Substituting equation (13) into equation (11) gives:

\[
J = \left\{ \frac{(2\pi mkT)^{3/2}}{h^5} \left( \frac{2\pi kT}{k_r} \right)^{3/2} \left[ 1 + n \left( \frac{V - V_s}{V_s} \right) \right] e^{-\frac{aE_v}{RT(V - V_d)}} \right\} \left[ \frac{E_v}{RT} \right]^{\frac{N}{V}}
\]

\[
\times \left\{ \frac{(2\pi mkT)^{3/2}}{h^5} \frac{eV}{N} \left( \frac{V}{V_s} \right)^{\frac{N}{V}} \right\}^{1/2} (17)
\]

where \( E_v \) and \( k_r \) are calculated from equations (14) and (16), respectively, and \( n = 10.7 \) and \( a = 0.0052 \) for all simple liquids.\(^{14}\) The modified solid-part of equation (17) is incorporated into \( f_1 \) and \( f_2 \) of equation (2), and mixture calculations are made for \( AR + NS \). The results are compared with those obtained from the Einstein oscillator.

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\(^{1}\) Eyring, H., T. Ree, and N. Hirai, these PROCEEDINGS, 44, 683 (1958); Eyring, H., and T. Ree, these PROCEEDINGS, 47, 526 (1961).

\(^{2}\) Fuller, E. J., A. T. Ree, and H. Eyring, these PROCEEDINGS, 45, 1594 (1959).


\(^{5}\) Carlson, C. M., H. Eyring, and T. Ree, these PROCEEDINGS, 46, 333 (1960).


\(^{9}\) Liang, K., H. Eyring, and R. Marchi, these PROCEEDINGS, 52, 1107 (1964).


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THE RELATIONSHIP BETWEEN THE DENSITOMETRIC AND DILATOMETRIC VOLUME CHANGES OF RIBONUCLEASE ACCOMPANYING A CHANGE IN pH

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The interpretation of the partial specific volume of a polyelectrolyte requires careful consideration of the components being measured, especially when the results of different types of experiments are compared. Ulrich, Kupke, and Beams\(^{1}\) have developed a sensitive magnetic densitometer for measuring the densities of small volumes of solutions. These workers found that the partial specific volume of ribonuclease decreased by 0.003 ml/gm when the pH was increased from 7.6 to
Rasper and Kauzmann measured the volume change, by dilatometry, for the reaction of ribonuclease with sodium hydroxide in going from pH 7.6 to pH 9.6 and found a volume increase equivalent to 0.0058 ml/gm. This apparent inconsistency was noted by Ulrich et al. We believe, however, that when properly compared, both findings are in excellent agreement.

For solutions whose densities increase linearly with protein concentration, the partial specific volume (ml/gm) of the protein is obtained experimentally from the relationship

\[ v_p = \frac{1}{d^o} \left[ 1 - 1000 \left( \frac{d - d^o}{g} \right) \right] \tag{1} \]

where \( d^o \) is the density of the solvent (gm/ml), \( d \) is the density of the solution (gm/ml), and \( g \) is the concentration of the protein (gm/liter).

Ulrich et al. dialyzed solutions of different protein concentrations against solvent and then determined the densities and concentrations of the dialyzed solutions. Because of the dialysis the Donnan membrane equilibrium must be taken into account. The system can be considered to have three components: a nondiffusible polyelectrolyte (ribonuclease), a diffusible salt (KCl), and water. The concentrations of hydrogen and hydroxide ions are assumed to be negligible. In the equations that follow, the superscript zero refers to species on the solvent side of the dialysis membrane and characters without superscripts refer to species on the side of the membrane containing the protein solution. Activities are assumed to be equal to concentrations. At equilibrium

\[ (C_+)(C_-) = (C_+^0)(C_-^0) = (C^0)^2 \tag{2} \]

where \( C_+ \) is the molar concentration of potassium ion and \( C_- \) is the molar concentration of chloride ion. If the protein concentration is small, the Donnan equilibrium gives

\[ C_+ = C^0 - (Z/2)C_{pz} \tag{3} \]
\[ C_- = C^0 + (Z/2)C_{pz}, \tag{4} \]

where \( C_{pz} \) is the molar concentration of protein ion, and \( Z \) is the net charge of the protein ion. Also,

\[ d = \frac{(C_{pz}M_{pz} + C_+M_+ + C_-M_- + C_wM_w)}{1000} \tag{5} \]
\[ d^o = \frac{(C^0M_+ + C^0M_- + C^0_wM_w)}{1000}, \tag{6} \]

where \( C_w \) is the molar concentration of water, and \( M_{pz}, M_+, M_- \), and \( M_w \) are the molecular weights of protein ion, potassium ion, chloride ion, and water, respectively.

Substituting equations (3) and (4) into equation (5), we obtain for the density difference

\[ d - d^o = \frac{[C_{pz}M_{pz} + (Z/2)C_{pz}(M_- - M_+) + (C_w - C^0_w)M_w]}{1000}. \tag{7} \]

The total volumes of a liter of solvent and solution are given by

\[ C_w^0V_w + C^0(V_+ + V_-) = 1000 \tag{8} \]
and
\[ C_w V_w + C_{PZ} V_{PZ} + C_+ V_+ + C_- V_- = 1000, \tag{9} \]
where \( V_w, V_+, V_-, V_{PZ} \) are the partial molar volumes of water, potassium ion, chloride ion, and protein ion, respectively. Inserting equations (3) and (4) into equation (9) and equating the left-hand sides of equations (8) and (9), we obtain
\[ (C_w^0 - C_w) V_w = C_{PZ} V_{PZ} + (Z/2) C_{PZ} (V_- - V_+). \tag{10} \]
Since \( V_w = M_w/d_w \), where \( d_w \) is the density of water (in gm/ml), equation (10) becomes
\[ (C_w - C_w^0) M_w = [(Z/2) C_{PZ} (V_+ - V_-) - C_{PZ} V_{PZ}] d_w. \tag{11} \]
Substituting equation (11) into equation (7), we obtain
\[ d - d^0 = [C_{PZ} M_{PZ} + (Z/2) C_{PZ} (M_- - M_+)] + (Z/2) C_{PZ} (V_+ - V_-) d_w - C_{PZ} V_{PZ} d_w] / 1000. \tag{12} \]
The expression for \( d - d^0 \) given above can then be inserted into equation (1) to give
\[ v_p = \frac{1}{d^0} \left\{ 1 - \frac{1}{g} \left[ C_{PZ} M_{PZ} + (Z/2) C_{PZ} (M_- - M_+) + (Z/2) C_{PZ} (V_+ - V_-) d_w - C_{PZ} V_{PZ} d_w \right] \right\}. \tag{13} \]
The value of \( g \) used by Ulrich et al. is ultimately based on the difference in dry weights of equal volumes of protein solution and dialyze,
\[ g = C_{PZ} M_{PZ} + C_+ M_+ + C_- M_- - C_0 M_+ - C_0 M_-; \tag{14} \]
which, in view of equations (3) and (4), gives
\[ g = C_{PZ} M_{PZ} + (Z/2) C_{PZ} M_+ - (Z/2) C_{PZ} M_. \tag{15} \]
Therefore,
\[ v_p = \frac{\left[ V_{PZ} + (Z/2) V_- - (Z/2) V_+ \right] d_w/d^0}{\left[ M_{PZ} + (Z/2) M_- - (Z/2) M_+ \right]}. \tag{16} \]
Recognizing the denominator as the total molecular weight, \( M_{PS} \), of the protein component as defined by Scatchard,\(^3\) the partial molar volume of the Scatchard protein component is
\[ V_{PS} = \left[ V_{PZ} + (Z/2) V_- - (Z/2) V_+ \right] d_w/d^0. \tag{17} \]
It follows that the difference in \( V_{PS} \) measured at two pH values, the net charge going from \( Z \) to \( Z' \), is
\[ V_{PS}' - V_{PS} = v_p' M_{PS}' - v_p M_{PS} = \left[ V_{PZ}' + (Z'/2) V_- - (Z'/2) V_+ \right] d_w/d^0 - \left[ V_{PZ} + (Z/2) V_- - (Z/2) V_+ \right] d_w/d^0. \tag{18} \]
The dilatometric procedure of Rasper and Kauzmann measures the change in volume for the reaction
\[ \text{Protein-NH}_3^+ + \text{OH}^- = \text{Protein-NH}_2 + \text{H}_2\text{O}, \]
or, when applied to the present case, for the reaction

\[ \text{Protein-NH}_3^+ + \text{OH}^- = \text{Protein-NH}_2 + \text{H}_2\text{O}, \]
\[ P_z + (Z - Z')OH^- = P_{z'} + (Z - Z')H_2O, \] (19)

where \( P_z \) represents the protein ion of net charge \( Z \). The dilatometric volume change for the titration of one amino group (i.e., \( Z - Z' = 1 \)) is given by

\[ \Delta V = V_{P(z-1)} - V_{Pz} + V_w - V_{OH}, \] (20)

where \( V_{OH} \) is the partial molar volume of the hydroxide ion. For the titration of \( Z - Z' \) groups we have

\[ (Z - Z')\Delta V = V_{Pz'} - V_{Pz} + (Z - Z')V_w - (Z - Z')V_{OH} \] (21)

or

\[ (V_{Pz'} - V_{Pz}) = (Z - Z')\Delta V + (Z - Z')V_{OH} - (Z - Z')V_w. \] (22)

In going from pH 7.6 to pH 9.6 (approximately the isoelectric point\(^4\)), ribonuclease takes up about three moles of hydroxide ion,\(^5\) so that \( Z \) and \( Z' \) have the values 3 and 0, respectively. (Figure 4 of Rasper and Kauzmann\(^2\) indicates that 5 moles of hydroxide are taken up in this pH range, but the pH scale in this figure is evidently in error.) Using these values in equations (18) and (22) we find

\[ v_p'M_pS_p' - v_pM_pS = V_{PpS_p'} - V_{PpS} = [(V_{PO} - V_{Ps}) - (\frac{1}{2})V_+ + (\frac{1}{2})V_+]d_{wr}/d_p \] (23)

and

\[ V_{PO} - V_{Ps} = 3\Delta V + 3V_{OH} - 3V_w. \] (24)

Substituting equation (24) into equation (23), we obtain

\[ v_p'M_pS_p' - v_pM_pS = V_{PpS_p'} - V_{PpS} = [(3\Delta V + 3V_{OH} - 3V_w) - (\frac{1}{2})V_+ + (\frac{1}{2})V_+]d_{wr}/d_p. \] (25)

In order to obtain a numerical solution to the above equation, the following additional information is needed:

\[ \Delta V = 16 \text{ ml/mole}\(^2\) \]
\[ V_w = V_{OH} + V_H + 21.3 \text{ ml/mole}\(^4\) \]

or

\[ 3V_{OH} - 3V_w = -3 \times 21.3 - 3V_H, \]

where \( V_H \) is the partial molar volume of the hydrogen ion,

\[ V_{KC1} = V_+ + V_- = 26.8 \text{ ml/mole}\(^5\) \]
\[ V_{HC1} = V_H + V_- = 17.8 \text{ ml/mole}\(^8\). \]

On making the proper substitutions, equation (25) becomes

\[ v_p'M_pS_p' - v_pM_pS = V_{PpS_p'} - V_{PpS} = [3 \times \Delta V - 3 \times 21.3 - 3V_H - (\frac{1}{2})V_+ + (\frac{1}{2})V_+]d_{wr}/d_p \]
\[ = [3 \times \Delta V - 3 \times 21.3 - 3V_{HC1} + (\frac{1}{2})V_{KC1}]d_{wr}/d_p \]
\[ = -29.1 \text{ (ml/mole)}(d_{wr}/d_p). \] (26)
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If we set \( M_{PS} \) equal to 13,683 (corresponding to \( Z = 0 \)), then \( M_{PS} = 13,686 + 1.5 \times 35.5 - 1.5 \times 39.1 = 13,681 \), where the molecular weight of the ribonuclease ion, \( M_{PS} \), is given the same value as the uncharged protein plus three, and 35.5 and 39.1 are the molecular weights of the chloride and potassium ions, respectively. The ratio \( d_{w}/d_{p} \) is 0.9928 for a 0.15 molar KCl solution. Taking the value of 0.6949 \( \pm \) 0.0015 ml/gm given by Ulrich et al. for \( v_{p} \), we calculate a value of 0.6927 ml/gm for \( v_{p}' \) from equation (26). The value of \( v_{p}' \) observed by Ulrich et al. is 0.6919 \( \pm \) 0.0017 ml/gm, which is in satisfactory agreement with the value just calculated. The difference, \( v_{p}' - v_{p} \), from the dilatometric measurements is, thus, \(-0.0022\) ml/gm which, within the experimental error, agrees with the value \(-0.003\) ml/gm measured by Ulrich et al.

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9 Personal communication from Dr. D. Kupke.

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THE OPTICAL DETECTION OF TRANSIENTS IN TRYSIN- AND CHYMOTRYPSIN-CATALYZED REACTIONS*

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A large group of enzymes which catalyze the hydrolysis of esters or the transfer of acyl or phosphoryl groups to water and other acceptors have one particularly reactive serine-OH group among their amino acid residues. Such diverse enzymes as trypsin, chymotrypsin, alkaline phosphatase, phosphoglucomutase, and subtilisin fall within this group, which might be called the "active serine enzymes." It has been postulated that these enzymes react with their substrates to form O-serine acyl enzymes or phosphoryl enzymes as intermediates:

\[
\begin{align*}
  E + AB & \rightleftharpoons EAB \xrightarrow{k_1} EA + B \\
  EA + C & \xrightarrow{k_2} E + AC
\end{align*}
\]

where \( C \) is either water or some other nucleophilic acceptor.

A number of lines of evidence are available for this generalization about the mechanism of chymotrypsin- and trypsin-catalyzed reactions: (1) Hartley and Kilby\(^1\) showed that the hydrolysis of nitrophenyl esters catalyzed by chymotrypsin proceeds in two distinct steps, an initial rapid nitrophenol liberation followed by a consequent acetate liberation. (2) The kinetics of the hydrolysis of a wide range of