left(1 - \frac{z M_B (1 - \bar{v}_B \cdot \rho)}{2 M_P (1 - \bar{v}_P \cdot \rho)} \right) \left(1 - \frac{z M_B \theta_B}{2 M_P \theta_P} \right) \quad (63)$$

Equation 62, like equation 45, will be suitable for use only for short columns of solution, because of the presence of the quantity c_P . However, interesting information can be obtained from a study of equation 62. In the first place, even though the system was considered ideal it can be seen that the apparent molecular weight depends upon the concentration of PX_2 . Furthermore, the correction term is inversely proportional to the concentration of the low-molecular-weight electrolyte. In the hypothetical case considered above, the quantity

$$\left(1 + \frac{z^2 M_B}{2 M_P} \cdot \frac{c_P}{c_B} \right)$$

would have the value 1.2 when the salt concentration was 0.1 M , and 1.02 when the salt concentration was 1 M . The limit approached by the apparent molecular weight at $c_2 = 0$ is also influenced. With the system considered above, if it is assumed that both components have the same partial specific volume and differential refractive-index increment, the apparent molecular weight will differ from the true value by about 2 per cent.

A somewhat different method for the analysis of the refractive index gradient has been developed by Johnson, Kraus, and Scatchard (5). They have chosen to use the experimentally measurable quantity S , defined as:

$$S = \frac{d \left(\ln \frac{1}{r} \frac{dn}{dr} \right)}{d(r^2)} \quad (64)$$

It is then shown that the degree of polymerization N of a polyelectrolyte is given by

$$N = \frac{S/A_2'}{1 - (z')^2 m_2' \cdot S} \quad (65)$$

$$\frac{2m_3 \cdot A_2'}{2m_3 \cdot A_2'}$$

The primed quantities refer to the monomer unit, and the components 2 and 3 are defined somewhat differently from the components PX_z and BX used above. If this equation is rearranged and rewritten in terms of our nomenclature, and in terms of concentration in grams per deciliter, it becomes

$$\frac{1}{\bar{S}} = \frac{1}{A_2} \left\{ \frac{1 + \frac{z^2}{2} \frac{M_B}{M_P} \cdot \frac{c_P}{c_B}}{c_B \left(1 + \frac{z}{2} \frac{M_B}{M_P} \cdot \frac{c_P}{c_B} \right)} \right\} \quad (66)$$

where

$$A_2 = A_P \left\{ 1 - \frac{z}{2} \frac{M_B}{M_P} \frac{(1 - \bar{v}_B \cdot \rho)}{(1 - \bar{v}_P \cdot \rho)} \right\} \quad (67)$$

To a good approximation, one may neglect the quantity

$$\frac{z}{2} \frac{M_B}{M_P} \frac{c_P}{c_B}$$

in the denominator of the second term on the right of equation 66, and obtain

$$\frac{\omega^2(1 - \bar{v}_P \cdot \rho)}{2RT} \cdot \frac{1}{\bar{S}} = \frac{1}{M^{**}} \left\{ 1 + \frac{z^2}{2} \frac{M_B}{M_P} \frac{c_P}{c_B} \right\} \quad (68)$$

where

$$M^{**} = M_P \left(1 - \frac{z}{2} \frac{M_B}{M_P} \frac{(1 - \bar{v}_B \cdot \rho)}{(1 - \bar{v}_P \cdot \rho)} \right) \quad (69)$$

This equation is simpler to use than equation 62, since terms involving the specific refractive-index increments are absent because of the choice of the quantity S . However, in practice it would be difficult to obtain the quantity S in an experiment with a short column of solution; on the other hand, if a long column is used the evaluation of the quantity c_P/c_B is uncertain. The situation with respect to macromolecular electrolytes may be summarized as follows: Unless a "supporting electrolyte" is added to the solution the molecular weight obtained will be only $1/(z + 1)$ of the true value. But the addition of another substance immediately complicates the picture by producing a second concentration gradient in the ultracentrifuge cell. Unless the concentrations of both components can be measured, it is necessary to resort to some degree of approximation. If the molecular weight of the macromolecule is high, or the charge is small, the error introduced will be small. Finally, there does not exist at the present time a theory which takes into account, besides the difficulties mentioned

above, the thermodynamic nonideality of the system. Presumably, such nonideality would modify the coefficient which multiplies the factor c_P/c_B in equations 62 and 66. This, however, has not been demonstrated.

V. SEDIMENTATION EQUILIBRIUM IN A DENSITY GRADIENT

A novel type of sedimentation equilibrium experiment has recently been described by Meselson, Stahl, and Vinograd (12). The system contains, in addition to the solvent, a dilute macromolecular component and a moderately concentrated low-molecular-weight salt of high density. The sedimentation equilibrium of the latter component produces a density gradient in the cell; roughly speaking, if conditions are properly chosen the quantity $(1 - \bar{v}\rho)$ for the macromolecular component will then be zero at some point in the cell; this component will then be distributed, at equilibrium, in a fairly narrow band about this point.

The theory which Meselson, Stahl, and Vinograd have developed for this type of experiment considers a macromolecular electrolyte, PX_n , and a low-molecular-weight 1-1 electrolyte, XY . The following assumptions have been made:

1. Deviations from thermodynamic ideality are neglected.
2. The concentration of XY and the solution density are linear over short distances in the cell:

$$c_{XY}(r) = c_{XY}(r_0) + \left(\frac{dc_{XY}}{dr}\right)_{r_0} (r - r_0) \quad (70)$$

$$\rho(r) = \rho(r_0) + \left(\frac{d\rho}{dr}\right)_{r_0} (r - r_0) \quad (71)$$

3. The solution is incompressible, and the partial specific volume and charge (n) of the macromolecule are independent of r , at least over short distances in the cell.

With the above assumptions, and a few minor approximations, the concentration distribution for the macroelectrolyte is calculated to be:

$$c_{PX_n}(r) = c_{PX_n}(r_0) \exp\left\{-\frac{(r - r_0)^2}{2\sigma^2}\right\} \quad (72)$$

The concentration of the macromolecular electrolyte is seen to be Gaussian, with a maximum at r_0 and a standard deviation of σ . The density of the solution at r_0 is shown to be

$$\rho(r_0) = \frac{1}{\bar{v}_{PX_n}} \left(\frac{1 - nRT(dc_{XY}/dr)_{r_0}}{M_{PX_n} \omega^2 \cdot r_0 \cdot c_{XY}(r_0)} \right) \quad (73)$$

and the standard deviation is given by

$$\sigma^2 = \frac{RT}{M_{PX_n} \bar{v}_{PX_n} \left(\frac{d\rho}{dr}\right)_{r_0} \cdot \omega^2 \cdot r_0} \quad (74)$$

Equations 73 and 74 indicate that from an experiment of this type both the molecular weight and the partial specific volume of the solute can be obtained.

For materials which are available in only small amounts, this is a great advantage. Furthermore, it is shown that for a heterogeneous solute both the number-average and the weight-average molecular weights (*cf.* Section VI) can be calculated.

It is apparent that Meselson, Stahl, and Vinograd have provided a new idea in sedimentation analysis. To develop it further one might write equations to describe the influence of "solvation" or "binding"; in other words, go back again to the thesis that the effects of thermodynamic nonideality generally require consideration. To treat the general case of a nonideal electrolyte system is beyond the scope of the present summary. However, a few remarks will be made for the analogous case where only neutral molecules are present. If the macromolecule is component 2, and the other solute called component 3, equations 33 and 34 may be applied. At the maximum in the m_2 vs. r curve,⁸ $dm_2/dr = 0$, and, dividing equation 33 by equation 34:

$$(1 - \bar{v}_2 \rho(r_0)) = (1 - \bar{v}_3 \rho(r_0)) \frac{M_3 a_{23}}{M_2 a_{33}} \quad (75)$$

Then, with equations 32 and 35 this equation becomes

$$(1 - \bar{v}_2 \rho(r_0)) = -(1 - \bar{v}_3 \rho(r_0)) \Gamma \frac{M_3}{M_2} \quad (76)$$

In order to use a gram-per-gram, rather than a mole-per-mole basis, $\Gamma' = \Gamma(M_3/M_2)$ may again be substituted and equation 76 takes the form

$$\frac{1}{\rho(r_0)} = \frac{\bar{v}_2 + \Gamma' \bar{v}_3}{1 + \Gamma'} \quad (77)$$

If Γ' grams of component 3 (and none of component 1) are bound to each gram of component 2 to produce a complex component, the partial specific volume of this complex component is given (2, 9) by

$$\bar{v}_{23} = \frac{\bar{v}_2 + \Gamma'(\bar{v}_3)}{1 + \Gamma'} = \frac{1}{\rho(r_0)} \quad (78)$$

Thus, the partial specific volume obtained by the method of Meselson, Stahl, and Vinograd must refer to the complex of macromolecule and component 3 and can be quite different from the value of \bar{v}_2 . It may be expected that the molecular weight obtained will be affected in a similar way.

VI. MULTICOMPONENT SYSTEMS

A. AVERAGE MOLECULAR WEIGHTS

Many macromolecular solutes are not homogeneous, but are mixtures of a large number of components differing in molecular weight. Recognition of this

⁸ It can be shown that for solutions dilute in component 2 (though not necessarily dilute in component 3) the quantity dm_2/dr will approach zero as dc_2/dr approaches zero. Thus, for solutions which are of the type normally studied the maximum will occur at the same point in the cell for both the c_2 vs. r and the m_2 vs. r curves.

fact led Lansing and Kraemer (8) to define several average molecular weights which can be calculated for such materials, as follows:

$$M_n = \frac{\sum w_i}{\sum w_i/M_i} \quad (79)$$

$$M_w = \frac{\sum w_i M_i}{\sum w_i} \quad (80)$$

$$M_z = \frac{\sum w_i M_i^2}{\sum w_i M_i} \quad (81)$$

Here w_i represents the weight of each component in the solute, and the summation is carried over all solute components. The three averages given above are referred to as the "number-average," "weight-average," and "z-average" molecular weights, respectively. It is possible to define higher average molecular weights (M_{z+1} , M_{z+2} , etc.) in an analogous manner, but they are largely inaccessible to present-day experiments.

The equations for describing the sedimentation equilibrium of a multicomponent system may be derived in a manner similar to that used for binary and ternary systems (2). It will be assumed that the density of the solution and the partial specific volumes of all components are independent of pressure and composition. Since many heterogeneous macromolecular solutes are mixtures of components differing only in molecular chain length, it will be assumed that partial specific volumes and the differential refractive increments are the same for all solute components. As before, the solvent will be component 1, and components 2 \cdots n will be termed solutes.

The chemical potential of each solute component will depend upon the pressure and the concentrations of all components. Then instead of the single equation (equation 8), the set of $n - 1$ equations is obtained:

$$M_i(1 - \bar{v}\rho)\omega^2 r = \sum_{k=2}^n \left(\frac{\partial \mu_i}{\partial c_k} \right)_{P, c_j \neq k} \frac{dc_k}{dr} \quad (i = 2, \dots, n) \quad (82)$$

Expressing the chemical potential of component i in terms of the concentration and activity coefficient

$$\mu_i = \mu_i^\circ + RT \ln c_i y_i$$

we have:

$$\frac{M_i(1 - \bar{v}\rho)\omega^2 r \cdot c_i}{RT} = \frac{dc_i}{dr} + c_i \sum_{k=2}^n \left(\frac{\partial \ln y_i}{\partial c_k} \right)_{P, c_j \neq k} \cdot \frac{dc_k}{dr} \quad (83)$$

Since it must in general be assumed that each activity coefficient is a function of all solute concentrations, the equations 83 are coupled in a very complex manner.

In order further to analyze the problem, simplifying assumptions must be made. For example, it can be assumed that the system is thermodynamically

ideal.⁹ In this case, the second term on the right side of equation 83 vanishes, and we obtain:

$$M_i c_i \frac{(1 - \bar{v}\rho)\omega^2 r}{RT} = \frac{dc_i}{dr} \quad (84)$$

Equations 84 now can be rearranged and summed to yield

$$M_{wr} = \frac{\sum_{i=2}^n c_i M_i}{\sum_{i=2}^n c_i} = \frac{RT}{(1 - \bar{v}\rho)\omega^2} \left(\frac{1}{rc} \frac{dc}{dr} \right) \quad (85)$$

where

$$c = \sum_{i=2}^n c_i$$

is the total solute concentration at the point r . The quantity M_{wr} is the weight-average molecular weight of the material at the point r . This quantity then can be integrated over the (sector-shaped) cell to yield the weight-average molecular weight of the whole solute.

$$M_w = \frac{\int_a^b M_{wr} \cdot cr \, dr}{\int_a^b cr \, dr} = \frac{2RT(c(b) - c(a))}{(1 - \bar{v}\rho)\omega^2 c^0 (b^2 - a^2)} \quad (86)$$

A similar scheme can be used for the evaluation of M_z . Wales (25) has developed a method for calculating successively higher average molecular weights, so that, in principle, it is possible to calculate as many average molecular weights as desired. In practice, it is found that the reliability of the results decreases rapidly for the higher average molecular weights. It should be pointed out as well that it is not in general possible to calculate the number-average molecular weight directly from sedimentation equilibrium data. As was shown by Lansing and Kraemer (8), this can be done only if the solute concentration approaches zero at some point in the cell.

The methods outlined above were used in the early experimental studies of the sedimentation equilibrium of heterogeneous materials. However, it soon became evident that the assumption of thermodynamic ideality sometimes could lead to gross errors. For example, Mosimann (13) and others (18, 19) found that the apparent molecular weight obtained from sedimentation equilibrium experiments with high polymers depended upon the solvent used, the concentration of the solution, and the rotor velocity, but they were not able to describe the cause. Furthermore, the quantity M_{wr} , which for a heterogeneous ideal solute should increase monotonically with distance from the center of rotation, was often found to pass through a maximum, or even decrease throughout the cell.

⁹ This will be the situation for polymer solutions at the "θ temperature" (1) and approximately true for many protein systems. These are the cases described by Lansing and Kraemer (8).

It was eventually recognized that these effects arose from the pronounced nonideality of the high-polymer solutions. A partial solution was offered by Schulz (17) in 1944 and independently by Wales, Bender, Williams, and Ewart (28) in 1946. The two methods were almost identical.¹⁰

The assumptions made by these workers were essentially the following:

- (1) Rather than assume that $\ln y_i = 0$ (ideal solution), it was suggested that

$$\ln y_i = BM_i c \quad \left(\text{where } c = \sum_2^n c_i \right) \quad (87)$$

- (2) It was considered that the coefficient B is independent of the molecular weight of the polymer. It would then follow that B could be evaluated once and for all for any polymer-solvent system from the dependence of the osmotic pressure or light scattering upon concentration.

Given these assumptions, equations 83 become

$$M_i c_i \frac{(1 - \bar{v}\rho)\omega^2 r}{RT} = \frac{dc_i}{dr} + BM_i c_i \frac{dc}{dr} \quad (i = 2 \cdots n) \quad (88)$$

and these equations can be summed to yield an expression for M_{wr} which represents a modification of equations 85 as follows:

$$M_{wr} = \frac{dc/dr}{\left\{ \frac{\omega^2(1 - \bar{v}\rho)}{RT} r - B \frac{dc}{dr} \right\} c} \quad (89)$$

This equation provided the means for calculating the average molecular weights in a straightforward manner.

The assumptions which were made to obtain equation 89 seemed justified by the theoretical and experimental knowledge which was available at the time. At present, both of these assumptions are open to criticism. In the first place, it now seems evident that for high polymers the expansion of $\ln y_i$ should be extended at least to terms in the squares and products of the solute concentrations, analogous to equation 13. Furthermore, it now appears that the single coefficient B must be replaced by an array of coefficients B_{ik} , which represent the interaction between the solute components i and k . Thus, a more rigorous formulation of equations 88 would take the form:

$$M_i c_i \frac{(1 - \bar{v}\rho)\omega^2 r}{RT} = \frac{dc_i}{dr} + M_i c_i \sum_{k=2}^n B_{ik} \frac{dc_k}{dr} + \cdots \quad (90)$$

Until more is known about the nature of the cross-coefficients and their relationships with the coefficients B_{ii} and B_{jj} , the analysis can be carried no further. Unfortunately, the information available (7) indicates that the B_{ik} are very complicated functions of M_i and M_k .

¹⁰ Perhaps it should be mentioned that Galén (3) in 1944 developed a method based upon a molecular kinetic analysis for the correction of the nonideality effects by making use of the dependence of the rates of sedimentation and diffusion upon concentration.

These difficulties have been summarized by Mandelkern, Williams, and Weissberg (10), who have suggested that the use of an ideal polymer-solvent system is essential in the study of high-molecular-weight heterogeneous polymers.

The authors believe that the situation at the present time can be described to be somewhat as follows: For organic high polymers of moderate to low molecular weight ($M_w < 100,000$) the nonideality effects are generally so small that the Schulz-Wales equation (equation 89) will give a good approximation to M_w if an estimate of B is available. On the other hand, for the measurement of higher molecular weights with polymeric solutes there seems to be required an ideal system. For the globular proteins the nonideality effects are smaller, and should cause less difficulty, if the salt concentration is sufficient to "swamp out" electrostatic effects.

B. DISTRIBUTION FUNCTIONS

In the discussion to this point of the sedimentation equilibrium in multi-component systems, methods are described whereby average molecular weights can be calculated. For many purposes, this information is sufficient. However, it should be pointed out that at least in principle the sedimentation equilibrium method is sensitive to the details of the distribution of molecular weight in the solute. For an ideal solution, the following relation must obtain between the molecular weight distribution and the concentration versus r curve:

$$c(r) = c(b) \int_0^{\infty} f_b(M) e^{-MS(r)} \cdot dM$$

where

$$c(b)f_b(M) = c^{\circ} \frac{MS(a)}{1 - e^{-MS(a)}} \cdot f(M) \quad (91)$$

and

$$S(r) = \frac{\omega^2(1 - \bar{v}\rho)}{2RT} (b^2 - r^2)$$

The function $f_b(M)$ is the molecular weight distribution of the material at the bottom of the ultracentrifuge cell; $f(M)$ is the overall molecular weight distribution. Equation 91, or rather its equivalent for a distribution of radii of spherical particles, was obtained by Rinde (16) in 1928. Several methods were proposed by Rinde for the solution of this equation for $f(M)$, if the c vs. r or dc/dr vs. r curve is given. The most satisfactory method involved expressing $f_b(M)$ in a series of the form

$$f_b(M) = e^{-KM} \sum_{i=1}^n a_i M^i \quad (92)$$

and evaluating the coefficients a_i from the coefficients of a corresponding series which is obtained for $c(r)$. The method involves considerable labor, and the result is often unsatisfactory, in the sense that the distribution function which is obtained may have negative values in some regions. A similar method developed

by Wales, Adler, and Van Holde (27) involves fitting $f(M)$ by a series of Laguerre polynomials. While the labor involved in the use of this method is considerably less, the resulting distribution may still be negative in some regions. Still another and similar method has been developed by Herdan (4).

Because of the difficulty in obtaining satisfactory solutions to equation 91 an entirely different procedure has been frequently employed (24, 26) to obtain some information about the molecular weight distribution. If several average molecular weights of a material are known, it may be possible to find a molecular weight distribution which would yield those averages. The process generally involves selecting a distribution function of a reasonable form, with as many adjustable parameters as there are reliable average molecular weights available. These average molecular weights are then used to calculate the parameters. A hazard in the use of this procedure is illustrated by figure 2, in which are shown three "distributions" each of which will yield the set of molecular weights $M_w = 200,000$, $M_z = 300,000$, and $M_{z+1} = 400,000$. If these average molecular weights were the only information available, it would be impossible to choose between these (or many other) distributions. In fact, it can be shown that it is always possible to fit three average molecular weights by an appropriate selection of the molecular weights and relative amounts of only two solute components.

If for some kinetic or statistical reason it is known that the molecular weight distribution of a substance is of a particular form, the above procedure may be

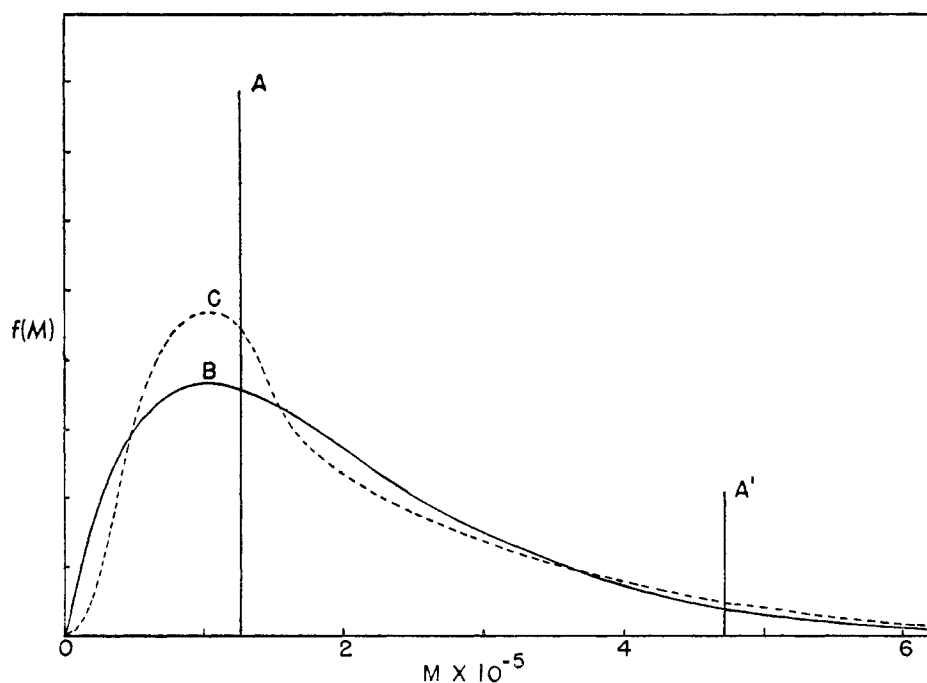


FIG. 2. Three "distributions," each of which will yield the set of molecular weights $M_w = 200,000$, $M_z = 300,000$, and $M_{z+1} = 400,000$. The dimensions of $f(M)$ are arbitrary.

used to great advantage. In other cases, however, this method has little to recommend it; it would seem preferable to simply give the mean of the distribution (M_w) and a measure of its breadth such as the standard deviation:

$$\sigma = M_w \left(\frac{M_z}{M_w} - 1 \right)^{1/2}$$

VII. THE TIME REQUIRED TO ATTAIN SEDIMENTATION EQUILIBRIUM

The time required to carry out a sedimentation equilibrium experiment is determined in most cases by the time necessary to approach to within a negligible error the equilibrium concentration gradient. Although several devices have been developed which allow several experiments to be carried on simultaneously, the long time frequently required always has been considered to be a disadvantage of the sedimentation equilibrium method. Thus, it is worthwhile to investigate the factors which determine this time.

Mason and Weaver (11), in 1924, solved the differential equation for the sedimentation of a single, ideal solute in the gravitational field. From this solution, Weaver (31) calculated the time to attain equilibrium to be not sensibly greater than twice the time required for the solute to sediment from the meniscus to the bottom of the cell. This rule was used for many years to estimate the time required for sedimentation equilibrium experiments. The problem has recently been reinvestigated by Van Holde and Baldwin (23), who point out that the conditions which Weaver assumed are not applicable to most sedimentation equilibrium experiments in the ultracentrifuge, and that Weaver's rule represents a limiting law. This point has been noted independently by Pasternak, Nazarian, and Vinograd (14).

The authors have developed a new procedure to estimate this time which is more appropriate to the problems at hand. Since the quantity desired from the sedimentation equilibrium experiments is the molecular weight of the solute, the quantity adopted as a measure of the displacement of the system from equilibrium is

$$\epsilon = \frac{M_2 - M_2^*(t)}{M_2} \quad (93)$$

Here $M_2^*(t)$ represents the value which would be obtained for the molecular weight if the experiment were stopped at the time t . It can be shown, by using Mason and Weaver's analysis,¹¹ that under conditions relevant to most experiments, the time required to reduce ϵ to any given value is

$$t \cong \frac{(b-a)^2}{D\pi^2 \cdot U(\alpha)} \ln \frac{\pi^2 \epsilon U^2(\alpha)}{4(1 + \cosh 1/2\alpha)} \quad (94)$$

where

$$U(\alpha) = 1 + \frac{1}{4\pi^2\alpha^2}$$

¹¹ The applicability of the Mason and Weaver treatment for a rectangular cell in a gravitational field to problems in the ultracentrifuge has been investigated by Yphantis and Waugh (33). It is found that in most cases of interest the error involved is negligible.

and

$$\alpha = \frac{2RT}{(1 - \bar{v}_2\rho)M_2 \cdot \omega^2(b^2 - a^2)}$$

In equation 94 D is the diffusion coefficient of the solute, and $(b - a)$ is again the length of the column of solution. This equation indicates that the equilibrium state can be attained most rapidly if:

(1) The solution column is made as short as possible.

(2) The ultracentrifuge is operated at as high a rotor velocity as possible.

With regard to the first condition, 2–3 mm. has been found to be convenient for many experiments, as contrasted to the 7–10 mm. commonly used. The principal disadvantage is that for the analysis of heterogeneous solutes some of the resolving power of the ultracentrifuge is lost. If the length of the solution column is decreased still further (to about 1 mm.), the equilibrium is attained very rapidly. In this case, it is convenient to measure the refractive index gradient at only one point, the center of the column of the solution. The molecular weight now can be calculated by assuming that the concentration is the same as the initial concentration. In these very short columns this approximation will be very good (23).

A practical limit to the second condition is imposed by the fact that if the rotor velocity is too high the refractive index gradients at the bottom of the cell will throw light completely out of the optical system.

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