Density-Gradient Sedimentation Equilibrium of DNA and the Effective Density Gradient of Several Salts

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Synopsis

The various treatments of sedimentation equilibrium are compared on a theoretical and an experimental basis. Particular attention is paid to the polyelectrolyte nature of the problem and the choice of a neutral component. The effective density gradients of several cesium salts for DNA are measured. Two previous theories for the effective density gradient are shown to be equivalent, and the experimental values are interpreted with respect to these theories. It is clear that sedimentation equilibrium in a density gradient may be used for the determination of unambiguous molecular weights.

INTRODUCTION

The determination of the molecular weight of high molecular weight DNA by sedimentation equilibrium is not possible because the high centrifugal fields currently available produce distributions too narrow to measure. To circumvent this problem, the sedimentation equilibrium experiment can be performed in a solvent with nearly the same density as the DNA, e.g., CsCl ($\rho \approx 1.70$ g/ml$^3$). This reduces the buoyant mass by about three orders of magnitude.

Molecular weights determined by this method, however, were low. The multicomponent nature of the problem, ignored by the first treatment, was later taken into consideration, as were the effects of pressure.$^{2-4}$ Even with this advanced understanding of the physics, the measured molecular weights were low.$^5$ The validity of this multicomponent treatment was later confirmed by a different formulation of the problem using a different experimental approach,$^6$ so that the answer to the discrepancy lay elsewhere. It is recognized that for DNA's of heterogeneous density resulting from base composition heterogeneity, the method yields low molecular weights;$^7$ this important criticism does not apply, of course, to homogeneous samples of intact viral DNA.

It has been recently shown that meaningful molecular weights can be obtained if simple virial corrections are applied to the data.$^8$ Since the method is reliable, we have obtained more accurate values for the effective gradient and have evaluated the effective gradient for several salts and temperatures. These values have been obtained by isotopic substitution of the
nitrogen in the DNA, a method discussed by both Vinograd and Hearst\textsuperscript{9} and Cohen and Eisenberg.\textsuperscript{6}

Since sedimentation equilibrium will be used as a standard tool to characterize DNA, it is important to compare critically the theories of density gradient equilibrium and to dispel some recent confusion.\textsuperscript{10} The treatments of Hearst and Vinograd,\textsuperscript{2,3} Cohen and Eisenberg,\textsuperscript{6} and Cassasa and Eisenberg\textsuperscript{11} for the multi-component effects are shown to be equivalent. The polyelectrolyte character of the DNA is treated and it is concluded that this merely allows many alternate definitions of neutral components, as previously recognized,\textsuperscript{12,13} and provides no new information. All models based on ion binding, partial specific volume of ions, and untenable assumptions of single-ion activities are therefore superfluous, at best, in particular, the treatment proposed by Daniel\textsuperscript{10} is proven to be erroneous.

It is easy to understand sedimentation equilibrium and the concept of the effective density gradient on an intuitive level. Concepts and derivations introduced here will be ignored; later they will be formally introduced in the Theory section. The DNA may have some net solvation, specifically it will be hydrated with $g$ grams of water per gram of DNA. This is depicted schematically in Figure 1 for two molecules in different positions in the density gradient. Although thermodynamic treatments are more general than this simple schematic picture since net solvation is not restricted to a well defined region about the DNA, the picture is useful for an understanding of the thermodynamic equations. The sedimenting mass is the DNA and its water of hydration, occupying the hydrated volume $s$. It is well known that for a homogeneous polymer in the ideal limit, a Gaussian concentration distribution in density gradient sedimentation equilibrium is obtained. The concentration distribution expected from considering the composition gradient of CsCl at equilibrium is shown in Figure 2 for a particular set of conditions. Any effect which increases the density gradient such as increasing the centrifugal speed, of course, narrows the observed concentration distribution. There are two experimental observations which indicate that the density gradient will be different from that predicted from the composition gradient due to the salt distribution, and that an effective density gradient must be considered instead. Since the value of the density gradient is changed, the observed concentration distribution will be different from that predicted with only the composition density gradient of the CsCl. The first experimental observation is that the extent of hydration decreases with decreasing water concentration (increasing salt concentration).\textsuperscript{12} The second experimental observation is that the buoyant density of the DNA decreases with increasing pressure.\textsuperscript{12} The first observation is consistent with our intuition and Le Chatelier's principle that, as the amount of available water is decreased, the extent of hydration should also decrease. The second observation is the result of the compressibility of the solvent being greater than that of the DNA.

From the second observation the molecule at higher pressure, (down field, in Fig. 1) must be shifted closer to the molecule at lower density by some
Fig. 1. Schematic of DNA in a density gradient.

\[ \rho_s = \frac{\rho_{\text{DNA}} + \rho_{\text{H}_2\text{O}}}{1 + g} \]

\[ M_s = M_{\text{DNA}}(1 + g) \]

Fig. 2. Influence of the effective density gradient on band width with various effects modifying the density gradient: (---) the concentration distribution expected from the composition density gradient; (----) the effect of pressure on the composition density gradient; (-----) the effective density gradient, including the variation of buoyant density with water concentration (this corresponds to the observed concentration distribution). The calculation is performed for 33.3 \times 10^6 dalton Cs DNA, (25.0 \times 10^6 dalton Na DNA) banding in CsCl 1.700 g/ml, 25°C, 6.50 cm from the center of rotation, at 25,000 rpm. The following parameters, explained in the later text, were taken to have the numerical values:

\[ \frac{1 + r'}{\beta_{\text{eff}}} = 7.87 \times 10^{-10} \]
\[ \frac{1 + r'}{\beta} = 11.1 \times 10^{-10} \]
\[ \frac{1 + r'}{\beta_B} = 11.9 \times 10^{-10} \]
\[ 1 + r' = 1.275 \]

amount \( \Delta \rho_B \). This has the effect of narrowing the concentration distribution or effectively increasing the density gradient. The distribution expected from the composition gradient will be sharpened by the effect of pressure as shown in Figure 2. The molecule at high density in Figure 1 is at a lower water concentration, and its hydration will be decreased. This decrease in hydration shifts it to a higher buoyant density by an amount of \( \Delta \rho_B \) relative to the molecule at lower density. This widens the concentration distribution and effectively decreases the density gradient. The observed concentration distribution will be the result of the composition gra-
dient, the pressure effect and the change of hydration with changing salt concentration. The concentration distribution that would be observed for a particular set of conditions is shown in Figure 2 and is considerably wider than that predicted with the composition density gradient. These observations may be summarized by the equation:

\[
\left(\frac{dp}{dr}\right)_{\text{eff}} = \frac{dp}{dr} - \frac{d\rho_s^0}{dr} + \left(\frac{dp}{dP} - \frac{d\rho_s}{dP}\right) \frac{dP}{dr}
\]

where \( \rho \) is the density of the solvent, \( \rho_s \) is the density of the solvated DNA, superscript zero denotes atmospheric pressure, and \( P \) is the pressure. The previous argument indicated the solvated density increased with increasing density and \( d\rho_s^0/dr \) is therefore positive. The effective density gradient is the composition gradient, \( dp/dr \), decreased by the change in solvated polymer density, \( d\rho_s^0/dr \), and increased by the compressibility difference,

\[
[(dp/dP) - (d\rho_s/dP)](dP/dr).
\]

The experimental determination of the effective density gradient in this work consists of measuring the distance between bands of normal DNA and density-labeled, \(^{15}\text{N}, \) DNA. The intuitive arguments presented in Figure 1 may be extended for the \(^{14}\text{N}, \) \(^{15}\text{N} \) substitution experiment. The density change \( \Delta \rho \) upon substitution of \(^{15}\text{N} \) is easily calculated assuming it contributes only mass and no change in volume or solvent interaction. The observed distance, \( \Delta r_{\text{obs}} \), between the bands will be the sum of three terms corresponding to the composition gradient, the second to the compression gradient, and the third due to the change in solvated polymer density. The experimental quantity, \( \Delta \rho/\Delta r_{\text{obs}} \), is therefore a measure of the effective density gradient as schematically depicted in Figure 1.

In the experimental part of this work, the effective density gradients are determined for a series of cesium salts using the method of isotopic substitution. By a method explained in the theory section, the contributions from the composition gradient and the pressure effects to the effective density gradients are also determined in this work. With this data it is then possible to evaluate the effect contributed by the change of hydration with water concentration. The values found for this parameter are then compared to the values found by Cohen and Eisenberg\(^6 \) and Hearst and Vinograd.\(^2 \) The experimental values found for the effect of the variation of hydration with water concentration by these three different methods are in agreement. Since these three approaches are thermodynamically equivalent, our understanding of the effective density gradient is quantitative.

THEORY

Sedimentation Equilibrium

For simplicity a three-component system of water, polymer, and a common ion univalent salt will be considered; the more general case has already been described.\(^1 \) Neutral components will be designated as 1, water;
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2, salt; and 3, some choice of neutral polymer; ionic species are represented as X, cation; Y, anion; and p, DNA polyanion with a charge of \(-n\).

The following definitions are employed: \( F \) = Faraday’s constant; \( M_i \) = molecular weight of \( i \); \( R \) = gas constant; \( T \) = absolute temperature; \( m_i \) = molality of \( i \); \( \bar{v}_i \) = partial specific volume of \( i \); \( \gamma_i \) = activity coefficient of \( i \); \( \rho \) = density; \( \psi \) = electrical potential; \( \mu_i \) = chemical potential of \( i \); \( \omega \) = angular velocity; where \( i \) refers to either a neutral component or an ionic species. The following abbreviations and identities will be used:

\[
A_i = M_i(1 - \bar{v}_i\rho)\omega^2r/RT
\]

\[
\mu_{ij} = \frac{1}{RT} \left( \frac{\partial \mu_i}{\partial m_j} \right)_{m_i} = \frac{1}{m_i} + \frac{1}{RT} \left( \frac{\partial \ln \gamma_i}{\partial m_i} \right)_{m_j} \quad j \neq i
\]

\[
\mu_{ij} = \frac{1}{RT} \left( \frac{\partial \mu_i}{\partial m_j} \right)_{m_i,m_k} = \frac{1}{RT} \left( \frac{\partial \ln \gamma_i}{\partial m_j} \right)_{m_i,m_k} = \mu_{ji} \quad k \neq i,j
\]

The condition for sedimentation equilibrium of the ionic species are expressed as four equations in four unknowns. There are gravitational terms\(^{12,14}\) for a uni-univalent salt and polymer with charge \(-n\) we have:

\[
\mu_{pX}(dm_p/dr) + \mu_{pX}(dm_X/dr) + \mu_{pY}(dm_Y/dr) - (nF/RT)(d\psi/dr) = A_p \quad (1)
\]

\[
\mu_{Xp}(dm_p/dr) + \mu_{XX}(dm_X/dr) + \mu_{XY}(dm_Y/dr) + (F/RT)(d\psi/dr) = A_X \quad (2)
\]

\[
\mu_{Yp}(dm_p/dr) + \mu_{YX}(dm_X/dr) + \mu_{YY}(dm_Y/dr) - (F/RT)(d\psi/dr) = A_Y \quad (3)
\]

and the electroneutrality condition

\[
-n(dm_p/dr) - (dm_Y/dr) + (dm_X/dr) = 0 \quad (4)
\]

There are many combinations of this system of equations which may be used to solve for the quantity of interest, the polymer concentration. Specification of the electrical potential gradient, however, requires a knowledge of single ion activity coefficients and partial specific volumes of single ions. Obviously there is no way to measure or approximate these quantities in high salt, e.g., \( 7m \) CsCl. The way around this is to merely eliminate the electrical potential terms by forming neutral components. These conclusions are not new since such problems were considered some time ago.\(^{15}\) The manner of eliminating the electrical potential tacitly defines a choice of neutral components. Adding the cation equilibrium expression in eq. (2) with the anion expression in eq. (3), the relation for a very familiar component, the neutral salt XY, is obtained.

\[
\mu_{zp}(dm_p/dr) + \mu_{zX}(dm_X/dr) + \mu_{zY}(dm_Y/dr) = A_z \quad (5)
\]
where for brevity the equality

$$\mu_{Xt} + \mu_{Yt} = \mu_{zt}$$

was used. A variety of methods of eliminating the electrical potential gradient might be considered. By adding $n$ times eq. (2) to eq. (1), an intuitive choice of neutral polymer, $\text{Cs}_n \text{DNA}$, is obtained. Similarly by adding $-n$ times eq. (3) to eq. (1), a somewhat more bizarre choice of neutrality, $\text{Cl}_{-n} \text{DNA}$, is obtained. Other choices might be the familiar Scatchard definition of neutral components, $\text{Cs}_{n/2} \text{Cl}_{-n/2} \text{DNA}$ or for the degree of ionization, $\theta$,

$$\text{Cs}_{\theta_n} \text{DNA} \quad \text{Cs}_{n(1-\theta)} \text{Cl}_{-n(1-\theta)} = \text{Cs}_{n/2} \text{Cl}_{-n/2} \text{DNA} \quad (\theta n/2) \text{CsCl}$$

Since no new physics is introduced in any of the manipulations, ultimately they must all be equivalent, as will be explicitly shown later. This derivation will be pursued with the Scatchard choice of neutrality to facilitate comparisons with other approaches. An identical derivation has already been performed for $\text{Cs}_n \text{DNA}$. By adding $n/2$ times eq. (2) and $-n/2$ times eq. (3) to eq. (1), the Scatchard definition of neutrality is obtained:

$$\left(\mu_{pp} + \frac{n}{2} \mu_{xp} - \frac{n}{2} \mu_{yp}\right) \frac{dm_p}{dr} + \left(\mu_{px} + \frac{n}{2} \mu_{xx} - \frac{n}{2} \mu_{yx}\right) \frac{dm_x}{dr}$$

$$+ \left(\mu_{py} + \frac{n}{2} \mu_{xy} - \frac{n}{2} \mu_{yy}\right) \frac{dm_y}{dr} = A_p + \frac{n}{2} A_x - \frac{n}{2} A_y$$

On designating the Scatchard definition of neutrality as $3^\prime$ and remembering

$$\mu_{3^t} = \mu_{pt} + (n/2)\mu_{xt} - (n/2)\mu_{yt}$$

this equation may be written in the more convenient notation:

$$\mu_{3^t} \frac{dm_p}{dr} + \mu_{3x} \frac{dm_x}{dr} + \mu_{3y} \frac{dm_y}{dr} = A_{3^t}$$

The concentration gradients of the ionic species appearing in eqs. (5) and (6) are interdependent because of the electroneutrality condition [eq. (4)]. To eliminate one dependent variable, the electroneutrality condition is first rewritten in two equivalent forms

$$dm_x = (dm_X + dm_Y)/2 + (n/2)dm_p = dm_z + (n/2)dm_p$$

$$dm_X = dm_z - (n/2)dm_p$$

which use the definition of neutral salt, component 2. The concentration gradients of the ionic species X and Y appearing in eqs. (5) and (6) may now be eliminated by using the alternate forms of the electroneutrality condi-
tion expressed in eq. (7). Performing these substitutions for \( dm_X \) and \( dm_Y \) we obtain

\[
[\mu_{2v} + (n/2)\mu_{2X} - (n/2)\mu_{2Y}](dm_v/dr) + (\mu_{3X} + \mu_{3Y})(dm_3/dr) = A_1, \tag{8}
\]

\[
[\mu_{2v} + (n/2)\mu_{2X} - (n/2)\mu_{2Y}](dm_v/dr) + (\mu_{2X} + \mu_{2Y})(dm_2/dr) = A_2 \tag{9}
\]

by using the additional regulations:

\[
\mu_{1X} + \mu_{1Y} = \mu_{1Z} \]

\[
\mu_{4v} + (n/2)\mu_{4X} - (n/2)\mu_{4Y} = \mu_{4Z}
\]

the two equations (8) and (9) in two unknowns may be rewritten as:

\[
\mu_{2v1}(dm_2/dr) + \mu_{22}(dm_2/dr) = A_2
\]

\[
\mu_{2v2}(dm_2/dr) + \mu_{22}(dm_2/dr) = A_2 \tag{11}
\]

There is obviously correlation between the equilibrium distributions of components 2 and 2', which is apparent in the cross terms appearing in this system of equations. Equation (11) is easily solved for \( dm_2/dr \), and this quantity is substituted into eq. (10) to obtain the equilibrium distribution of the polymer [eq. (12)]:

\[
(\mu_{2v1} + \Gamma_{22}\mu_{22})(dm_2/dr) = A_{2'} + \Gamma_{22}A_2
\]

where

\[
\Gamma_{22} \equiv (\partial m_2/\partial m_2)_{\mu_2} = -\mu_{2,2'}/\mu_{2,2'}
\]

The significance of this step is explained below. By using the definition of \( \Gamma_{22} \), eq. (12) may be rewritten in the more useful form

\[
(\partial \mu_{2v}/\partial m_2)_{\mu_2} dm_2/dr = A_{2'} + \Gamma_{22}A_2
\]

In the derivation of eq. (13) it was unnecessary to make any assumptions about single ion activity coefficients or single ion partial specific volumes. Most of the derivation was devoted to merely defining components in a systematic fashion to eliminate the dependent terms \( dm_X, dm_Y, \) and \( d\psi \) appearing in the original set of four equations in four unknowns in eqs. (1)-(4). The interesting step is the elimination of \( dm_2 \) appearing in eqs. (10) and (11) to obtain eqs. (12) and (13). Equations (12) and (13) are analogous to the result that would be obtained for a two-component treatment of sedimentation equilibrium. This arises essentially from defining a component that may be added to the solution without changing the chemical potential of the other components, which is the reason that \( \mu_2 \) is held constant on the left-hand side of eq. (13). This is the familiar membrane equilibrium component, 13 formulated so that upon addition of a mole of component 3, the concentration of component 2 is changed by the factor \( \Gamma_{23} \), such that the change in chemical potential of component 2 is zero.
Identical equilibrium statements have been obtained by defining Csₙ DNA as the neutral component and considering the membrane equilibrium component in combination with CsCl¹¹⁻¹² or water.² For all the concern with the electrical potential and the behavior of ions in the preceding derivative, we merely arrived at a different choice of neutral DNA and obtained no new information.

In the remainder of this section we shall use Hearst and Vinograd’s²,³ choice of components Csₙ DNA, 3, and water 1, and consider the concept of the effective density gradient. The derivations of Hearst and Vinograd²,³ and Hearst, Ifft, and Vinograd⁴ will be repeated here to make the experimental results more meaningful and to facilitate comparisons with Cohen and Eisenberg’s⁶ and Casassa and Eisenberg’s¹¹ treatments. For this choice of neutral components analogous to eq. (12), we may write

\[(\mu₂₂ - \Gamma\mu₂₁)dm₂/dr = A₂ + \Gamma A₁\]  

(14)

or substituting the definition of \(A₁\),

\[(\partial\mu₂/\partial m₂)μ₁ dm₂/dr = Mₛ(1 - \tilde{v}_s\rho)\omega² r/RT\]

(15)

where

\[Mₛ = M₂(1 + \Gamma')\]

\[\tilde{v}_s = (\tilde{v}_₂ + \Gamma'\tilde{v}_₁)/(1 + \Gamma')\]

(16)

\[M₂\Gamma'/M₁ = - (\partial m₁/\partial m₃)μ₁\]

\(Mₛ\) may, for convenience, be thought of as a hydrated molecular weight, and the hydration \(\Gamma'\) as the number of grams of water that must be added with a gram of DNA to hold \(μ₁\) constant.

Experimentally the initial solution density is chosen such that \((1 - \tilde{v}_s\rho) = 0\) and the DNA bands at some point \(r₀\). It is assumed that the following linear expansions about \(r₀\) are sufficient. This assumption is justified for all the DNA samples studied here but is somewhat less justified for very broad bands such as protein bands.

\[\rho = \rho₀ + (d\rho/dr)δ\]

\[\tilde{v}_s = \tilde{v}_s,₀ + (d\tilde{v}_s/dr)δ\]

\[Mₛ = Mₛ,₀ + (dmₛ/dr)δ\]

for \(δ = r - r₀\)

These expansions are substituted into eq. (15), only first order terms of \(δ\) being retained. The equation for sedimentation equilibrium in an isopycnic density gradient and the definition of the effective density gradient are obtained.

\[-Mₛ,₀(d\rho/dr)ₜt = 0 = (d\rho₀/dr) + (d\tilde{v}_s,₀)\tilde{v}_s,₀ d\tilde{v}_s,₀ d\tilde{v}_s,₀\]

(17)

\[(d\rho/dr)ₜ₀ = (d\rho₀/dr) + (d\tilde{v}_s,₀)\tilde{v}_s,₀ d\tilde{v}_s,₀\]

(18)
Effective Density Gradient

To evaluate the effective density gradient, we consider the effects of pressure and salt concentration on $\rho$ and $\bar{\rho}_s$ in terms of experimental quantities. In the first term of eq. (18) the density is expanded about atmospheric pressure, $P = 0$. The symbol $P$ is defined as the difference between cell pressure and atmospheric pressure.

$$\rho = \rho^0/(1 + KP)$$

$$K = -(1/v)(\partial v/\partial P)$$

$$\frac{d\rho}{dr} = \frac{1}{1-KP} \left[ \frac{d\rho^0}{dr} + \frac{K(\rho^0)^2}{(1-KP)^2} \right] = \left[ \frac{1}{\rho^0} + 1 + K(\rho^0)^2 \right] \omega^2 r \quad (19)$$

The approximation in eq. (19) is the neglect of salt redistribution caused by the pressure gradient $1-KP = 1-0.01$. The density gradient at 1 atm is calculated from thermodynamic data at that pressure:

$$\frac{d\rho}{dr} = (1/\rho^0)\omega^2 r$$

The second term of the effective gradient is expanded in terms of pressure and water activity. Water activity was originally chosen as a common concentration parameter to represent the effects of many salts, but any concentration variable reflecting the changes in salt concentration will suffice.

$$\frac{d\bar{\rho}_s}{dr} = (\partial\bar{\rho}_s/\partial P)_w(dP/dr) + (\partial\bar{\rho}_s/\partial a_w)(da_w/dr) \quad (20)$$

An isothermal compressibility is defined for the hydrated polymer, and the water activity at 1 atm is expanded with the salt density at 1 atm.

$$\frac{d\bar{\rho}_s}{dr} = -\bar{\rho}_s aK_s\rho_s \omega^2 r - \frac{1}{\rho_s a^2} \left( \frac{\partial \rho_s a}{\partial a_w} \right)_P \left( \frac{da_w}{da_s} \right) \left( \frac{d\rho}{dr} \right)^0 \quad (21)$$

The complete description of the effective gradient is obtained by substituting equations (21) and (19) into equation (18).

$$\left( \frac{d\rho}{dr} \right)_{e_{tt}} = \left( \frac{d\rho}{dr} \right)^0 + (K - K_s)(\rho^0)^2 \omega^2 r = \left( \frac{\partial \rho_s a}{\partial a_w} \right)_P \left( \frac{da_w}{da_s} \right) \left( \frac{d\rho}{dr} \right)^0 \quad (22)$$

The effective gradient may also be written in another form which relates the pressure dependent terms $\psi(\rho_s, \rho^0)$ to the slope of a plot of buoyant density versus pressure.

$$\left( \frac{d\rho}{dr} \right)_{e_{tt}} = [ (1/\beta^0) + \psi(\rho_s, \rho^0)^2 ] (1 - \alpha) \omega^2 r$$

$$= (1/\beta_B)(1 - \alpha) \omega^2 r$$

where

$$1/\beta_B = (1/\beta^0)^2 + \psi(\rho_s, \rho^0)^2$$

$$\psi = (K - K_s)/(1 - \alpha)$$
The buoyancy gradient \((1/\beta_B)\omega^2r\) is a composite of the salt gradient \(1/\beta^0\omega^2r\) and the compression gradient \(\psi\rho_s,0^2\omega^2r\). It has been shown that the buoyancy gradient may be measured directly by banding the polymer in solutions of two different densities \(\rho_{s,1}^0\) and \(\rho_{s,2}^0\) and measuring the distance between the two bands, \(r_{0,1} - r_{0,2}\), and the average distance from the center of rotation \(r_0 = (r_{0,1} + r_{0,2})/2\),

\[
(1/\beta_B)\omega^2r_0 = \frac{(\rho_{s,0} - \rho_{s,1}^0)/(r_{0,1} - r_{0,2})}{[(1/\beta^0) + \psi(\rho_{s,0})^2]\omega^2}
\]

Equation (24) is valid only for equal column lengths. If unequal column lengths are employed, the isoconcentration points in the two cells will be unequal; and there will also be a different compression gradient. The result for this condition is derived with no additional assumptions\(^5,19\) except equidistant cell bottoms for convenience. We omit the details of this simple derivation.

\[
\frac{1}{\beta_B} = \omega^2\frac{\rho_{s,0} - \rho_{s,1}^0}{\bar{r}_0(r_{0,2} - r_{0,1}) - \bar{r}_m(r_{m,2} - r_{m,1})/2} + \text{Error}
\]

\[
\text{Error} = \frac{\psi\rho_s^0\bar{r}_m(r_{2,m} - r_{1,m})}{2\omega^2\bar{r}_0(r_{2,0} - r_{1,0}) - \bar{r}_m(r_{2,m} - r_{1,m})} < \frac{\psi\rho_s^0(r_{2,m} - r_{1,m})}{\omega^2(r_{2,0} - r_{1,0})}
\]

The position of the menisci are \(r_{1,m}\) and \(r_{2,m}\) and \(\bar{r}_m\) is given by

\[
\bar{r}_m = (r_{1,m} + r_{2,m})/2
\]

This more general result corrects for the difference of the positions of the two isoconcentration points. The effect of the compressibility gradient however can be either eliminated or doubled by having the menisci displaced by the same amount as the distance between the bands. For CsCl and DNA the compressibility gradient is less than 10% of the salt gradient,\(^4\) and errors will be tolerable if the menisci are reasonably coincident.

It is possible as mentioned earlier to measure the effective compression density gradient \(\psi(\rho_{s,0})^2\omega^2r\) by following the buoyant density with pressure.\(^4\) Since the composition gradient is known from thermodynamic data, it is then possible to calculate the buoyancy gradient. Calculated buoyancy gradients will be compared to experimental buoyancy gradients evaluated with eq. (25) in a later section.

Since the buoyancy gradient may be measured by either of two methods, it is only necessary to measure \((1 - \alpha)\) to evaluate the effective gradient. The quantity \(\alpha\) may be found by plotting \(\rho_{s,0}\) against water activity \(a^0\) for a series of salts. The ratio of the slope of this plot to the slope of solvent density \(\rho\) with water activity is formally \(\alpha\) [eq. (23)]\(^1\). This was the approach taken by Hearst and Vinograd.\(^3\) This assumes that there are no specific anion effects.

It is also possible to evaluate \(\alpha\) from the pycometric data of Cohen and Eisenberg\(^5\) and the theory of Casassa and Eisenberg.\(^9\) Cohen and Eisenberg’s data is at atmospheric pressure and to compare this data with the
Casassa and Eisenberg's treatment with $\alpha$ appearing in the effective density gradient it is necessary to eliminate all pressure-dependent terms in the effective gradient. This can be accomplished by replacing $(dp/dr)$ in Cohen and Eisenberg's eq. (8) with the buoyancy gradient. On performing this substitution and assuming their results [eq. (10)\textsuperscript{4}] equivalent to Hearst and Vinograd's results [eq. (15)\textsuperscript{4}], the following identity must hold:

$$-(1 + \Gamma_{s,0})\bar{v}_{s,0}(1 - \alpha) = \frac{d}{d\rho_0} \left[ \left( \frac{\partial \rho_0}{\partial C_s} \right)_{\mu} \right]$$  \hspace{1cm} (27)

In our notation their pycnometric parameter becomes

$$\left( \frac{\partial \rho_0}{\partial C_s} \right)_{\mu} = (1 + \Gamma_{s,0})(1 - \bar{v}_{s,0})$$

Performing the indicated differentiation in eq. (27) at the buoyant density $(1 - \bar{v}_{s,0} \rho_0) = 0$, we find

$$-(1 + \Gamma_{s,0})\bar{v}_{s,0}(1 - \alpha) = -(1 + \Gamma_{s,0})[\bar{v}_{s,0} + \rho_0 \left( \frac{d\bar{v}_{s,0}}{d\rho_0} \right)]$$

$$= -(1 + \Gamma_{s,0})\bar{v}_{s,0}(1 - \left( \frac{dp_0}{d\rho_0} \right))$$  \hspace{1cm} (28)

This proof of eq. (27) confirms the equivalence of the two solutions of the multicomponent problem. It is possible to test the numerical accuracy of our experimental value of $\alpha$ by comparing it to Cohen and Eisenberg's pycnometric data with eq. (27); alternately with $\rho_0$ from this data, we may calculate $\alpha$ with eq. (28).

We now have two different ways to measure both $\alpha$ and $\beta_0$; however, $\alpha$ is determined as the slope of a plot in both cases and will be inaccurate. There is yet another and more accurate way to find the effective gradient. By substituting an isotope, $^{15}$N, into the DNA, a shift in the buoyant density is observed and the effective gradient is measured from the change in mass and the distance shifted.

$$\frac{(1 + \Gamma')}{\omega^2 \bar{r}_0} \left( \frac{dp}{dr} \right)_{\text{ett}} = \frac{\Delta m \rho_{s,0}}{m \Delta \omega^2 \bar{r}_0} = \frac{(1 + \Gamma')}{\beta_{\text{ett}}}$$  \hspace{1cm} (29)

where $\Delta r$ is the distance between the two bands $\bar{r}_0 = (r_{0,1} + r_{0,2})/2$, $m$ is the mass of the neutral component (which for convenience is taken as the mass of a cesium nucleotide component 3), and $\Delta m$ is the change in mass upon substitution and is independent of the definition of neutrality. This approach was first suggested by Vinograd and Hearst\textsuperscript{8} and later verified by Cohen and Eisenberg,\textsuperscript{6} and by Eisenberg.\textsuperscript{30} To appreciate the usefulness of this approach, notice eq. (17) may be written as

$$-M_{s,0} \left( \frac{(1 + \Gamma')}{\beta_{\text{ett}}} \right) \bar{v}_{s,0} \delta \omega^2 \rho_0 \delta = (\partial \mu_s / \partial m_0)_{\mu} dm_0$$  \hspace{1cm} (30)

All the parameters appearing in eq. (30) are observable in the experiment except $(1 + \Gamma')/\beta_{\text{ett}}$, which is precisely the quantity measured with isotopic substitution [eq. (29)].\textsuperscript{6,30} If we momentarily substitute eq. (29) into eq. (30), we find the combination $(M_{s,0}/m)$. The term $M_{s,0}/m$ is the number of nucleotides which is the parameter measured in the experiment.
Obviously \( m \) must have the same definition of neutrality as \( M \). This work will be based on component 3 being Cs DNA; and, correspondingly, we must take \( m \) as the mass of a cesium nucleotide for consistency. Since the only quantity measured is the number of nucleotides, even unusual choices of neutral components such as Cs\(_{n/2}\)Cl\(_{n/2}\) DNA \( \cdot \) \( \theta(n/2) \)CsCl, \( \theta \) any number, can only give consistent results. This concept has previously been explicitly pointed out for multicomponent sedimentation equilibrium in general.\(^6\) We may observe that Daniel's laborious estimation\(^8\) of \( \theta \) for this problem is therefore irrelevant.

**Molecular Weight Determination**

The well-known conclusion that the concentration distribution is Gaussian is derived by assuming thermodynamic ideality for the solvated polymer,

\[
(\partial \mu_3/\partial m_3)_{\mu_1} = RT/m_3
\]

The connection between this assumption and the multicomponent nature of the problem has been previously discussed.\(^3\) Assuming thermodynamic ideality, eq. (30) is easily integrated and we find

\[
m_3 = m_{3,0} \exp\left\{ -M_3(1 + \Gamma')\hat{\nu}_{s,0}^3 \sigma_0^2/2\beta_{et1}RT \right\}
\]

\[
= m_{3,0} \exp\left\{ -\hat{\nu}^2/2\sigma^2 \right\}
\]

(31)

where

\[
M_3(1 + \Gamma')\hat{\nu}_{s,0}^3 \sigma_0^2/\beta_{et1}RT = 1/\sigma^2
\]

and \( m_{3,0} \) is the concentration at band center.

It has been demonstrated in a previous publication that thermodynamic nonideality of the solvated DNA is appreciable.\(^6\) Various methods of obtaining apparent molecular weights as a function of concentration were discussed, and alternate extrapolations to infinite dilution to correct for nonideality were considered in that work. It was found that evaluation of the moments

\[
\langle m_3 \rangle = \int_{-\infty}^{+\infty} m_3 d\delta / \int_{-\infty}^{+\infty} m_3 d\delta
\]

and

\[
\langle \delta^2 \rangle = \int_{-\infty}^{+\infty} \delta^2 m_3 d\delta / \int_{-\infty}^{+\infty} m_3 d\delta
\]

and an equation to correct for thermodynamic nonideality

\[
\ln[\beta_{et1}RT/\langle \delta^2 \rangle (1 + \Gamma')\hat{\nu}_{s,0}^3 \sigma_0^2] = \ln M_3 - B'(m_3)
\]

(32)

gave a suitable analysis of the experimental data.

**EXPERIMENTAL**

*E. coli* 3100 was grown with aeration on a synthetic medium with NH\(_4\)Cl as the only nitrogen source.\(^11\) For density labeling, \(^{15}\)NH\(_4\)Cl

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\( ^6 \) Schmid AND HEARST

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\( ^8 \) Schmid AND HEARST
(International Chemical and Nuclear Corp.) with a manufacturer’s assay of 99.1% atom $^{15}$N was used. Mass spectroscopy assayed the sample as being at least 99% atom $^{15}$N. Overnight cultures of synthetic medium with the appropriate isotope were infected with $E. coli$ from a slant. Small aliquots of overnight culture were used to infect subsequent overnight cultures or the main growth medium. This was done to dilute out $^{14}$N contaminants from the slant. Three independent samples of $^{15}$N $E. coli$ and two of unlabeled $E. coli$ were grown to test for complete incorporation. Cells were grown to late log or early stationary phase for high yield.

The phenol extraction procedure of Smith was used to isolate DNA from these samples. After two or three phenol extractions and exhaustive dialysis, the samples were incubated with deoxyribonuclease free ribonuclease; and the phenol extractions and dialysis were repeated. The final product was dialyzed against 0.15 M CsCl, 0.01 M Tris, pH 7.2, and stored in this solvent. High shear was avoided to assure narrow bands which facilitate locating band center.

Solutions of cesium chloride, Harshaw optical grade, and cesium sulfate, Gallard-Schlesinger optical grade, were prepared with 0.01 M Tris and buffered at pH 7.2. A concentrated stock of cesium formate, Harshaw optical grade, 1.98 g/ml, pH 6.95, was diluted with doubly distilled water to the desired density. Cesium bromide (A. B. Mackay, Inc.) and cesium trifluoroacetate$^{21}$ stock solution of density 1.69 and 2.0 g/ml and pH 8.6 and 8.4, respectively, were diluted with 0.01 M Tris, pH 7.2 to pH 7.9 and 8.2, respectively. Alkaline CsCl was prepared by adding a known mass of cesium hydroxide, Harshaw optical grade, sufficient to adjust the pH to 12. Measured pH’s in the alkaline CsCl solutions were 11.9 to 12.0, on a Beckman Expandomatic pH meter with a Corning combination pH electrode.

All centrifugation was performed on a Beckman Model E ultracentrifuge with a scanner and electronic speed control,$^{22}$ charcoal-filled Epon or titanium$^{23}$ double-sector centerpieces being used. Equilibrium was verified by the constant distance between two peaks and the constancy of band shape. Equilibrium is achieved in about 48–96 hr, depending on the salt and speed. Normally the temperature was changed after equilibrium and the system was reequilibrated at the new temperature. Two or three temperatures could be studied during a run.

For the buoyancy gradient work it was desirable to measure small density differences $\sim 0.02$ g/ml on small quantities of sample. A Linderstrom-Lang density gradient column was used for this purpose. It consisted of an 80-cm water-jacketed column. The temperature was held at 25°C with a temperature bath and circulator. A linear composition gradient of $\alpha$-dichlorobenzene (Eastman Organic) and a mixture of $\alpha$-dichlorobenzene and $m$-dibromobenzene (Eastman Organic), 1.82 g/ml, was poured to form a density gradient of 0.006 g/cm$^4$. The gradient was allowed to stabilize for 1 hr. Drops of 10–20 $\mu$l of the cesium salt-DNA solutions were placed on the column and the distances between drops were measured with a cathetometer (Griffin and George Ltd., London). All solutions in the buoyancy
gradient determinations had a separation of greater than 2.5 cm on the column. The densities of several solutions were measured with calibrated 1-ml Dumas bottles at 25°C in the circulating bath. These served to calibrate the density gradient. The density differences were constant with time over a 3-hr period. This was not true for several other organic solvents, including iodobenzene, bromobutane, and dibromobutane; the drops slowly dehydrated in these solvents.

To measure the buoyancy gradients of the cesium salts solutions of the two different densities are loaded in opposing sectors of the double-sector cell, and the bands in both densities are superimposed on the same trace (see Fig. 5).

Densities used in eq. (29) for the effective gradients were measured with calibrated 1-ml Dumas bottles or 100-μl pipes at room temperature (21°C) with no attempt to regulate temperature. The root-mean-square position in the liquid column is taken as the isoconcentration point.9

RESULTS

A typical trace of the isotopic substitution experiment is shown in Figure 3. The band centers are located by extrapolating the mean to the top of the peak, and the distance between the peaks is easily measured. The average cesium nucleotide molecular weight is 441 for a 50% GC DNA. The fractional change in mass is therefore \((0.991 - 0.004)3.75/441 = 8.39 \times 10^{-3}\), where we have applied the assay for \(^{15}\)N and a small correction for the natural abundance of this isotope. With these data the quantity \((1 + \Gamma')/\beta_{\text{eff}}\) of eq. (29) is evaluated. For the CsCl alkaline gradients, pH 12.0, the average nucleotide weight is 506.6, for which \(\Delta m/m = 7.31 \times 10^{-3}\). This is found by allowing the guanine and thymine to be neutralized with cesium since they are titrated at this pH.24 This is a new choice of neutral component. This new choice of component for the alkaline condition is \(\text{Cs}_{0.52}^4\text{DNA}\) where the thymine and guanine groups in the DNA are deprotonated.

To test the possibility of concentration dependence of \((1 + \Gamma')/\beta_{\text{eff}}\), the concentration of \(^{16}\)N DNA was held constant at 0.1 OD265; and the concentration of unlabeled DNA was systematically varied from 0.07 to 0.8 OD265 over five samples. No trend appeared, and \((1 + \Gamma')/\beta_{\text{eff}}\) is independent of DNA concentration. Allowing the samples to stand one week at room temperature resulted in broader bands, but the values of \((1 + \Gamma')/\beta_{\text{eff}}\) were unaffected. Speeds of 21,000–30,000 rpm were used in a cesium sulfate study and \((1 + \Gamma')/\beta_{\text{eff}}\) showed no dependence on the angular velocity. Combinations of the three labeled DNA preparations and the two unlabeled preparations gave the same values of \((1 + \Gamma')/\beta_{\text{eff}}\).

In Table I the values of \((1 + \Gamma')/\beta_{\text{eff}}\) at 20°C, the densities, and number of determinations are tabulated. The precision of these measurements is about ±2%. It is interesting that the CsCl and alkaline CsCl gradients have identical values of \((1 + \Gamma')/\beta_{\text{eff}}\). The density of a saturated solution of cesium bromide is 1.69 g/ml and it is possible to achieve this density in
the centrifuge cell giving shifts in band positions and noisy optics. This salt is not recommended for routine work. The value of $(1 + \Gamma^\prime)/\beta_{\text{eff}}$ for CsCl in the presence of 1% Sarkosyl (Geigy Chemical Co.) is about 3% lower than the value in Table I.

![Fig. 3. Raw data, employed in determining the effective density gradient by $^3$H isotope substitution, found for Cs$_2$SO$_4$, 25,000 rpm at 8.5°C.](image)

**Table I**

Values of $(1 + \Gamma^\prime)/\beta_{\text{eff}}$ at 20°C

<table>
<thead>
<tr>
<th>Salt</th>
<th>$(1 + \Gamma^\prime)/\beta_{\text{eff}} \times 10^{10}$</th>
<th>$\rho_{\text{so}, \text{g/ml}}$</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsFo</td>
<td>4.02 ± 0.08</td>
<td>1.752</td>
<td>6</td>
</tr>
<tr>
<td>CsCl</td>
<td>8.21 ± 0.17</td>
<td>1.705</td>
<td>20</td>
</tr>
<tr>
<td>CsTFA</td>
<td>11.46 ± 0.02</td>
<td>1.600</td>
<td>3</td>
</tr>
<tr>
<td>CsBr</td>
<td>16.4 ± 0.02</td>
<td>1.628</td>
<td>3</td>
</tr>
<tr>
<td>CsSO$_4$</td>
<td>19.0 ± 0.36</td>
<td>1.426</td>
<td>20</td>
</tr>
<tr>
<td>CsCl—OH</td>
<td>8.08 ± 0.08</td>
<td>1.763</td>
<td>3</td>
</tr>
</tbody>
</table>

To calculate $(1 + \Gamma^\prime)/\beta_{\text{eff}}$ at various temperatures the buoyant density was assumed constant at its 20°C value. Although the band positions are a dramatic function of temperature in CsCl the dominant term is the thermal expansion of the solvent so that the buoyant density is constant to 1% over the temperature considered.$^{25}$ The temperature studies for CsCl and Cs$_2$SO$_4$ are plotted in Figure 4. The data are adequately represented by a linear fit. Only two temperatures were studied for cesium trifluoroacetate (CsTFA) and cesium formate (CsFO). Empirical equations for the temperature dependence and the temperature range studied are summarized below:

CsFo:  $(1 + \Gamma^\prime)/\beta_{\text{eff}} = 4.308(1 - 0.00334t) \times 10^{-10}$, range 8–20°C

CsCl:  $(1 + \Gamma^\prime)/\beta_{\text{eff}} = 9.849(1 - 0.00802t) \times 10^{-10}$, range 7–33°C

CsTFA:  $(1 + \Gamma^\prime)/\beta_{\text{eff}} = 13.83(1 - 0.00855t) \times 10^{-10}$, range 8–20°C

Cs$_2$SO$_4$:  $(1 + \Gamma^\prime)/\beta_{\text{eff}} = 23.01(1 - 0.00824t) \times 10^{-10}$, range 8–33°C
For cesium chloride at 25°C, $(1 + \Gamma')/\beta_{\text{eff}} = 7.87$ is in disagreement with the previously measured value\textsuperscript{2-4} of 8.82. To determine the source of this large discrepancy, eqs. (23) and (29) may be examined term by term. Evaluation of $\Gamma'$ with eq. (15) requires a value of $\theta_3$. For $\theta_3 = 0.471$,\textsuperscript{3} we find $\Gamma'$ is $0.28$.\textsuperscript{3} This value has been confirmed with pycnometric data.\textsuperscript{6} The compressibility gradient is only 9%, and uncertainties in this term could not contribute significantly to the 12% error. Improved values of the density gradient of the salt\textsuperscript{18} are in the wrong direction and raise the error to 15%. The only quantity remaining is $(1 - \alpha)$. Large uncertainties were suspected when this parameter was determined.\textsuperscript{3}

To obtain accurate values of $(1 - \alpha)$ and to test the representation of the effective gradient with this parameter, we evaluated the buoyancy gradient for several salts. Coupled with the effective gradient data, the buoyancy gradient permits evaluation of $(1 - \alpha)$ [eq. (23)].

The experimental determination of the buoyancy gradient was performed by putting DNA cesium salt solutions of two different densities in

![Graph](image-url)
Fig. 5. Experimental trace used to determine the buoyancy density gradient, formed by putting DNA solutions of different densities in opposing sectors of a double sector cell. This particular run is at 35,000 rpm and 23°C in CsCl having a density difference of 0.0192 g/ml.

the opposing sectors of a double-sector centerpiece (Fig. 5). The distance between the peaks, as well as the positions of the menisci are easily measured from Figure 5. This data with the density difference from the density gradient column determines the buoyancy gradient [eq.(25)]. The buoyancy gradients for cesium chloride and cesium bromide were calculated from salt gradient salt by using the compressibility gradient observed for cesium chloride. The significance of the compressibility is less for salts with high gradients such as cesium bromide, and the use of the cesium chloride compressibility gradient for the cesium bromide calculation is a fair assumption.

It is necessary to correct for hydration to compare $(1 + \Gamma')/\beta_{eff}$ with $1/\beta_B$. The hydration is calculated with the reported buoyant densities by taking $\bar{v}_2 = 0.471^{15,16}$ and $\bar{v}_1 = 1.00$ and using the definition of the buoyant density [eq. (15)]. These parameters are summarized in Table II.

The agreement between the values of $\alpha$ found and here those of Hearst and Vinograd, $(\partial \rho_{s,0}/\partial a_v')_p (da_v^0/d\rho^0)$, is fair. Hearst and Vinograd estimate their uncertainty to be approximately \pm 20%, and a similar value is applicable to our values. In the case of CsCl, the buoyancy gradients found by two different methods are in agreement and, correspondingly, $\alpha$ is confirmed for this salt since $\beta_{eff}$ is accurately known. This value $\alpha = 0.35$ is in agreement with an estimate taken from Cohen and Eisenberg’s data. This estimate was taken from the slope of $(1 + \Gamma')/(\bar{v}_2 + \Gamma'\bar{v}_1)$ plotted against $\rho$ for their three highest densities. The agreement is probably somewhat fortuituous, as will be seen later.

To further test the notion of expressing $\alpha$ in terms of the derivative $(\partial \rho_{s,0}/\partial a_v^0)_p$, this quantity has been evaluated in Table II on using the salt values previously reported. These slopes are plotted on Figure 6. They give an adequate representation of the the trend of $\rho_{s,0}$ with water activity. This indicates both the validity of the approach and its inherent
<table>
<thead>
<tr>
<th>Salt</th>
<th>$(1 \times 10^5 \beta_{eff})$</th>
<th>$\rho_{20}(25^\circ C)$, g/ml</th>
<th>$1 \times 10^{10} \frac{1}{\beta_{eff}}$</th>
<th>$1 \times 10^{10}\left(\frac{1}{\beta_0} + \psi_{p+0.5}\right)$</th>
<th>$10^5 \frac{1}{\beta_0}$ g-sec$^2$/cm$^6$</th>
<th>$\Delta \rho \cdot \delta$</th>
<th>$\frac{d \rho}{d \alpha}$</th>
<th>$\frac{d \alpha}{d \rho}$</th>
<th>$\alpha$</th>
<th>$\frac{\partial \rho_{eff}}{\partial \alpha}$</th>
<th>$\frac{\partial \alpha_{eff}}{\partial \rho}$</th>
<th>$\alpha_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cs$_2$SO$_4$</td>
<td>18.3</td>
<td>1.423</td>
<td>10.28</td>
<td>16.8</td>
<td>10.0 ± 1.4d</td>
<td>0.39</td>
<td>0.46</td>
<td>2.7</td>
<td>0.36</td>
<td>0.53 ± 0.1c</td>
<td>0.67</td>
<td>0.840</td>
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<tr>
<td>CsTFA</td>
<td>10.9</td>
<td>1.600</td>
<td>7.73</td>
<td>9.40a</td>
<td>0.23</td>
<td>0.35</td>
<td>0.40</td>
<td>0.81</td>
<td>0.24</td>
<td>0.31</td>
<td>0.622</td>
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<tr>
<td>CsCl</td>
<td>7.87</td>
<td>1.790</td>
<td>6.17</td>
<td>9.35</td>
<td>0.22</td>
<td>0.24</td>
<td>0.62</td>
<td>0.757</td>
<td>0.31</td>
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<td>CsFo</td>
<td>3.95</td>
<td>1.781</td>
<td>4.21</td>
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<td>0.24</td>
<td>0.62</td>
<td>0.757</td>
<td>0.31</td>
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<td>(50-50)</td>
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<td>0.470</td>
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<td>CsFo-CsAc</td>
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<td>1.863</td>
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<td></td>
<td>0.39</td>
<td>0.46</td>
<td>2.7</td>
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<td>CsBr</td>
<td>15.6e</td>
<td>1.637</td>
<td>11.5</td>
<td>14.4</td>
<td>0.20</td>
<td>0.26</td>
<td>0.76</td>
<td>0.856</td>
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</table>

* The buoyancy gradient, $(dp/dr)_B = \omega^2/\beta_B$, is the gradient which is used in calculating the density difference between two DNA bands from their spacings in the density gradient. This is a frequent procedure when the density of a new variety of DNA is desired relative to a marker or standard DNA.

b Values of $\alpha$ found by the method of Hearst and Vinograd.

c Calculated from the experimental values of $\alpha$ by using the salt data of Hearst and Vinograd, their definition of this parameter, and the data of Tunis and Hearst.

d Unpublished data of Tunis and Hearst.

f Estimated from Figure 6 using the method of Hearst and Vinograd and the salt data of Tunis and Hearst.

g Data of Tunis and Hearst.

h Assuming the same temperature coefficient as CsCl.

i Found from the data of Cohen and Eisenberg as explained in the text.
Fig. 6. Buoyant density of *E. coli* DNA plotted against water activity: (O) from the data of Table II; (Δ) values of T-4 DNA for other salts; (——) slopes calculated from experimental values of α for the particular salt as explained in Table II.

Fig. 7. Pycnometric results of Cohen and Eisenberg for CsCl DNA (O) plotted against solution density. The slope of the line is calculated from the experimental value of α, as explained in text, and the line is drawn through the buoyant density of *E. coli* DNA (×). The buoyant density has been corrected to 1 atm pressure, although the magnitude of this correction is trivial. The line is theoretically the slope of the curve at the buoyant density.
inaccuracy. The plotted slopes are for individual anions, and this tests
the adequacy of assuming no special anion effects and the representation of
different salts using water activity as the common concentration unit.

To examine the usefulness of Cohen and Eisenberg's pycnometric ap-
proach, the slope of their experimental parameter with density [eq. (27)],
has been calculated with the data for CsCl in Table II. The line has been
positioned at the zero pressure buoyant density in Figure 7.

The agreement between the calculated slope and the curved experimental
data reflects the equivalence of the theories and experimental agreement be-
tween the two sets of data. There is no reason to expect linear behavior of
\((\partial \rho / \partial C_i)_m\) over an extended density range, however only the slope at the
buoyant density is of interest. It is difficult to obtain data in this region as
Cohen and Eisenberg point out, and their data do not adequately predict
the buoyant density. To circumvent the paucity of data in this region, the
authors chose to infer the slope from isotopic substitution data as was done
here.

**DISCUSSION**

To better understand density-gradient sedimentation equilibrium, it is
interesting to compare the various physical descriptions which have been
proposed.

The original two-component approach predicted a Gaussian band shape
with the inverse square of the band width proportional to molecular weight. While this is essentially observed, the description is, of course, an inadequate one. Preferential interaction of the polymer with a solvent component per-
sists to the limit of infinite dilution and it is necessary to correct for this
effect. Since preferential interactions will vary somewhat with binary sol-
vent composition, the effect is complicated; and it is necessary to quantita-
tively specify the variation of interactions over the composition range
covered by the band. The experiment is performed near the buoyancy con-
dition and pressure effects on the partial specific volume of the polymer
must be considered, although pressure effects of this magnitude would be
negligible for conventional sedimentation equilibrium.

Yeandle's treatment, which was essentially an inclusion of electrolyte
effects on the original two-component approach, resulted in a new definition
of the neutral component, the Scatchard definition. This definition ad-
dresses itself to preferential interaction of an electrostatic nature. How-
ever, Yeandle specifically omits all nonelectrical interactions by assuming
the activity coefficients of cesium, chloride, and DNA ions are constant.
This assumption neglects the cross terms in eqs. (10) and (11). For the
case of DNA in particular, it has been shown the electrolyte contribution to
the preferential interaction is less than the contribution from other terms, and, therefore, the cross terms are non-negligible. Choosing a Scatchard
definition for neutral DNA does not completely solve a problem and is of no
special virtue, although it can easily be considered as in the Theory Sec-
ton.
A discussion of density-gradient sedimentation equilibrium has been recently presented by Daniel\textsuperscript{18} based partially on Yeandle's treatment, ion binding, an arbitrary introduction of hydration, and the results of isotopic substitution. As mentioned in the Theory Section, selection of a component such as Cs\(_{n/2}\)Cl\(_{-n/2}\) DNA \(\theta(n/2)\)CsCl would be just as good as any other choice of neutral component. This choice corresponds to partially ion bound, \(\theta_n\), polyion; the rest of the charges being neutralized with a Scatchard definition which results from using Yeandle's approach. Choice of a fraction \(\theta\) of ion bound does not influence the observed degree of polymerization, and any numerical value of \(\theta\) would be acceptable. For 95\% \(^{15}\)N substitution, Daniel observes a density shift of 0.0155 g/ml; this is in agreement with a shift of 0.0152 ± 0.0003 g/ml expected for a 95\% labeled DNA from our 25°C data. However, Daniel's treatment is in error for two serious reasons: his use of numerical values for the partial specific volume of ionic species and postulation of a hydration of an ionic species. Neither concept is necessary or experimentally measurable. Yeandle's treatment specifically defines an electrically neutral species with the neutrality condition and it is therefore unwarranted to interpret his results with an undefined ionic volume. The molar volume of the Scatchard definition of a neutral component in our notation is merely

\[
M_{3'}\theta_{3'} = M_3\theta_3 - (n/2)M_2\theta_2
\]

Daniel's casual introduction of the hydration corresponds to neglecting \((n/2)(\mu_{2X} - \mu_{2Y})\) in the first term of eq. (9), rewriting the second term on the left-hand side of eq. (8) as \(\mu_{pX} + \mu_{pY}\), and then eliminating \(d\mu_{pY}/dr\) in eq. (8) with eq. (9). This error results in an incomplete elimination of \(d\mu_{pY}/dr\), and the pseudo two-component equilibrium, eq. (13) is incomplete. The whole point of defining either a hydration or a negative absorption of salt is to precisely describe the DNA in membrane equilibrium with bulk solvent; by not eliminating \(d\mu_{pY}\), this component has not been described. This is the reason Daniel had to consider the ill-defined concept of the hydration of an ion.

It should also be noted that recalculation of Thomas and Pinkerton's\textsuperscript{26} data by Daniel\textsuperscript{10,27} is unjustified. Thomas and Pinkerton omitted virial corrections,\textsuperscript{8} whereas Daniel observes virial effects.

It has already been pointed out that the treatments of Hearst and Vinograd\textsuperscript{2,3} and Casassa and Eisenberg\textsuperscript{11} are in agreement. Both theories describe a preferential interaction of the DNA with a solvent component either water, hydration\textsuperscript{2} or equivalently with salt, negative absorption.\textsuperscript{11} Both theories recognize the composition gradient through the band and allow the extent of interaction to vary with composition, expressed as either density\textsuperscript{11} or water activity.\textsuperscript{8}

Use of water activity to describe the composition of many different cesium salts assumes the results are independent of the anion. In particular this assumption seems questionable for cesium trifluoroacetate. The trifluoroacetate ion has definite effects on the helix stability of the DNA,\textsuperscript{2} and
the buoyant density of DNA in this salt is somewhat lower than what is expected for this water activity\textsuperscript{28} (Fig. 6). The agreement between the value of \(\nu_2\) found by extrapolating buoyant densities to zero water activity\textsuperscript{3} and the pycnometric value found in CsCl\textsuperscript{6} indicates the assumption is a reasonable approximation. Hearst and Vinograd were able to measure \(\alpha\) for many different salts with this concept.

Determining the variation of preferential interaction with the pycnometric method\textsuperscript{6} is laborious and limited to only one salt, while the water activity approach could easily be applied to new salts as they are developed. Of course, the pycnometric method obviates the question of special anion effects.

Either approach requires accurately evaluating a derivative and the difficulty of this is seen in Figures 6 and 7. This difficulty is avoided in the isotopic substitution experiment where the experimental parameter is directly related to the effective density gradient.

Since all the quantities contributing to the effective density gradient have been introduced theoretically and experimentally, it is possible to quantity the intuitive arguments concerning the band width in Figure 2. From eq. (30) it is apparent the band width is inversely proportional to the square root of the effective density gradient. Since pressure shifts the buoyant density to lower values, DNA on the denser side of band center is shifted closer to band center and the distribution is narrowed. The compressibility term appearing in eq. (33) modifies the composition density gradient \((1/\rho_0)\alpha^2r\) by the factor \(\psi(\rho_0, \rho')\alpha^2r\). It increases the effective density gradient and therefore narrows the concentration distribution. From the buoyancy-density gradient data in Table I for CsCl and the composition-density gradient data,\textsuperscript{18} it may be shown that the effects of pressure increase the effective density gradient by 7\% and decreases the band width by 4\%.

The buoyant density is seen to increase with decreasing water concentrations (Fig. 6). DNA on the dense side of band center will be in a region of lower water concentration and its buoyant density will increase widening the band. It is seen in eq. (23) that this effect decreases the effective density gradient by the factor \(\alpha\) or 0.35 in CsCl, and increases the band width by 20\% (Fig. 2).

In evaluating molecular weights with density-gradient sedimentation equilibrium, the only parameter not experimentally observable is \((1 + \Gamma')/\beta_{eff}\), which is the parameter reported for several temperatures and salts in this study. It is therefore possible to substitute \((1 + \Gamma')/\beta_{eff}\) into eq. (31) and evaluate \(M_3\), the molecular weight of dry cesium DNA.

It is our contention that the values of \((1 + \Gamma')/\beta_{eff}\) will be applicable to DNA samples having base compositions and buoyant densities different than DNA \textit{E. coli} which was used to determine this quantity. The composition density gradient is a weak function of density\textsuperscript{17,18} and should not vary appreciably over the buoyant densities of different DNA's. The compressibility term \(\psi\) is dependent on the isothermal compressibility of the DNA. The isothermal compressibilities of protein, virus, and DNA appear similar;
and, therefore, differences in base composition should have a negligible effect on $\psi$.

Tunis and Hearst have measured the variation of the buoyant density with water activity for DNA's of various base composition. From their results $\alpha$ appears to be independent of base composition. Cohen and Eisenberg's data for calf thymus DNA (39% GC) in Figure 7 are adequately represented with the results for DNA E. coli (50% GC).

The hydration $\Gamma'$ is dependent on base composition. For the salts studied here it can be estimated from the data of Tunis and Hearst that $1 + \Gamma'$ will vary by less than 3% in the range 50 ± 25% GC. For a DNA with a base composition in this range it is recommended that $(1 + \Gamma')/\beta_{att}$ be used without correction. If desired or if a DNA of an extreme base composition is being studied, suitable corrections of $(1 + \Gamma')$ may be estimated from the data of Tunis and Hearst.

The molecular weights of the coliphage DNA's previously reported are recalculated with the more accurate value of $(1 + \Gamma')/\beta_{att}$ for CsCl at 20°C reported in this work and are reported in Table III. These values are in good agreement with recent physical-chemical measurements of the molecular weights. Since the theory is well understood and accurate values of the effective density gradient are available, density-gradient sedimentation equilibrium may be used with confidence in determining the molecular weights of DNA.

<table>
<thead>
<tr>
<th>DNA</th>
<th>$M \times 10^{-4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-7</td>
<td>24.8 ± 0.4</td>
</tr>
<tr>
<td>T-5+</td>
<td>68.7 ± 6</td>
</tr>
<tr>
<td>T-4</td>
<td>113 ± 6</td>
</tr>
</tbody>
</table>

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References


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