THE PARTIAL SPECIFIC VOLUMES OF MACROMOLECULES

A THESIS

by

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INTRODUCTION

Of the many properties of interest in the vast and important domain of macromolecules, one of the most essential is the molecular weight or distribution of molecular weights. In several of the methods that are available for determining these quantities, an accurate value for the partial specific volume, $\bar{V}$, (1) of the macromolecular species is necessary. The present study is concerned with the critical evaluation of $\bar{V}$ for several different polymeric materials by different techniques.

High polymers or macromolecules are formed from atoms, or groups of atoms linked by primary valences to form chains, two-dimensional laminated sheets, or three-dimensional networks. Only the first type will be considered in this work since it is difficult to envisage a discrete molecular weight in the other types. The chain molecules include synthetic polymers, natural polymers and globular proteins. Globular proteins may be thought of as chains linked together by primary and secondary valences in a discrete structure. Synthetic polymers generally possess a distribution of molecular weights that results from the methods of preparation, while naturally occurring polymers may display a distribution

in molecular weights, as in natural rubber, or possess singular molecular weights, at least to a first approximation as in the globular proteins. Despite a distribution of molecular weight, a given species of macromolecule does not possess a distribution of $\bar{V}$.

One of the better methods to determine the molecular weight of a macromolecule is by sedimentation velocity. In this method the rate of sedimentation of the macromolecule under an intense centrifugal field is measured. The molecular weight may then be computed from Svedberg's formula (2):

$$M = RTs / D(1-\bar{V}d) \qquad \ldots\ldots\ldots(1)$$

where R is the gas constant, T the absolute temperature, D the diffusion constant, s the sedimentation coefficient, d the density of the medium and $\bar{V}$ the partial specific volume.

Sedimentation equilibrium is another useful method by which the molecular weight may be determined. According to Svedberg (2) if the centrifugal field is applied long enough to enable the solute to reach a state of equilibrium between sedimentation and diffusion then:

$$M = \frac{2\,RT \ln (c_2/c_1)}{(1-\bar{V}d)\omega^2(x_2^2 - x_1^2)} \qquad \ldots\ldots\ldots(2)$$

where ω is the angular velocity and c_1 and c_2 are the concentrations at distances x_1 and x_2 from the centre of rotation.

A third method of molecular weight determination is due to Archibald (3). Since the net flux of solute molecules

across the liquid meniscus and the bottom of the ultracentrifug cell is zero, transport of solute by sedimentation must be equal to that by diffusion. Therefore for these two positions in the cell the following equation is valid at all times during the experiment:

$$M_{m,b} = \frac{RT}{(1-\bar{V}d)\omega^2} \quad \frac{(dc/dx)_{m,b}}{x_{m,b}\, c_{m,b}} \qquad \ldots\ldots\ldots(3)$$

where m refers to the position of the meniscus and b refers to the position of the base of the cell, and c and dc/dx are the concentration and concentration gradient respectively.

$\bar{V}$ is also useful in deriving the frictional ratio of a macromolecule f/f_o, from which in turn, the shape of a macromolecule may be inferred. When the molecular weight is known (from sedimentation equilibrium, sedimentation velocity plus diffusion, light scattering etc.)the frictional constant, f_o, may be calculated for a compact spherical and unhydrated molecule of the same mass:

$$f_o = 6\pi\eta N \left(\frac{3M\bar{V}}{4\,N\pi}\right)^{1/3} \qquad \ldots\ldots\ldots(4)$$

The actual frictional constant, f, possessed by a macromolecule may be evaluated from (4) :

$$f = M\,(1-\bar{V}d)/s \qquad \ldots\ldots\ldots(5)$$

or

$$f = RT\,/\,D \qquad \ldots\ldots\ldots(6)$$

If the ratio f/f_o is equal to 1.0 for a macromolecule, it must have a compact, spherical shape and cannot be hydrated. Values of the frictional ratio greater than 1.0 may be due to asymmetry, hydration, or both. Theories are available (4) which assume the

molecule to be an ellipsoid of revolution for which axial ratios may be calculated from f/f_o.

THEORETICAL DESCRIPTION OF $\bar{V}$

The volume of an aqueous solution in general is not equal to the sum of the volumes of its individual components. Indeed, some salts when added to water cause a decrease in the volume of the system to less than that of the water alone. Frank and Evans (5) have shown that both charged and uncharged molecules exert forces on water molecules which tend to organize the dipole water molecules locally and results in a change in the volume of a system. The effect would be large in macromolecules possessing charged groups. Dipolar ions such as occur in amino acids and proteins would also affect the dipoles of the water molecules.

Since the contribution to the volume of a solution of each component cannot be isolated, the part played by each component is described in an operational manner by its partial specific volume. From the postulations of Lewis and Randall (1):

$$V_{1,2,\ldots,k} = f(g_1, g_2, \ldots . g_k) \quad \ldots\ldots\ldots (7)$$

where $V_{1,2,\ldots,k}$ is the volume of a solution which contains $g_1, g_2, \ldots, g_k$ grams of components $1,2,\ldots,k$. The partial specific volume $\bar{V}_i$ of component i can be designated by the equation:

$$\bar{V}_i = \left(\partial V_{1,2,\ldots,k} / \partial g_i\right)_{T,P,g_1,\ldots,g_k} \quad \ldots . (8)$$

That is, the partial specific volume of the i th component is defined as the rate of change of the number of grams of component i, all other components being held constant at constant pressure and temperature. The partial specific volume of component i may also be regarded as the change in volume caused by the addition of one gram of component i to a quantity of solution so large that the addition of one gram of component i makes no significant change in the composition at constant temperature and pressure. The specific volume of the solution $v_{1,2.....,k}$ is accordingly:

$$v_{1,2....,k} = \frac{f(g_1, g_2, ... g_k)}{(g_1 + g_2 + ... + g_k)} . \qquad .....(9)$$

Usually, aqueous solutions of macromolecules are considered binary with the macromolecule as component 2 and the aqueous medium as component 1.

From equation (8) it is apparent that $\overline{V}$ can be obtained by plotting the volume of the solution $V_{1,2}$ against g_2. The slope of the curve obtained at each concentration of g_2 is the partial specific volume of the second component. Since it is difficult to measure such a slope with the required precision, it is convenient to introduce another quantity termed the apparent specific volume ϕ (1)of the macromolecule. This is the increase in the volume of a solution caused by the addition of one gram of solute.

Now, from equation (7) for a binary solution, partial differentiation gives:

$$dV_{1,2} = (\partial V_{1,2}/\partial g_1)_{g_2}\, dg_1 + (\partial V_{1,2}/\partial g_2)_{g_1} dg_2$$

$$= \bar{V}_1 dg_1 + \bar{V}_2 dg_2 \qquad \text{.....(10)}$$

Equation (10) by integration at constant composition, pressure and temperature gives:

$$V_{12} = \bar{V}_1\, g_1 + \bar{V}_2\, g_2. \qquad \text{.....(11)}$$

In the infinitely dilute solution or pure solvent

$$V_1 = g_1\, \bar{V}_1 = g_1\, v_1. \qquad \text{.....(12)}$$

By definition

$$\phi = (V_{1,2} - V_1)\, /g_2. \qquad \text{.....(13)}$$

$\therefore$ from equation (12)

$$\phi = (V_{1,2} - v_1 g_1)\, /\, g_2. \qquad \text{.....(14)}$$

Transformation of Equation (14) gives:

$$\phi = \frac{(V_{1,2}/g_{1,2}) - v_1\, (g_1/g_{1,2})}{g_2/g_{1,2}}. \qquad \text{.....(15)}$$

If w_2 is the weight fraction of the macromolecule and w_1 is equal to $(1 - w_2)$, equation (15) can be rearranged to:

$$\phi = v_1 + (v_{1,2} - v_1)\, /\, w_2\, . \qquad \text{.....(16)}$$

And since specific volume is the reciprocal of the density equation (16) may be written as:

$$\phi = \frac{1}{d_1}\left[1 - \frac{d_{1,2} - d_1}{d_{1,2}\, w_2}\right]. \qquad \text{.....(17)}$$

Equation (17) is very suitable for the evaluation of ϕ from density measurements and with proteins (6) ϕ equals $\bar{V}_2$ within the error of measurement. The same is probably true of other macromolecules when ϕ does not vary with concentration.

The apparent specific volume, ϕ, may be used to obtain $\bar{V}_2$ (1). If ϕ is plotted against log g_2/g_1, the slope of the curve $d\phi$ / d log g_2/g_1, provides a means of calculating $\bar{V}_2$ since:

$$\frac{d\phi}{2.303\ d\ g_2/g_1} = \bar{V}_2 - \phi. \qquad \text{.....(18)}$$

As an alternate method of obtaining $\bar{V}_2$ from ϕ (7), a powers series in $g_2/g_1 = \gamma$ may be employed.

From equation (14):

$$\phi = \frac{(V_{1,2/g_1}) - v_1}{\gamma} = a_1 + a_2\gamma + a_3\gamma^2 + \text{.....(19)}$$

Also

$$V_{1,2/g_1} = v_1 + a_1\gamma + a_2\gamma^2 + a_3\gamma^3 + \text{. .....(20)}$$

Therefore

$$\bar{V}_2 = (\partial V_{1,2}/\partial g_2)_{g_1} = \left[\partial(V_{12}/g_1)/\partial\gamma\right]_{g_1}$$

$$= a_1 + 2a_2\gamma + 3a_3\gamma^2 . \qquad \text{.....(21)}$$

Subtraction of equation (19) from (21) gives:

$$\bar{V}_2 = \phi + a_2\gamma + 2a_3\gamma^2 + \text{.} \qquad \text{.....(22)}$$

The constants a_2, a_3, ... a_n may be evaluated from equation (19) by a least squares analysis of ϕ as a function of γ.

SURVEY OF METHODS

Probably $\bar{V}$ is most often estimated from density measurements with pycnometers. The densities are measured and ϕ or $\bar{V}$ calculated from equations similar to (17) or (22). The method is simple but tedious and time consuming. However, all that is required is a good balance, calibrated weights, pycnometers and a thermostat. Pycnometers of the double capillary type (8) and of capacity of about 30 ml. are probably most suitable for solutions of macromolecules. It is extremely difficult to obtain better accuracy than ten parts per million with pycnometers. Reproducible weighing of flasks with considerable glass surface is difficult owing to water adsorption on the glass, which demands a standard procedure for wiping the surface. Inherently the accuracy of buoyancy corrections probably limits the accuracy of the usual pycometric procedure to ten parts per million.

A different method (9), in which two very similar pycnometers are employed is probably capable of accuracy to a few parts per million. Varying humidity effects are balanced owing to the similarity of the two pycnometers. The correction for air buoyancy affects only the small differences in the volumes of liquid held in the capillary stems because of the differential method employed. A modification of this method appears to be quite accurate in the work of Rosen (10). However, MacInnes et al. (11) on the basis of their work on the density of salt solutions by the submerged float method, suggest that the pycnometer method has undiscovered sources of

error.

The method of measuring liquid densities by submerged float, although not new (12), has recently been applied to the determination of $\bar{V}$ of macromolecules (6, 13). The basis of this method is that a totally submerged object will neither rise nor fall when its mass volume ratio is equal to the mass to volume ratio of the liquid it displaces. To extend the range of density that may be measured by a particular float several modifications have been employed. One of the more successful types was that of Lamb and Lee (14) in which a small iron bar was enclosed in a glass stem attached to the float. By a combination of weights added to the top of the float, and magnetic force from a solenoid exerted on the iron bar in the float, a wide range of density was covered. The current through the solenoid at which the float neither rose nor sank could be converted to density units. An accuracy of about $\pm$ 1 x 10^{-7} in Δd was obtained. Geffcken et al. (15) used two similar magnetic floats in a differential manner to obtain an accuracy of $\pm$ 2 x 10^{-8} in Δd.

MacInnes et al.(11) modified the method of Lamb and Lee (14) by inserting a small bar magnet in the tail of the float and measuring the velocity of movement of the float as a function of current through an encircling solenoid. The precision obtained was about one part per million in density.

Another method of some value in determining the density of solutions of macromolecules involves the use of a density gradient column. The development of this method is largely

due to Linderstrøm - Lang (16). The method depends upon the fact that a drop of solution when immersed in an immiscible liquid medium, which has a linear gradation in density, will move to an equilibrium level in the gradient equivalent to its own density. The gradient is usually formed by layering a liquid of lesser density over a liquid of greater density and then mixing symmetrically on each side of the interface for a short time. Diffusion of the two miscible liquids then produces a linear density gradient for some distance centered on the original interface. The equilibrium position of drops in the gradient define their density when compared with the position of drops of known density. This method has been applied to the measurement of $\bar{V}$ of macromolecules by Taylor and Lowry (17) who used a kerosene-bromobenzene gradient.

Traube (18)concluded that the apparent molar volumes of organic molecules in water were the sums of the atomic increments plus a "covolume" of about 13 cc./mole. The atomic increments in cc./mole deduced for a temperature of 15°C. were:

C	H	N	O ether or carboxyl	O alcoholic hydroxyl	O carboxylic hydroxyl
9.9	3.1	1.5	5.5	2.3	0.4

$-CH_2-$	$-NH_2$	-OH	$-CO_2H$	-CO. NH-
16.1	7.7	5.4	18.9	20.0

To correct for the presence of ring structures Traube has pro-

vided "ring factors". Thus, the apparent molar volumes may be calculated if the type of constituents of the macromolecule are known. The partial specific volume may then be obtained by dividing by the molecular weight.

Cohn and Edsall (19) have made use of Traube's method to calculate $\bar{V}$ of several proteins. With modern methods the amino acid contents of many proteins are now known (20). The molecular weight is not necessary for the calculation. The apparent specific volume of each amino acid residue, ϕ_i, (a water molecule is lost upon formation of each peptide bond) can be calculated from Traube's increments or from the apparent specific volumes of the amino acids. Each amino acid loses the elements of water. Loss of charges on terminal groups gives rise to an expansion of 13.3 cc./mole (electrostriction). The covolume (14.1 cc.) is negligible for the large protein molecule but not for an amino acid.

$$\therefore \phi_i \text{ (residue in protein)} = \phi \text{ (amino acid in solution)} - 6.6 + 13.3 - 14.1$$
$$= \phi \text{ (AA in sln)} - 7.4 .$$

The molal volumes divided by their respective equivalent weights provide the apparent specific volumes of the residues. Values for various residues calculated in this manner are listed by Cohn and Edsall (19). From these values, ϕ of the protein may be calculated since:

$$\phi \text{ protein} = \sum \phi_i W_i / \sum W_i \qquad \text{.....(23)}$$

where ϕ_i is the apparent specific volume of the i th residue and W_i is its weight percent in the protein.

McMeekin and Marshall (21) have compiled a table which shows good correspondence between calculated and observed values of $\emptyset$ of proteins, However, the details of the experimental procedures are not adequate to assess probable errors. Charlwood's measurements (13) on $\emptyset$ of gelatin and edestin have brought their values into correspondence with the calculated values from the residues.

The suggestion was made by McBain (22) that $\overline{V}$ for macromolecules could be determined by changing the density of a medium in which their sedimentation is brought about until a point is reached at which no sedimentation occurs. The density of the medium at zero sedimentation rate would then be the reciprocal of $\overline{V}$.

The operation and theory of particle sedimentation in high centrifugal fields was developed by Svedberg (2). Sedimentation of particles in a suspension under the influence of gravity is commonly used industrially to obtain estimates of particle size. However, as the size decreases the opposing transfer of material by diffusion balances that which occurs by sedimentation under gravity until a limit is reached at about one μ. By use of strong centrifugal fields it was possible to sediment particles with diameter much less than one μ at a rate much greater than that of diffusion. Svedberg (2) developed an ultracentrifuge in which an intense centrifugal field could be applied to a small quantity of solution and convectionless sedimentation could be observed optically while it was in progress. The solution is held in a transparent sectorial cell

mounted in a rotor which can be revolved at a speed sufficient to produce a centrifugal force of about 250,000 times that of gravity.

The motion of a particle in a centrifugal field can be defined by the centrifugal potential, the chemical potential, and the frictional resistance to motion of the particle. Experimental conditions are chosen so that the centrifugal potential can be equated with either the chemical potential as in sedimentation equilibrium, or with the frictional resistance as in sedimentation velocity. The basic theory assumes that the laws of dilute solution hold and that the motion of the particle is not influenced by convection or electrostatic fields. In modern ultracentrifuges the latter conditions hold, as temperature may be controlled, and proper buffering can reduce electrostatic potentials in most cases to a negligible value. However, many macromolecule solutions deviate from ideal behaviour and, much recent work has been done to develop methods for extrapolation of values obtained at finite concentrations to values which would apply at infinite dilution.

Under the influence of a centrifugal field macromolecules starting from an equilibrium position are accelerated for a short time but soon move with a constant velocity; the centrifugal force being opposed by the frictional resistance of the medium. On N particles suspended in a rotating liquid the centrifugal force is:

$$N\phi\ (d_p - d)\omega^2 x \qquad \ldots\ldots\ldots(24)$$

where ϕ is the volume of one particle, d_p the particle density,

d the density of the solvent, ω the angular velocity, and x the distance from the centre of rotation. If N is taken as the Avogadro constant, expression (24) becomes:

$$M (1 - \bar{V}d) \omega^2 x \qquad \ldots\ldots\ldots(25)$$

where M is the molecular weight and $\bar{V}$ is the partial specific volume of the solute. The opposing frictional force is:

$$f dx/dt \qquad \ldots\ldots\ldots(26)$$

where f is the frictional coefficient per mole and t is the time. The centrifugal force can be equated to the frictional force to give:

$$M (1 - \bar{V}d) \omega^2 x = f \, d \, x / d \, t$$

or

$$M (1 - \bar{V}d) = \frac{f dx/dt}{\omega^2 x} \qquad \ldots\ldots\ldots(27)$$

The sedimentation velocity under unit centrifugal field is:

$$(dx/dt) / \omega^2 x \qquad \ldots\ldots\ldots(28)$$

and is usually designated by the symbol s (2). Insertion of this symbol into equation (27) results in:

$$M (1 - \bar{V}d) = fs . \qquad \ldots\ldots\ldots(29)$$

For an unhydrated spherical molecule with the partial specific volume $\bar{V}$, the molar frictional constant f_o is:

$$f_o = 6\pi\eta N (3M\bar{V} / 4\pi N)^{1/3} = \eta F_o (M,\bar{V}) \ldots\ldots(30)$$

where η is the viscosity of the medium and $F_o (M,\bar{V})$ is a function of M and $\bar{V}$. When solvation and nonspherical shape are involved the function takes the form:

$$f = \eta F (M,\bar{V},h) \qquad \ldots\ldots\ldots(31)$$

where h refers to the hydration of the molecule. Assuming that $F (M,\bar{V},h)$ is a constant, f^1, for a given substance, equation

(29) can be expressed as:

$$M\,(1 - \bar{V}d) = s\eta f^{1} \quad \text{.....(32)}$$

If sedimentation of a particle occurs in media of different density a linear relation can be established between ηs and d from equation (32).

$$s_1 \eta_1 = M\,(1 - \bar{V}d_1) / f^{1}$$

$$s_2 \eta_2 = M\,(1 - \bar{V}d_2) / f^{1}$$

and

$$s_1 \eta_1 - s_2 \eta_2 = -\frac{M\bar{V}}{f^{1}}\,(d_1 - d_2) \quad \text{.....(33)}$$

At zero sedimentation rate $\eta s = 0$ and therefore

$$M\,(1 - \bar{V}d) / f^{1} = 0$$

and

$$\bar{V} = 1/d \quad \text{.....(34)}$$

Several attempts have been made to apply the above theory to obtain $\bar{V}$ of macromolecules. The usual procedure has been to add increasing amounts of some additional solute such as glycerol or sucrose to the buffered solution of the macromolecule to increase the density of the medium. However, the procedure only provides with certainty the density of the sedimenting unit in the particular medium at which zero sedimentation occurs. Schachman and Lauffer (23) have shown that a linear relation can be obtained experimentally even if the composition of the sedimenting entity does not remain constant while the density of the medium is being altered. From their considerations if h is the amount of liquid transported with the macromolecule, d_s the density of this liquid, and d the

density of the medium the accelerating centrifugal force applied to the hydrated molecule can be equated to the frictional force as previously to give:

$$\omega^2 x \left[(M + hd_s) - (M\bar{V} + h)d\right] = f^1 \eta (dx/dt). \quad .....(35)$$

Equation (35) can be shortened by inserting the symbol s to obtain:

$$M \left[1 - \bar{V}d + (h/M)(d_s - d)\right] = \eta s f^1. \quad .....(36)$$

It can be seen that if h is equal to zero or if the transported liquid has the same density as the medium, equation (35) reduces to equation (32) and the reciprocal of the medium density at zero sedimentation equals $\bar{V}$. The possibility exists that d_s might be a linear function of d of the form $d_s = d_{s_o} + k\,(d - d_{s_o})$ which transforms equation (36) to:

$$\left[M + hd_{s_o}(1 - k)\right] \left\{1 - \left[M\bar{V} + h(1 - k)\right] d / \left[M + hd_{s_o}(1 - k)\right]\right\}$$

$$= \eta s f^1. \quad .....(37)$$

A quantity $\bar{V}_h$ can be defined as $\left[M\bar{V} + h(1 - k)\right] / \left[M + hd_{s_o}(1 - k)\right]$ and insertion into equation (37) gives:

$$\left[M + hd_{s_o}(1 - k)\right] (1 - \bar{V}_h d) = \eta s f^1 \quad .....(38)$$

Therefore it can be seen that if the volume h and coefficient f^1 of the solvated particle remain constant, the product of viscosity times sedimentation velocity will be a linear function of the density of the medium and at zero sedimentation velocity the density of the medium will equal the reciprocal of $\bar{V}_h$ in that particular medium.

A further complication to the interpretation of sedimentation in multicomponent systems was described by Kauzmann (24). In effect a medium containing added components to alter the

density could not be considered a continuum with regard to the sedimenting particles. Steric exclusion of these components would occur in the region immediately surrounding the sedimenting particles. Of course the extent of this region would be a function of the size of the added molecules. This could be interpreted as a preferential binding of the water of the medium. Taking into consideration the views of Kauzmann, an interpretation was evolved by Katz and Schachman (25) for systems in which additions were made to alter the density of the medium. To outline their views, a few changes will be made in the symbols used but not in the main argument.

For systems containing a solvent, macromolecule, and a component added to alter the density, the subscripts 1,2, and 3 will refer to solvent, dry macromolecule, and added component respectively. A buffer is also usually part of the medium but it is maintained at a constant amount in each media and its contribution may be ignored. For such systems, an equation similar to equation (32) is:

$$f^{1}\eta s = M(1 - \bar{V}d) \qquad \text{.....(39)}$$

with the symbols having the same meaning except that $\bar{V}$ refers to the sedimenting unit which may have associated with it components of the media. A molecule of component 2 may be considered to have $(k\, n_1 + w)$ molecules of component 1 and $k\, n_3$ molecules of component 3 bound to it in an unspecified manner. In the centrifuge cell n_1 is the total number of molecules of component 1 and n_3 is the total number of molecules of component 3. The preferential adsorption coefficient of component 1 is w and k

is a proportionality constant. It is possible for k to have any value, $k \geq 0$, and w to be positive, negative or zero. Now the chemical potential μ of the sedimenting unit can be expressed as:

$$\mu = \mu_2 + (kn_1 + w)\mu_1 + kn_3\mu_3. \quad \ldots\ldots\ldots(40)$$

Equation (40) may be differentiated with respect to pressure to obtain:

$$\bar{V} = \frac{M_2\bar{V}_2 + (kn_1 + w)M_1\bar{V}_1 + kn_3 M_3\bar{V}_3}{M_2 + (kn_1 + w) M_1 + kn_3 M_3} . \quad \ldots\ldots\ldots(41)$$

Insertion of this value of $\bar{V}$ in equation (39) gives:

$$f^1\eta s = M_2(1-\bar{V}_2 d)+wM_1(1-\bar{V}_1 d)+kn_1M_1(1-\bar{V}_1 d)+kn_3M_3(1-\bar{V}_3 d). \quad \ldots(42)$$

At infinite dilution of component 2 the sum of the last two terms of equation (42) equals zero since they are equivalent to $Nk(g_1 + g_3) - Nkd(g_1\bar{V}_1 + g_3\bar{V}_3)$ where g_i is the total number of grams of i in the centrifuge cell and thus equation (42) reduces to:

$$f^1\eta s = M_2(1 - \bar{V}_2 d) + wM_1 (1 - \bar{V}_1 d) \quad \ldots\ldots\ldots(43)$$

If the superscript zero denotes the values at $s = 0$ then

$$w^o = - M_2 (1 - \bar{V}_2^o d^o) / M_1 (1 - \bar{V}_1^o d^o) \quad \ldots\ldots\ldots(44)$$

It can therefore be seen that only preferential adsorption of component 1 or 3 can be determined in this type of experiment unless one assumes that $w = 0$. Also it is noticed that kn_1 and kn_3 have no effect on the product $f^1\eta s$. Any effect on f^1 of additional associated liquid is compensated by a change in s so that $f^1\eta s$ remains constant. If w is zero then equation (43)

reduces to:

$$f^1\eta s = M_2 (1 - \bar{V}_2 d) . \qquad \ldots\ldots\ldots(45)$$

In this circumstance it can be seen that at zero sedimentation rate $\bar{V}$ of the macromolecule is obtained and not $\bar{V}$ of the sedimenting unit.

The greater part of the work on the determination of $\bar{V}$ by sedimentation methods, has been applied to virus particles as described in a review by Lauffer and Bendet (26). In 1922 MacCallum and Oppenheimer (27) tried to use the specific gravity, obtained by sedimentation, as a fundamental characteristic of virus particles. The viruses were swung in glycerol media of several densities until two media were found which bracketted the apparent density of the viruses. Other investigators (28, 29, 30) used similar methods. However, with more refined techniques it became apparent that the additives to the media affected the rate of sedimentation in unexpected ways. Smadel et al. (31) studied vaccine virus in different media in an air driven centrifuge by the light absorption method. Sucrose, glycerol, and urea were used to vary the density of the media. Each additive gave a different relation between sedimentation rate, corrected for the viscosity, and density of the medium. Also the relation was not linear and in some cases the sedimentation rate changed with the length of time the virus was in contact with the medium. Among the suggested reasons for these results was extraction of water from within the particles, partial replacement of occluded water, and detachment of bound water from the surface of the virus.

Several other investigations of sedimentation in sucrose solutions also displayed non-linear relations between sedimentation rates, corrected for the viscosity of the solvent, and the density of the solvent (32, 33). Schachman and Lauffer (24) employed measured values of viscosity and density of the solution in their investigation of tobacco mosaic virus in sucrose solutions and found a linear relation between the product of solution viscosity and sedimentation rate, and density of the solution. However, use of viscosity and density of the solution did not result in a linear relation between ηs and d with southern bean mosaic virus (34).

Since Smadel (31) had found that the sedimentation rate of viruses in sucrose, glycerol, and urea solution was time dependent and suggested that it might be caused by the high osmotic effects of the additive, Sharp (35) tested the effect of a sucrose solution (11 %) and a bovine serum albumin solution (12.5 %) on the sedimentation rate of influenza virus. The sedimentation rate was less time dependent in the bovine serum albumin solution than in the sucrose solution over a period of about six hours. Although the densities of the solutions were approximately equal the albumin had a much lower osmotic effect. A linear relation was obtained between ηs (solvent) and d (solvent) for the virus in bovine serum albumin solutions. The solvent density at the extrapolated zero sedimentation rate was 1.104 g./ml. whilst the reciprocal of $\bar{V}$ obtained by use of pycnometers was 1.215 g./ml. With sucrose as additive the data extrapolated to an intermediate value of

solvent density at zero sedimentation rate. Other experiments using bovine serum albumin to vary the solution density also gave linear relations (36). However, Lauffer et al. (37) showed that bovine serum albumin interacted very strongly with virus protein over a range of pH values.

In view of the difficulties experienced with the various agents employed to alter the density of the solution some investigators used deuterium oxide for this purpose. Svedberg and Eriksson - Quensel (38) had used deuterium oxide in the sedimentation of hemocyanin to check the corrections used to reduce experimental values to those of a standard medium eg. water at 20° C. This experiment indicated that deuterium oxide did not change the solvation of the sedimenting unit. Sharp et al. (39) measured the sedimentation rates of swine influenza virus in media in which the density was altered by the addition of either bovine serum albumin, sucrose, or deuterium oxide. The data extrapolated to solvent densities, at zero sedimentation rate, which increased in value from bovine serum albumin, to sucrose, to deuterium oxide, with the value obtained with deuterium oxide being equal to the reciprocal of $\bar{V}$ obtained by the use of pycnometers. Of the three media only the sucrose gave a marked non-linearity. The swine influenza virus and two other species of influenza virus A and B, were also sedimented in Ringer's solution in which increasing amounts of deuterium oxide replaced water. All three viruses showed a linear ηs (solvent) against d (solvent) relation. The reciprocals of the solvent density at zero sedimentation rate in ml./g.

were 0.76 for swine, 0.70 for A species, and 0.72 for B species, while the $\bar{V}$ obtained by the use of pycnometers were 0.76, 0.75, and 0.76 ml./g., respectively. In another study Sharp and Beard (40) and Cheng and Schachman (41) measured the sedimentation rates of polystyrene latex spheres in media of low ionic strength in which the concentration of deuterium oxide was varied to alter the solvent density. The latex spheres were also sedimented in bovine serum albumin solutions (40) and in sucrose solutions (41). In each instance linear relations were observed between ηs (solvent) and d (solvent). Also, the intercepts at zero sedimentation rate were the same in each medium and equal to $1 / \bar{V}$ obtained by other methods. Katz and Schachman (25) measured the rate of sedimentation of deoxyribonucleic acid in solutions in which the density was altered by the addition of either sucrose or deuterium oxide. In both types of media linear relations were observed between ηs (solvent) and d (solvent). The reciprocals of the density of solvent at zero sedimentation rate were 0.59 and 0.70 ml./g. for sucrose and deuterium oxide solutions respectively.

It would appear that for most macromolecules, attempts to interpret the density of the medium at zero sedimentation rate in terms of partial specific volume or hydration will not be successful if substances such as sucrose, bovine serum albumin, or salts (34) are employed to alter the density of the medium. As shown by Schachman (23) and Katz (25), only the preferential adsorption of water or substance added to alter the medium density can be determined. If the preferential adsorption is zero

then $\bar{V}$ can be evaluated. This is shown for polystyrene latex spheres where hydration did not occur and therefore $\bar{V}$ was obtainable in sucrose, bovine serum albumin solution or deuterium oxide solution.

The use of deuterium oxide to alter the density of the medium should be of value because, although hydration probably occurs, there is no preferential adsorption of constituents of the medium by the macromolecule. However, a significant and rapid exchange of deuterium atoms for labile hydrogen atoms (42) in the macromolecules will occur in solutions containing deuterium oxide. The result would be an increase in molecular weight and since the volume of the macromolecule probably does not change, $\bar{V}$ will decrease. Thus according to Martin et al. (43) the molecular weight M in aqueous solution will become Mk in deuterium oxide and $\bar{V}$ will change to $\bar{V}/k$ where k is the ratio of the molecular weights in the two media. Also k may be derived from:

$$k = 1 + \left[\frac{\text{(weight increase per gram of macromolecule)}}{(d_1 - d_o)/\Delta d}\right] \qquad \text{.....(46)}$$

where $(d_1 - d_o)$ is the increment in density of the medium due to D_2O and Δd is the increment in density of 100 % D_2O over the density of H_2O.

The sedimentation rate in aqueous and heavy water solutions may be expressed as:

$$f^1 \eta_1 s_1 = Mk_1 (1 - \bar{V}k_1^{-1} d_1) \qquad \text{.....(47)}$$

$$f^1 \eta_2 s_2 = Mk_2 (1 - \bar{V}k_2^{-1} d_2) \qquad \text{.....(48)}$$

Now, since the value of $\bar{V}$ in aqueous solution is required for the evaluation of molecular weight, k_1 is of course equal to unity, and therefore division of equation (47) by equation (48) and rearrangement gives:

$$\bar{V} = (\eta_2 / \eta_1 - ks_1/s_2)(d_1\eta_2 / \eta_1 - d_2 s_1/s_2)^{-1} \quad ..(49)$$

The linearity of equation (47) can be shown by a plot of $\eta s/k$ versus $k^{-1}d$. Extrapolation of the curve to zero value of s results in $\bar{V} = k/d$. However, the values of d and k at zero sedimentation rate represent an unrealizable situation since k cannot be greater than the value at 100% D_2O concentration and at the density of 100% D_2O zero sedimentation will not occur. Therefore equation (49) should be employed if linearity can be assumed or established experimentally.

From the observed standard errors of s_1 and s_2, an approximation of the corresponding standard error of $\bar{V}$ may be obtained by way of the conventional device of regarding mathematical differentials as statistical differentials. Partial differentiation of equation (49) results in:

$$\partial \bar{V} / \partial s_i = Fs_j \quad .....(50)$$

where i = 1 when j = 2 and vice versa, and where $F = (kd_1 - d_2)\eta_1\eta_2 / (d_1s_2\eta_2 - d_2s_1\eta_1)^2$. Now assuming that η and d have negligible errors and that the joint error of s_1 and s_2 is the root - sum - square of the individual errors, equation (50) can be transformed to give:

$$\Delta\bar{V} = F(s_1 + s_2)\left[(\Delta^2 s_1 + \Delta^2 s_2) / 2\right]^{\frac{1}{2}} \quad .....(51)$$

where Δ is to be read "the standard error of".

Morowitz and Chapman (44) found by the analysis of

the vapour from deuterium oxide solutions with a recording spectrometer, that at room temperature all hydrogen atoms not involved in carbon hydrogen bonds were exchangeable with deuterium in solution. However, Linderstrøm - Lang (42) has shown by density measurements of the D_2O recovered by cryo-sublimation from deuterated proteins that at room temperature the exchange would not be quite complete for some proteins. Indeed Blout (45) has shown by infrared spectroscopy that some bonds in native BPA in solution are able to deter the penetration of D_2O into the molecular structure and therefore some of the hydrogen atoms do not exchange. Upon denaturation of the BPA a ready exchange occurs with all labile hydrogen. Haggis (46) examined the infrared absorption spectra of dry protein films. Two percent protein solutions were reacted with D_2O for various time intervals and several temperatures in sealed vials. By inserting the body of the vials in a bath at -70°C. a dry film formed in the neck of the vial at 20°C. In some experiments the neck was also kept at 60°C. to ensure dryness. The neck was sealed off by heating and the spectrum of the dry film recorded. Some of the protons of the backbone N-H groups of insulin were slow to exchange at room temperature but, as found also by Linderstrøm - Lang (42), the exchange at 37°C. was more rapid and complete. With ribonuclease in which Morowitz and Chapman (44) had found 110% of the theoretical exchange at room temperature, Linderstrøm-Lang found almost the theoretical exchange at 39°C. and only 85% exchange at 0°C. However, Haggis did not find complete exchange even at 60°C. He also observed that at 37°C.,

β-lactoglobulin, egg albumin, and γ-globulin exchanged approximately 70% of the backbone N-H protons after four days in solution. Serum albumin exchanged about 85% of its backbone N-H protons at 20°C. and at 37°C. Therefore some doubt exists as to the number of labile hydrogen atoms which would exchange with deuterons in a solution of deuterium oxide.

SCOPE OF THE INVESTIGATION

It is important to be able to measure $\bar{V}$ for biological macromolecules as an aid to their characterization. For the easily purified macromolecules this is no major problem, with the exception that even here there is some uncertainty about the concentration. However, there are frequently problems of scarcity of sample, impurities, presence of more than one component, and difficulty in determination of concentration due to lability of the biological material.

The preceding outline has indicated briefly the main methods by which the value of $\bar{V}$ might be determined. The pycnometer method suffers not only from the difficulties already mentioned but with some macromolecule solutions there is difficulty also from foaming and denaturation caused by drawing the solution through the capillary. Rather large quantities of pure solution of accurately known composition and concentration are required. The float method, although very sensitive to density variation, does not permit separation of the contributions to density due to the presence of several components. Also large quantities of the macromolecule solutions of accurately known

concentration are needed. The density gradient column method requires only small quantities of macromolecule solution of accurately known concentration, but does not differentiate components. Also, in some cases, it is difficult to select gradient fluids which do not interact with the macromolecule system. The atomic increment method of Cohn and Edsall (19) is only valid when the composition of the macromolecule is known. This method might become more valuable as modern methods of amino acid analysis become more prevalent.

In view of the above considerations it was thought worthwhile to investigate the possibility of employing the differential sedimentation method to obtain $\overline{V}$ of macromolecules first with solutions containing only one macromolecular constituent and then with solutions containing more than one macromolecular constituent. It was proposed to investigate the method with the view that only an estimate of concentration would be required. Also, although comparatively large volumes of solution were to be used in many of the aspects of the investigation it can be shown that the method requires only a small amount of the macromolecular component, which is subject to recovery.

To make the investigation deuterium oxide was used as the preferred way to alter the density of the solution. As mentioned earlier, this involves the problem of isotope exchange. This exchange was investigated on a quartz spiral balance. Also accurate measurements were made with a float apparatus of the density of aqueous and deuterium oxide solutions of macromolecules.

Two macromolecules of moderate molecular weight, bovine plasma albumin, and polyvinyl alcohol were selected for the most intensive study of the differential sedimentation method of evaluating partial specific volume. Bovine plasma albumin is a typical globular molecule and its physical and chemical characteristics are fairly well established (6,13,47, 48). Polyvinyl alcohol, one of the few water-soluble synthetic polymers, is a linear, coiled, chain type of molecule and its chemical and physical properties have been studied to some extent (49,50,51). Sodium alginate (52,53) a seaweed extract, a typical polyelectrolyte, was investigated to a lesser extent. Some doubt existed as to its purity and constitution due to a tendency to degrade with elimination of CO_2. β-lactoglobulin (54) was selected to investigate mixtures because the equipment available had just enough resolution to separate it from bovine plasma albumin, when the two macromolecules were sedimented as a mixture. The two other mixtures of macromolecules investigated were α- and β-livetin and γ-livetin and lipovitellin of hen's egg yolk. They were selected because the author was investigating their characteristics for other purposes (55,56) and they were good subjects to illustrate the use of the sedimentation method on impure material.

Since the materials mentioned above would be expected to exchange labile hydrogen atoms only to the extent of one to two weight percent, glycine and triglycylglycine were investigated with the float apparatus in deuterium oxide and aqueous solutions.

EXPERIMENTAL

MATERIALS

Crystalline bovine plasma albumin lot # A 1201 (Pentex Inc.), crystalline bovine β-lactoglobulin lot # 0053 B (Armour and Co.) and triglycylglycine lot # 7484 (Nutritional Biochemicals Corp.) were used without further purification and will be referred to as BPA, β-LG, and TGG respectively. The polyvinyl alcohol (Shawinigan Chemicals Ltd.) and sodium alginate (Alginate Industries Ltd.) termed PVA and AC_1 respectively, were dissolved in distilled water, centrifuged and filtered to remove insoluble material, and then lyophilized. The PVA had a remnant of 12 acetate groups per 100 repeating units of the polymer owing to incomplete alcoholysis. Glycine (Eastman) was dissolved in warm water and filtered from a slurry of charcoal and celite, then precipitated with methanol, washed with methanol-ether, and dried at 45°C. *in vacuo*.

The water soluble protein of hen's egg yolk termed livetin (57) was separated into its α-β- and γ-components by the procedures of Martin *et al.*(55,56). Lipovitellin of yolk was prepared by the method of Joubert and Cook (58). It was possible to store the α- and β-livetin in a lyophilized condition but the lipovitellin and γ-livetin had to be stored at 5°C. in solution. The deuterium oxide was purchased from Merck & Co., Inc., lab. # 57CD251. A quartz spiral was purchased from Microchemical Supply Co.

A stock of distilled water was prepared by adding

potassium permanganate to alkaline tap water and distilling with a minimum of reflux. The distillate was redistilled, again with a minimum of reflux. This water was assumed to have a density of 0.997074 g./ml. at 25.000°C.

METHODS

The sedimentation procedures consisted of two distinct phases. The first experiments were made with solutions of about 0.4% concentration and sedimentation was undertaken in media of several densities to investigate the linearity of the relation between $\eta s/k$ and d/k. A concentration of 0.4% was selected because this concentration gave easily measured sedimentation peaks without serious overlapping of peaks when two cells were used in one sedimentation experiment. The second experimental series was made to determine $\bar{V}$ from sedimentation coefficients extrapolated to zero concentration in only two densities of media, one medium being aqueous and the other containing deuterium oxide.

To produce measurable sedimentation with particles of the size of macromolecules, the centrifugal fields are usually of the order of 250,000 gravities. With such fields it is possible to obtain sedimentation rates of molecules as small as 10,000 in molecular weight. As the molecular weight decreases the diffusion process increases and causes blurring of the boundary region until a point is reached when the position of the boundary cannot be established. The sedimentation rate, s, as defined in equation (28), can be determined from the change in the position of the boundary between solution and solvent,

measured at regular time intervals during the sedimentation. At the sedimentation boundary the concentration is half the value found in the sedimentation cell in the plateau region of the cell. The plateau region is that part of the solution in the cell in which the concentration of solute is constant with distance from the centre of rotation. The concentration of solute in the plateau region has the value:

$$c_t = c_o \, (x_o/x_t)^2 \qquad \text{.....(52)}$$

where c_t is the concentration at the time t, c_o the initial concentration, x_o the distance of the meniscus from the centre of rotation, and x_t the distance from the centre of rotation of the portion of the plateau region concerned at the time t. This relation was derived by Svedberg (2) and is due to the sector-shaped cell, which is required to permit sedimentation along radial lines, and also to the fact that the centrifugal force changes with distance from the centre of rotation. The actual position of the boundary is therefore measured at the point $c_t/2$ rather than $c_o/2$ as is customary in diffusion measurements. To evaluate s, equation (28) is integrated to give:

$$s = \ln (x_2 / x_1) / \omega^2 (t_2 - t_1) \qquad \text{.....(53)}$$

where x_1 is the position of the boundary at time t_1 and x_2 is the position at time t_2. It is customary to reduce the measured value of s to the value it would have in some medium chosen as standard e.g. water at 20°C. and refer to it as $s_{20,w}$ as follows:

$$s_{20,w} = s \, \frac{\eta_t}{\eta^o_{20}} \left(\frac{1 - \bar{V}_{20} d^o_{20}}{1 - \bar{V}_t \, d_t} \right) \qquad \text{.....(54)}$$

where η_t is the viscosity of the medium at temperature t, η^o_{20} is the viscosity of water at 20°C., $\bar{V}_{20}$ is the partial specific volume at temperature 20°C. in water, $\bar{V}_t$ is the partial specific volume in the medium at temperature t, d^o_{20} is the density of water at 20°C. and d_t is the density of the medium at temperature t.

The value of s for each experiment was calculated from equation (53) by taking the average value over a number of time intervals except in experiments in which the boundary had moved an appreciable distance from the meniscus before measurements could be made. With increasing distance from the meniscus, increases occur in both the density and viscosity of the medium, both increases being small in aqueous solution. Dilution of the solution also occurs, owing to the sector-shaped cell and, if there is interaction between solute molecules, the sedimentation rate may be affected. Oth and Desreux (59) have examined these features and show that in aqueous solution the two effects partially compensate each other and that lnx as a function of t is thus linear. The coefficient of sedimentation, s, was evaluated from the slopes of these curves when measurements were made after the boundary had moved some distance from the meniscus.

The sedimentation coefficient is usually a function of the concentration of the solution, markedly so for linear polymers such as PVA. For this reason, the sedimentation coefficient at zero concentration, in the various media, s^o_t, medium, has been determined from least squares analysis of s versus c or 1/s versus c.

In the preliminary experiments the densities of solutions at 25°C. were measured in a density gradient column or with a small magnetic float (60). In later experiments, including those with mixed solutes, densities were measured at 20°C. with a Mohr - Westphal type of balance which gave better than 1 part per 1000 accuracy with about 10 ml. of solution.

The viscosity of solutions was determined in an Ostwald - Fenske viscometer, size 50, with a flow-time with water of 325 seconds at 20°C. The solutions were filtered directly into the viscometer through a coarse sintered glass filter. Flow-times were reproducible to about 0.1 second. In some cases the viscosities of salt solutions were obtained from the solvent viscosities plus the increments due to the salts, by appropriate calculations (2).

Two centrifuges were used for the experiments, both of which were model E analytical machines (61) manufactured by the Specialized Instruments Corporation, now Beckman Instrument Co. In this model the rotor, suspended from a piano-wire of 0.1 inch diameter, is driven through a train of gears. One of the features of this suspension is that the rotor adjusts itself to unequal loads, thus requiring no precise balancing of rotor cells.

The analytical rotor usually holds one measuring cell and one dummy cell as a counter-balance. However, during the period of this work it was necessary to use two measuring cells in each experiment as the equipment was in constant use on other projects. A hole through the rotor provides a reference distance from the axis of rotation. The mid-point of a cell when placed

in a rotor is 6.5 cm. from the axis of rotation. In preliminary experiments it was only possible to measure the rotor temperature by thermocouple before and after each experiment. Subsequently it was possible to control and record the rotor temperature as the rotor revolved. The rotor may be operated in vacuum at 60,000 r.p.m., thereby developing centrifugal forces of about 250,000 gravities.

The 12 mm. cell which was used contains a centrepiece with a sector-shaped slot, each end of which is sealed by a quartz disc. This forms a sector-shaped cavity which will hold about 0.8 ml. of liquid plus a small air space. The sector shape is essential to convection-free sedimentation (2). When using two cells in one rotor one of the cells was loaded with slightly less liquid to avoid superimposed boundary regions. However, with one of the ultracentrifuges it was possible to use a prism-shaped window for one of the cells thus displacing the image vertically.

The optical system employed in the ultracentrifuge records changes in the refractive index gradient in the cells, caused by the sedimenting material according to the method of Philpot (62). The refractive index gradient is a measure of change of concentration in the cell. In the boundary region between solvent and solution, the gradient is highest and thus forms a peak on the photographic plate. The wire usually employed as a schlieren diaphragm was replaced by a modified Wolter phaseplate (63) for the later experiments.

In the usual procedure for sedimentation experiments,

the solute in a suitable solvent was dialysed against the solvent. Dilutions were then made from the dialysed solution using diffusate as the diluent. Viscosity and density measurements were made on the diffusate and sometimes also on the solutions. Refractive index increments were also obtained with a differential refractometer (64), from which solute concentration was calculated from previously determined values of the specific refractive index increments. The specific refractive index increments were based upon dry weight measurements of concentration. In some experiments the solvent was added to a weighed amount of solute and the solution analysed without prior dialysis. With the type of materials involved it was necessary to make the dialysis in a cold room and to store the various solutions in a refrigerator when they were not under study. When using deuterium oxide it was necessary to use syringes or pipettes equipped with drying tubes during transfer of solutions to prevent exchange with hydrogen atoms of water vapor in the atmosphere. The deuterium oxide was recovered from the solutions by cryosublimation.

The operations were essentially of a standard type, and involved the usual location of cells in the rotor, suspension of the rotor from the drive mechanism, determination of the rotor temperature, evacuation of the rotor chamber, and manipulation of the controls to bring the rotor to operating speed. The sedimentation peaks were observed on the viewing screen as they separated from the menisci.

When the peaks indicative of sedimentation were

adequately resolved and separated from the position of the meniscus, the sequence of photographic exposure was begun. Depending upon the sedimentation rate of the material, photographic exposures were taken at multiples of two minute intervals until diffusion of the macromolecules had spread the peaks to such an extent that further photographic record was of little use. The rotor was then brought to a stop and the vacuum released.

The data recorded in a typical experiment, in which the rotor temperature was not controlled, were:

Date of Experiment: March 9/55. U.C. #9

Cell A : 0.73 ml. of 0.406% PVA, solvent D_2O

Cell B : 0.70 ml. of 0.399% PVA, solvent H_2O

8:19 PM	reference 25.1°, zero 0.1, free couple -1.2
8:21	vacuum pump on
8:33	acceleration of rotor
8:42	rotor reaches 52,640 r.p.m. vacuum 2 microns
9:01	photo #1, wire angle 50°, exposure 6 sec.
9:17	" 2, " " " , " " "
9:33	" 3, " " " , " " "
9:49	" 4, " " " , " " "
10:05	" 5, " " " , " " "
10:21	" 6, " " " , " " "
10:21	deceleration of rotor
10:30	rotor comes to rest
10:33	reference 25.1°, zero 0.1, free couple -0.2

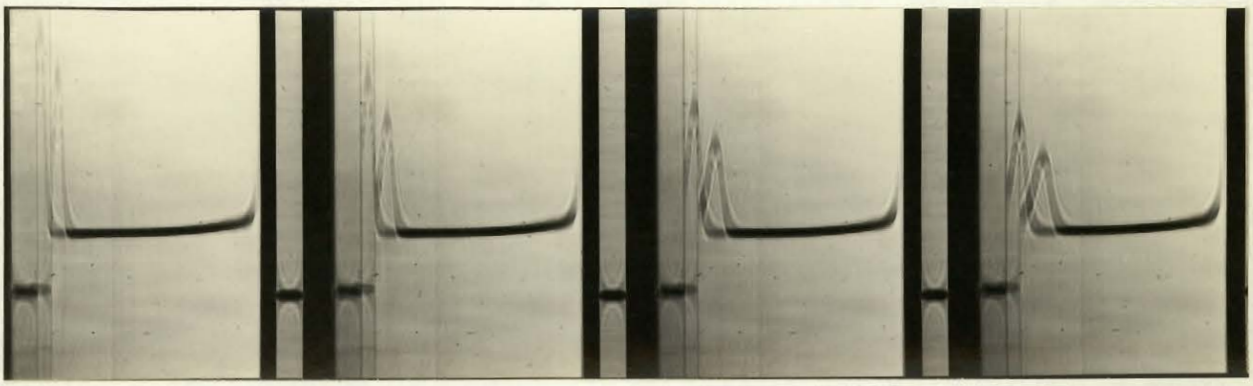
FIGURE I

Typical Sedimentation Patterns with two Cells in the Rotor

1 (a) top, Polyvinyl Alcohol

1 (b) bottom, Bovine Plasma Albumin

Peaks at the left in each frame are in deuterium oxide solution. Peaks at the right in each frame are in aqueous solution.



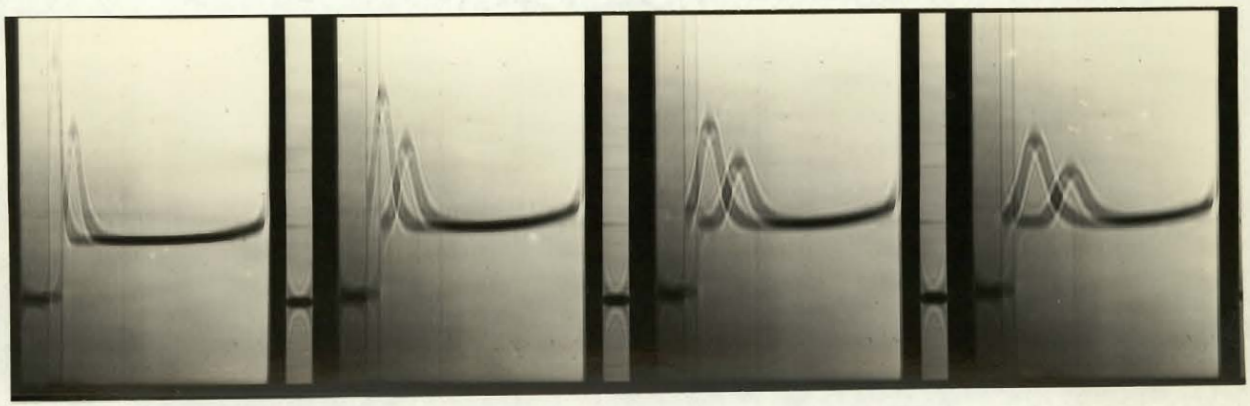
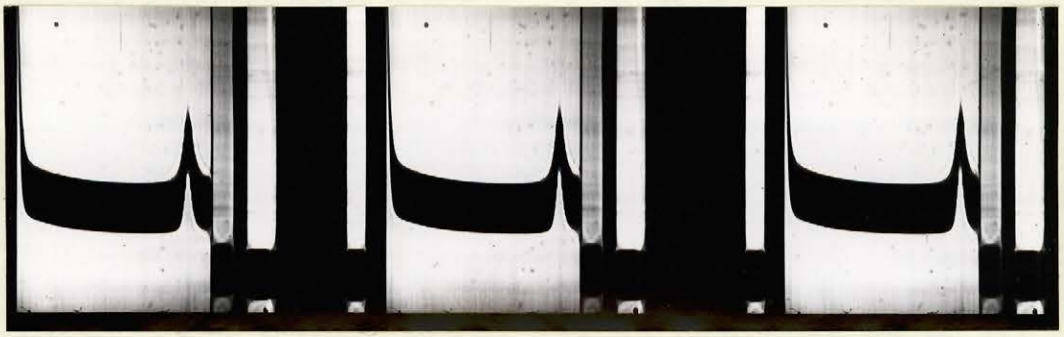


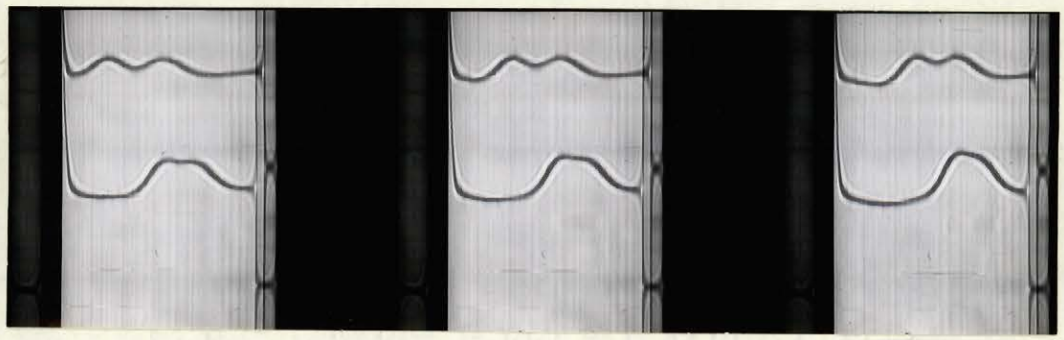
FIGURE 2

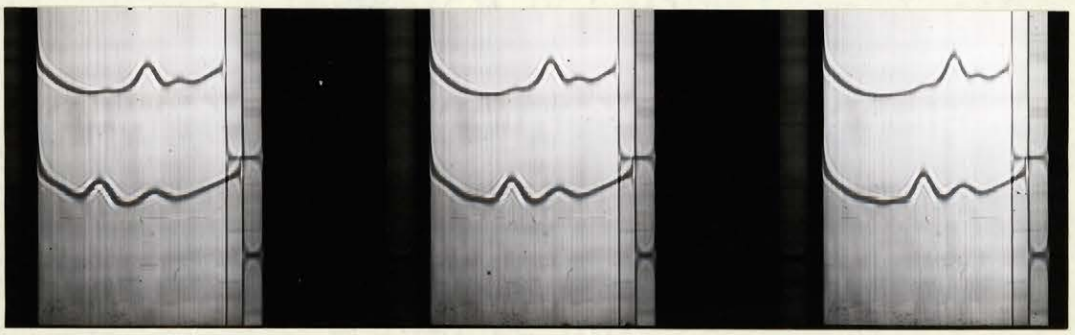
Typical Sedimentation Patterns with two Cells in the Rotor

Photo sequences from top to bottom are:

(a) AC_1, sedimentation proceeds from left to right
(b) BPA + β-LG " " " " " "
(c) Lipovitellin + γ-livetin " " " " " "
(d) λ- + β-Livetin " " " " " "







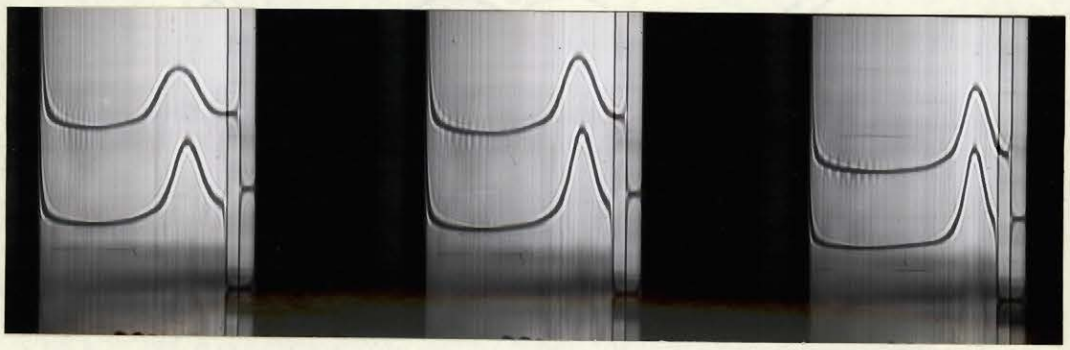


Figure 1a illustrates the schlieren refraction pattern from the above experiment. The influence of the different density of the medium in the two cells can be noticed by the increase in the separation of the two peaks with each exposure. It can also be noticed that the peaks are skewed in relation to the meniscus, owing to the concentration dependence of PVA. Comparison with Figure 1b illustrates the difference in the rate of sedimentation between PVA and BPA. In Figure 2 are shown typical patterns obtained with the other materials of this investigation. In Figures 1a and 1b a wire diaphragm was used, in Figure 2a, a bar, and in Figures 2b,c, and d the phase plate method was used.

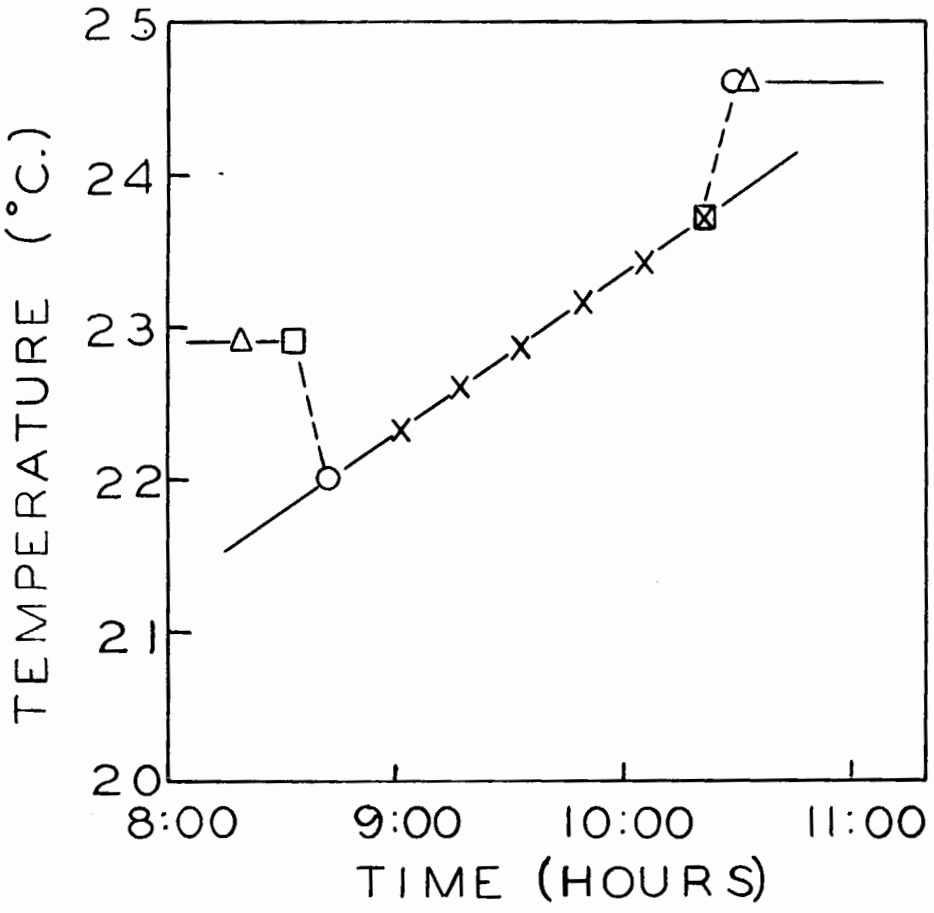
Sedimentation coefficients were computed with the aid of tracings of enlarged schlieren patterns on graph paper. In some experiments however, a Gaertner comparator was used. The peak movement estimated from the enlarged tracings had to be related to the actual boundary movement in the cell. Exposures of a ruled glass disc held in a cell in a suspended rotor were made. From the known distance between the rulings on the disc and on the exposed plate, the magnification factor for the ultracentrifuge camera was obtained. For the magnification factor of the enlarger, a glass scale was placed with its etched face in the same plane of the enlarger as the emulsion of the exposed plates from the ultracentrifuge. The position of the enlarged scale markings were marked on the graph paper. It was then possible to calculate the enlarger magnification factor either in graph units or in cms.

The hole in the rotor with its reference edge at a

FIGURE 3

Graphical Interpolation of Rotor Temperature

△	Thermocouple measurements
□	Start of acceleration and deceleration
○	Completion of acceleration and deceleration
×	Exposures


25
24
23
22
21
20
TEMPERATURE (°C.)
8:00
9:00
10:00
11:00
TIME (HOURS)

distance of 7.30 cm. from the axis of rotation provided a reference line on the exposed plates. The images of the schlieren patterns were projected so that the edge of the reference hole in each exposure fell, in turn, on the same line of the graph paper. Once this adjustment had been made, the graph paper was fixed in place with cellulose tape, and the pattern traced on the paper. The location of each meniscus was marked so that cell leakage might be detected by a movement of the image of the meniscus towards the location of the reference line. The distance from the estimated centre of each peak was recorded for each exposure for use in computing the sedimentation rate.

Curves were drawn as shown in Figure 3 for each sedimentation experiment in which the temperature was not controlled. The temperatures of the rotor, derived from the thermocouple measurements, were plotted against time. Temperature of the rotor was assumed to be constant while the rotor chamber was being evacuated. Since adiabatic expansion during acceleration of the rotor (65) lowers the temperature about $0.9^{\circ}C.$, the temperature when full speed was reached was 0.9° below the initial rotor temperature. Upon deceleration the temperature of the rotor rises $0.9^{\circ}C.$, and this was subtracted from the final rotor temperature to estimate the temperature of the rotor at the time when deceleration began. The plotted point at which full speed was reached was joined to the point at which deceleration began by a straight line. The time of each exposure was marked on the line and the temperature of the rotor was then read from the curve.

To calculate the sedimentation constant, and the viscosity and density of the medium, the curves of Figures 4, 5, 6, and 7 were drawn from the data in Table I. Since data were not available for 0.2 M NaCl, this curve was drawn by interpolation between curves for 1% NaCl and 2% NaCl. The increments between the density curves for water and for 0.2 M NaCl aqueous solution were used to plot the curve for 0.2 M NaCl in D_2O solution. The data for the viscosity of 0.2 M NaCl aqueous solution were not available but the curve was easily interpolated between the curves for 0.1 M and 0.25 M NaCl in water solution. The increments in viscosity between the curve for 0.2 M NaCl aqueous solution and that for water were used to plot the viscosity curve for 0.2 M NaCl in D_2O solution. The curves for 0.5 M NaCl were also plotted to aid in estimating the viscosity of the medium when the solutions were not exactly 0.2 M.

The curves of Figures 4, 5, 6, and 7 were used to estimate the density and viscosity of the medium at the average temperature of the exposure interval. Water presented no difficulty. However, for D_2O the concentration was calculated from the density measured by the small magnetic float (60) at 25°C. When the concentration was nearly 100% D_2O, extrapolation to the density and viscosity at the required temperature was not difficult. The density and viscosity of intermediate concentrations of D_2O were calculated from the increment in density or viscosity between H_2O and D_2O, since Longsworth (66) has found that H_2O and D_2O form ideal solutions.

FIGURE 4

Density: Temperature Relation of Aqueous Sedimentation Media

○ Water

△ 1% NaCl in water

● 0.2 M NaCl in water

□ 2% NaCl in water

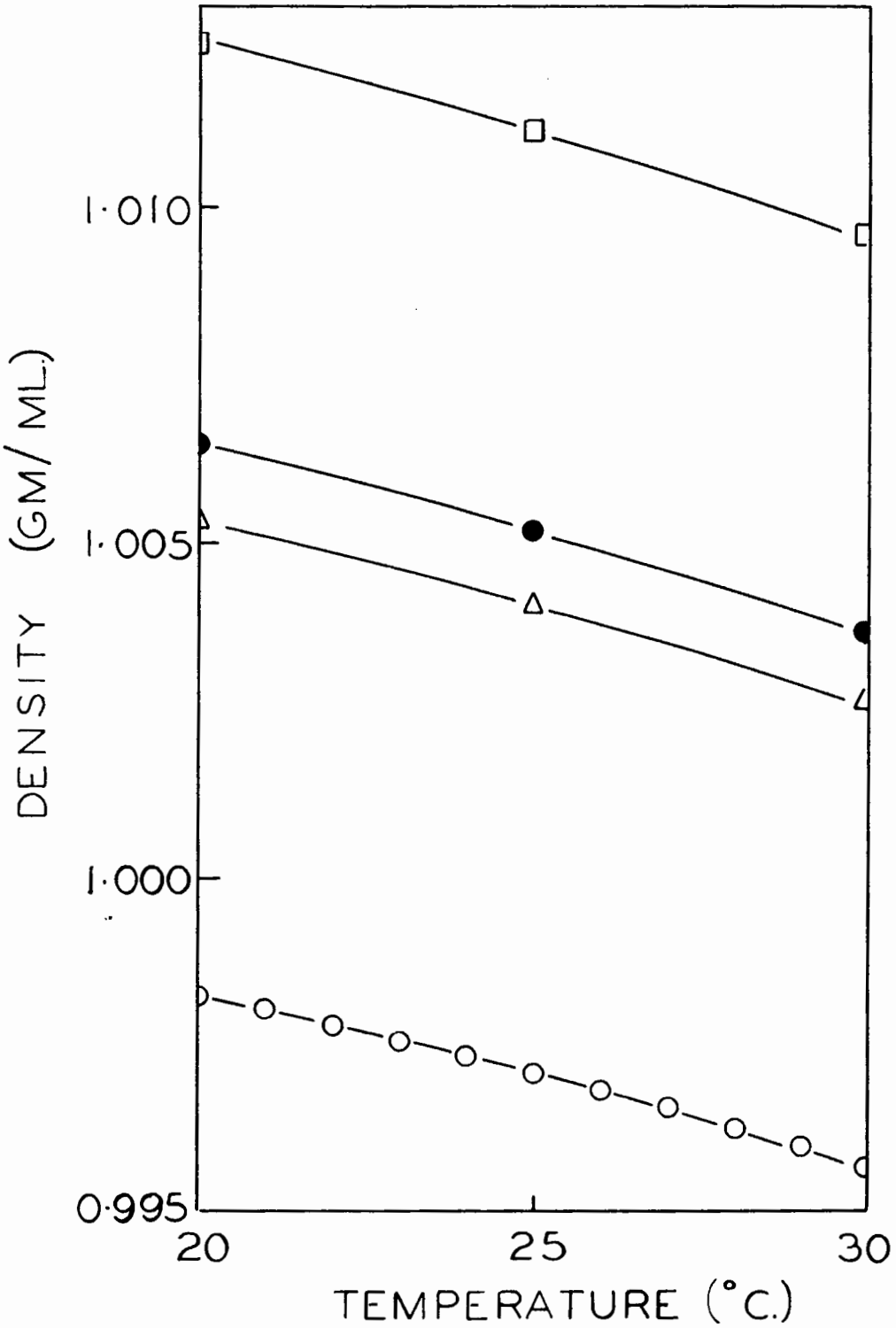

1·010
1·005
1·000
0·995
DENSITY (GM/ML.)
20
25
30
TEMPERATURE (°C.)

FIGURE 5

Density: Temperature Relation of

Deuterium Oxide Sedimentation Media

○ 100% D_2O

△ 0.2 M NaCl in D_2O (100%)

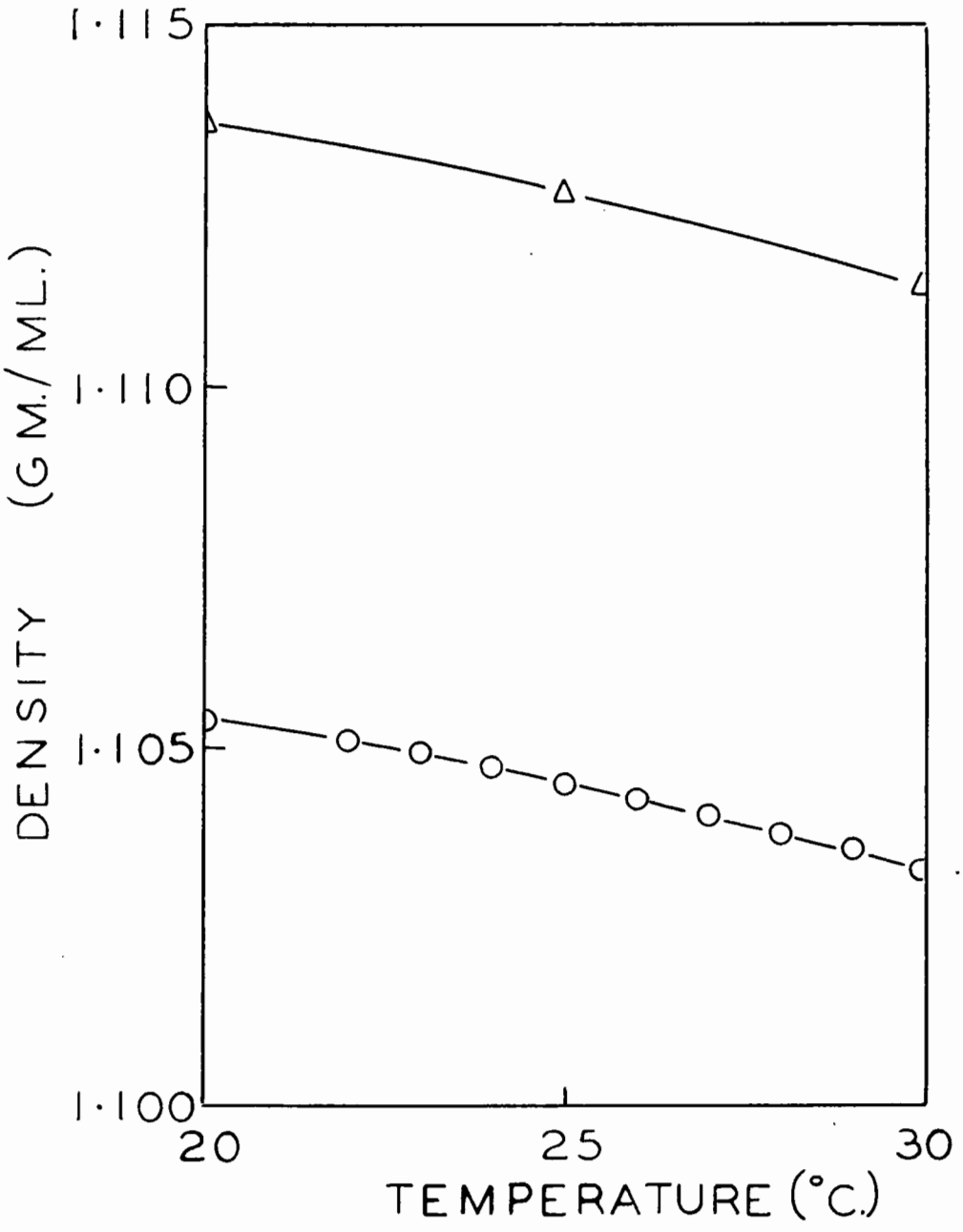
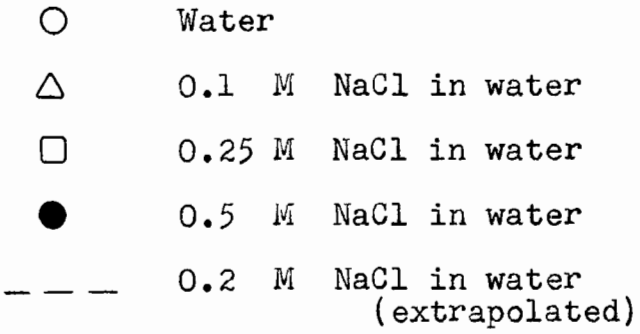

1·115
1·110
1·105
1·100
DENSITY (GM./ML.)
20
25
30
TEMPERATURE (°C.)

FIGURE 6

Viscosity: Temperature Relation of Aqueous Sedimentation Media



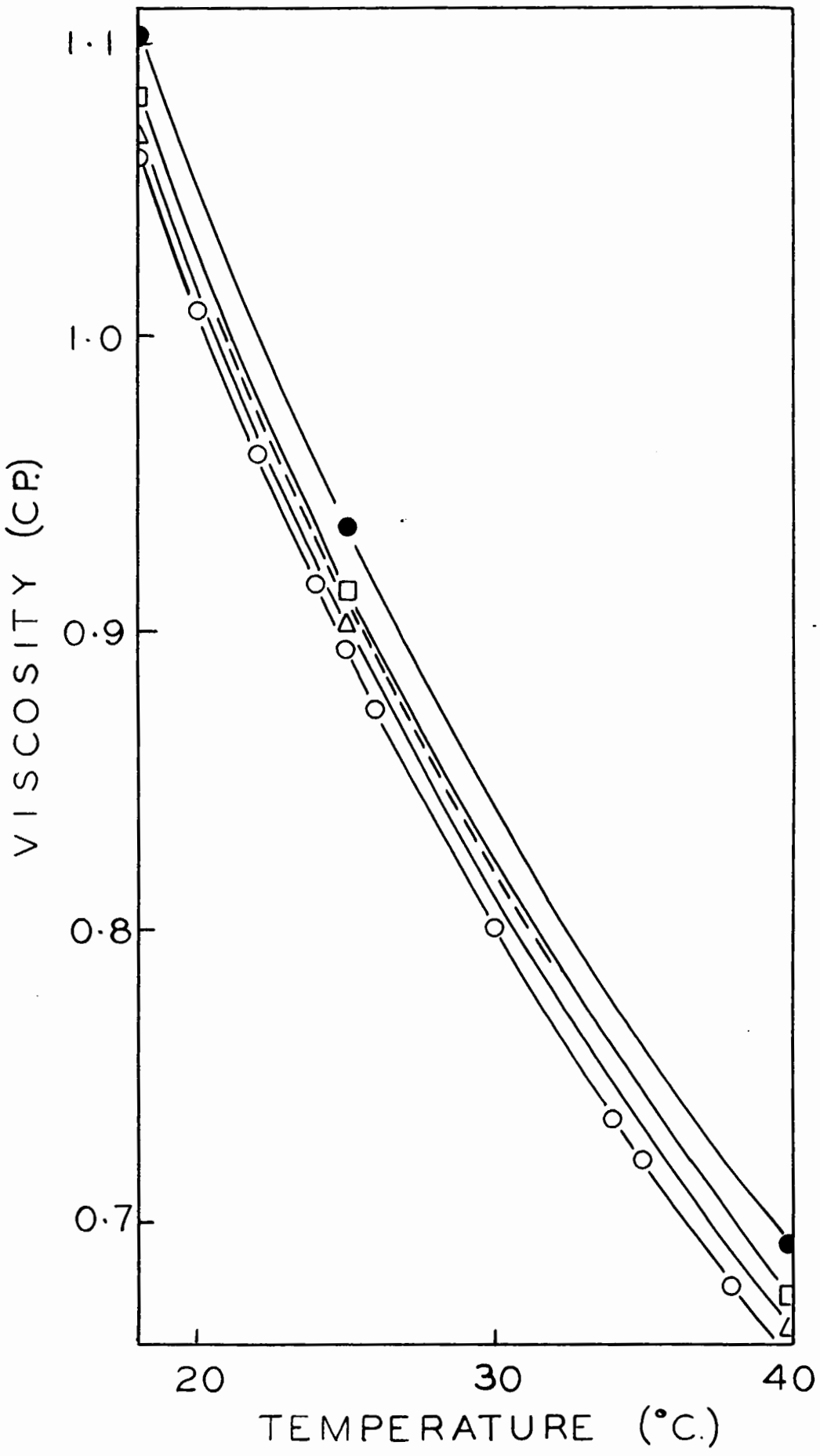

1·1
1·0
0·9
0·8
0·7
VISCOSITY (C.P.)
20
30
40
TEMPERATURE (°C.)

FIGURE 7

Viscosity: Temperature Relation of Deuterium Oxide Sedimentation Media

O	100% D_2O
- - - -	0.2 M NaCl in D_2O (extrapolated)
- ——— -	0.5 M NaCl in D_2O (extrapolated)

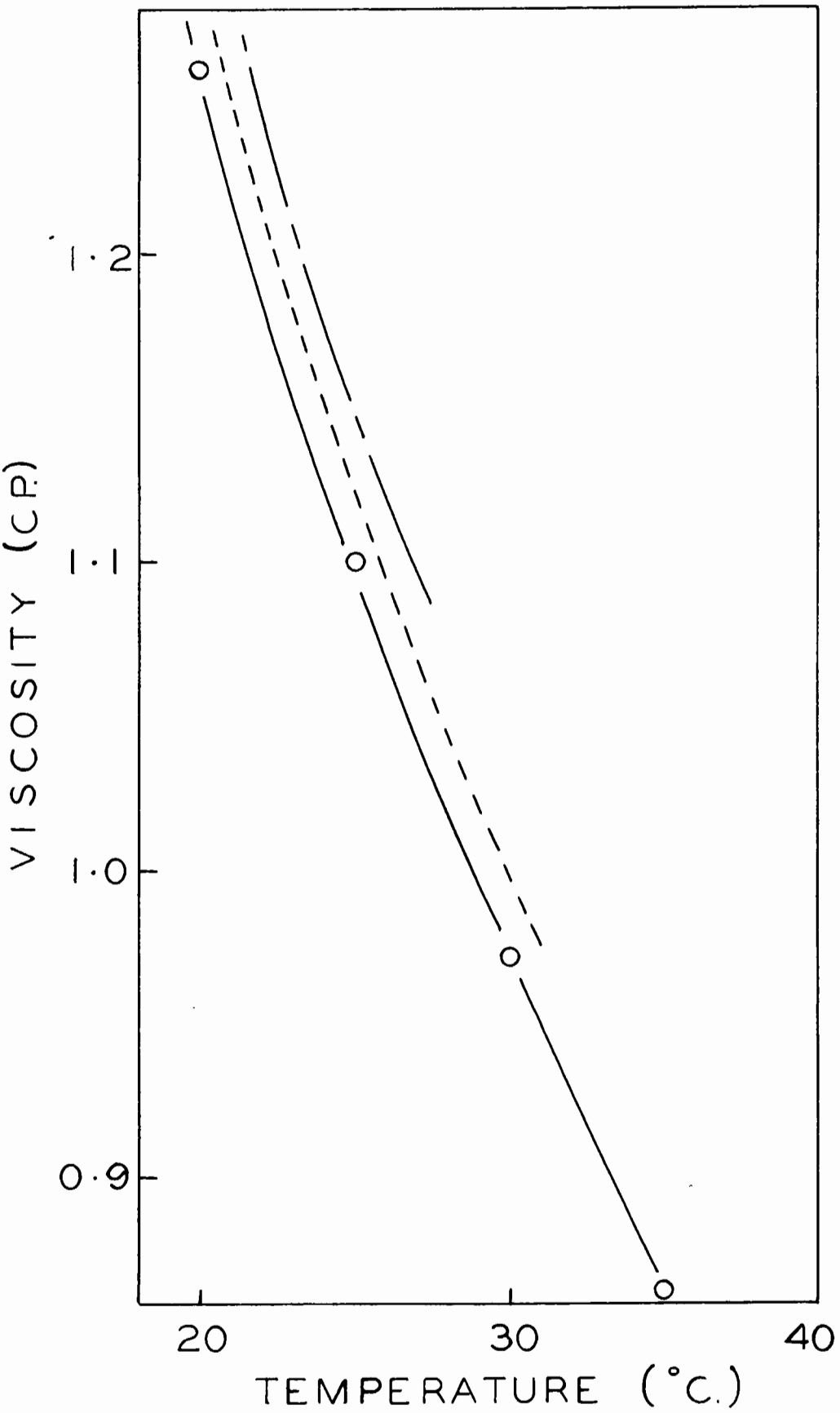

VISCOSITY (C.P.)
1·2
1·1
1·0
0·9
20
30
40
TEMPERATURE (°C.)

TABLE I

Viscosity and Density Data for the Sedimentation Media

Temp. °C.	H_2O (I.C.T.)		NaCl (I.C.T.)				
	d^t gm./ml.	η^t cp.	d^t gm./ml. 1%	d^t gm./ml. 2%	η Referred to H_2O same t. 0.1M	0.25M	0.5M
19		1.060			1.008	1.020	1.040
20	0.99823	1.008	1.00534	1.01246			
21	0.99802						
22	0.99779	0.960					
23	0.99756						
24	0.99732	0.916					
25	0.99707	0.894	1.00409	1.01112	1.009	1.022	1.046
26	0.99681	0.874					
27	0.99654						
28	0.99626						
29	0.99597						
30	0.99567	0.800	1.00261	1.00957			
34		0.735					
35		0.721					
38		0.679					
40		0.658			1.010	1.026	1.053

TABLE I (contd.)

Viscosity and Density Data for the Sedimentation Media.

Temp. °C.	NaCl ηcalc. Cp. 0.1M	0.25M	0.5M	$\frac{\eta(D_2O)}{\eta(H_2O)}$	D_2O (8) $\eta(D_2O)$ Calc. Cp.	$\frac{d^t}{d^{25}}$	d^t Calc. gm./ml.
18	1.068	1.081	1.102				
20				1.249	1.260	1.00081	1.10540
21							
22						1.00053	1.10509
23						1.00036	1.10491
24						1.00019	1.10472
25	0.902	0.914	0.935	1.232	1.100	1.00000	1.10451
26						0.99980	1.10429
27						0.99959	1.10406
28						0.99936	1.10380
29						0.99915	1.10357
30				1.215	0.972	0.99890	1.10329
34							
35				1.198	0.864		
38							
40	0.665	0.675	0.693				

Salt solutions of intermediate D_2O - H_2O concentrations were not so easily estimated. The increment in density and viscosity due to the salt was added to the density and viscosity of the liquid estimated from the curves. A sample calculation from experiment U.C. #14 is shown below. The average temperature for the exposure interval was 24.0°C.

d^{25} of the H_2O-D_2O	1.04757	d^{25} of the H_2O-D_2O	1.04757
d^{25} of the salt sln.	1.05663	d^{25} H_2O	.99707
increment due to salt	0.00906 gm./ml.	Δd	.05050 gm./ml.

d^{25} of 100% D_2O	1.10452	d^{24} of 100% D_2O	1.10472
d^{25} of H_2O	.99707	d^{24} of H_2O	0.99732
Δd	0.10745 gm./ml.	Δd	0.10740 gm./ml.

$\therefore$ increment in d^{24} of D_2O - H_2O over $d^{24}H_2O$ =

$$\frac{.05050}{.10745} \times .10740 = 0.05048 \text{ gm./ml.}$$

$\therefore$ density at 24.0° of medium = 0.05048 + 0.99732 + 0.00906

= 1.05686 gm./ml.

η 24 of 100% D_2O	1.130
η24 of H_2O	.916
$\Delta\eta$	0.214 cp.

$\therefore$ increment in η over $H_2O = \frac{0.05050}{0.10745} \times 0.214 = 0.1006$ cp.

An increment of 0.014 cp. due to the 0.2 M salt was estimated from the viscosity curve of Figure 6.

$\therefore \eta^{24.0}$ medium $= 0.1006 + 0.916 + 0.014 = 1.031$ cp.

Equation (53) was used in computing all of the sedimentation coefficients and its use is illustrated by the data from experiment #9 in Table II. Column (1) of Table II is the distance from the reference boundary to the centre of the peak, measured in cm. from the enlarged film. Column (2) is the actual distance in cm. in the cell. Column (3) gives the actual distance x of the centre of the peak from the axis of rotation. Column (5) is the natural logarithm of this distance. In column (9) s is the sedimentation constant in unit field or in cm. X sec.$^{-1}$ X dynes^{-1}. The average temperature was 23.0° estimated from the curve of Figure 3. The density and viscosity of the medium at the temperature of 23.0° were calculated as described above.

There was one more factor to consider before ηs could be used to obtain the density of the medium at zero sedimentation rate. The sedimentation rate is dependent upon the concentration of the sedimenting particles because of frictional forces and interaction of the particles. For a globular type of particle such as BPA no correction is needed if the concentration remains essentially constant, since the sedimentation rate of BPA shows very little concentration dependence (2, 48).

TABLE II

Computation of ηs from Experimental Data

Cell A

Photo No.	(1) Peak to reference	(2) x 0.9224	(3) 7.30-(2) cm.	(4) Log x	(5) Ln x	(6) $\triangle$ Ln x	(7) $\triangle t$ sec.	(8) $\triangle t\,\omega^2$	(9) s X 10^{13}
1	14.57	1.3440	5.9560	0.77495	1.78440	#4-#1			
2	14.37	1.3255	5.9745	.77627	1.78744	.00988	48X60	8.751	1.129
3	14.14	1.3043	5.9957	.77786	1.79110	#5-#2			
4	13.93	1.2850	6.0150	.77924	1.79428	.00932	"	"	1.065
5	13.77	1.2701	6.0299	.78032	1.79676	#6-#3			
6	13.59	1.2535	6.0465	.78147	1.79941	.00831	"	"	0.949

$t = 23.0^{\circ}C.$ $d^{23.0} = 1.1047$ $\eta^{23.0} = 1.162$ s Av. 1.048

$\therefore \eta s = 1.217$

Cell B

1	13.52	1.2471	6.0529	0.78197	1.80056	#4-#1			
2	13.20	1.2176	6.0824	.78405	1.80535	.01541	48X60	8.751	1.761
3	12.81	1.1816	6.1184	.78661	1.81125	#5-#2			
4	12.50	1.1530	6.1470	.78866	1.81597	.01534	"	"	1.753
5	12.19	1.1244	6.1756	.79071	1.82069	#6-#3			
6	11.85	1.0930	6.2060	.79281	1.82552	.01427	"	"	1.631

$t = 23.0^{\circ}C.$ $d^{23.0} = 0.9976$ $\eta^{23.0} = 0.937$ s Av. 1.715

$\therefore \eta s = 1.607$

However, coiled chain molecules such as PVA display considerable concentration dependence (2,67), and sedimentation experiments were therefore made with PVA at several concentrations in water solution and the data plotted on a $^{1}/_{s}$ (2) versus concentration curve to permit correction for the concentration dependence effect on the sedimentation rate. As the concentrations were not far removed from the selected value of 0.4%, the slope from the $^{1}/_{s}$ plot in water solution was also used to correct for concentration dependence in the D_2O solutions.

It might be noted at this point that the addition of a new rotor temperature control and indicating unit to the ultracentrifuge made constant temperature sedimentation possible. For this reason, the experiments with mixtures of macromolecules were conducted at 20°C. and not at approximately 25°C. as with the experiment with the single solutes. The viscosities and densities of the sedimentation media and solutions for the experiments on mixtures were determined at 20°C. Also, the sedimentation coefficients of the macromolecules from the experiments on mixtures were determined from the slope of ln x as of function of t, because the boundaries were measured after they had moved some distance from the meniscus.

To estimate the change in $\bar{V}$ due to deuteration of the macromolecule, several of the materials investigated by sedimentation were examined on a McBain - Bakr quartz spiral balance. A quartz spiral balance was adopted because of its inherent stability, commercial availability, simple manipulation, and adaptability to vacuum operation.

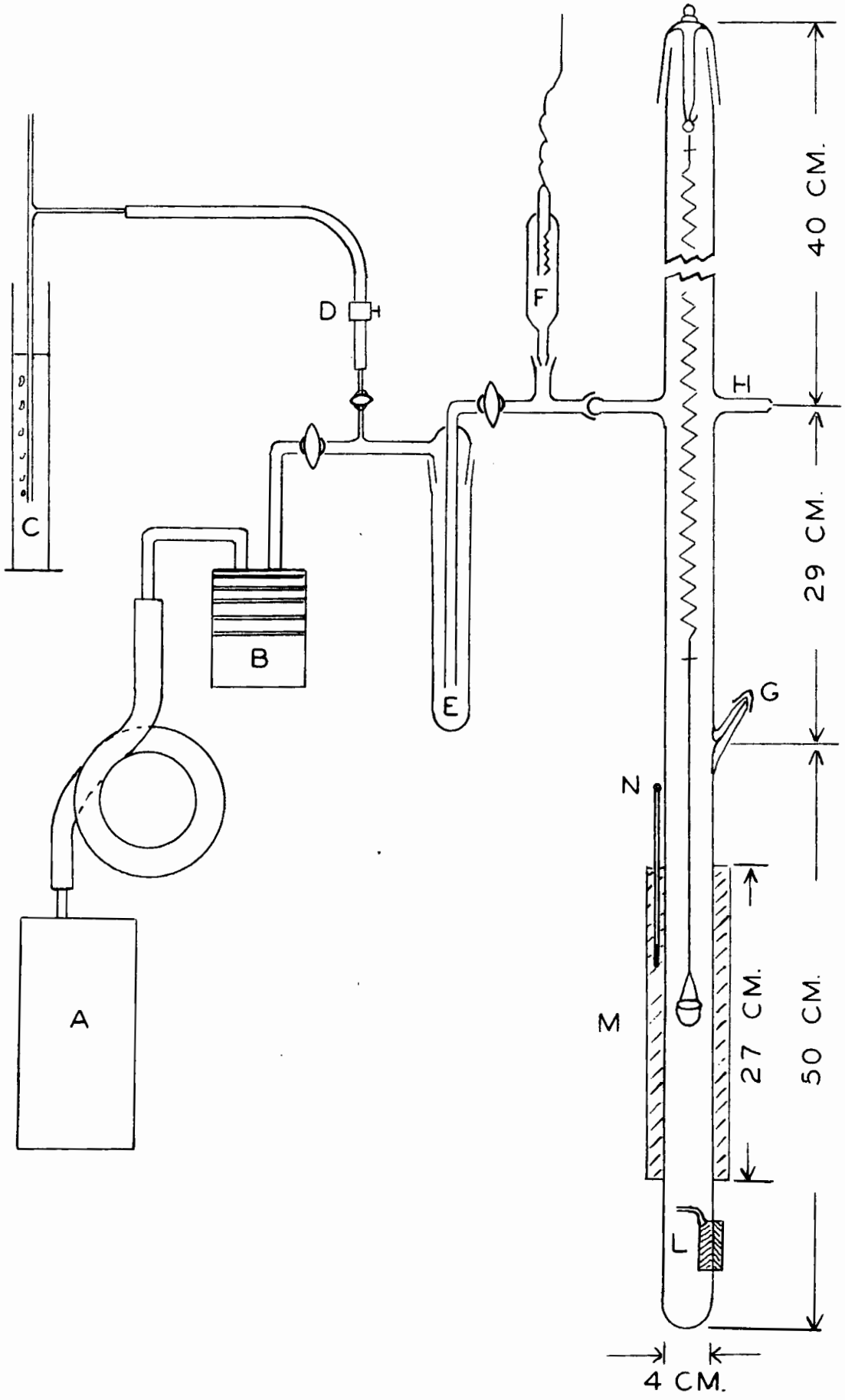
A schematic diagram of the spiral balance assembly is shown in Figure 8. A Welch Duo Seal vacuum pump A was connected to a Cenco air-cooled metal diffusion pump B by rubber vacuum tubing looped to diminish vibrations. Silicone oil DC 702 was used in the diffusion pump. Small bore heavy wall gum rubber tubing controlled by a pinchcock D was used to allow nitrogen gas to enter the system as desired. Pressure was controlled by a water head at C. A trap was connected at E, followed by a connection to the gauge head of an Edwards ionization gauge at F. The glass balance case was connected to the vacuum system by a ball joint and a connection was available at H for ionization gauge calibration against a Mac Leod gauge. The arrangement of the quartz spiral and basket is evident from the diagram. Reference glass fibres were fastened above and below the quartz spiral with glyptal cement diluted with acetone. It was possible to raise the basket in vacuo by manipulation of the two permanent magnets L to the position of the capillary tube G, where liquids could be added to the basket by means of a syringe and needle. A removable furnace M consisted of heavy wall dural tubing, nichrome wire insulated and held in position by Sauerensen cement, asbestos fibres, asbestos paper, and an outer metal container. A thermometer N was placed in a deep well, containing ethylene glycol, in the dural tubing. The temperature of the oven was controlled from a variac. Vacuum stopcocks were used throughout. With the exception of D and G, the minimum diameter path was 10 mm. Joints and stopcocks were greased with Apiezon L grease.

Many designs of baskets were tested in an effort to

FIGURE 8

Quartz Spiral Balance

Apparatus


A
B
C
D
E
F
G
H
L
M
N
40 CM.
29 CM.
27 CM.
50 CM.
4 CM.

achieve lightness with maximum capacity. Solutions invariably crept up any fold or crevasse of the baskets by capillary action. The problem was solved by forming baskets of aluminum foil and dipping them into a dispersion of silicone grease in benzene. The baskets were dried at 110° C. Baskets with capacities of one to two ml. weighed about 40 to 60 mgm. This capacity was required to make the best use of the particular spiral available.

The spiral was calibrated in vacuo by placing calibrated platinum weights in a pan suspended from the spiral. The spiral extension was measured with a Wild cathetometer at 25° C. The glass scale of the cathetometer was free standing and ruled in 0.1 mm divisions, from which it was possible to read to 0.01 mm. by a vernier attachment. The precision of the readings was about ± 0.02 mm.

Baskets suspended from the spiral were lowered into the glass balance case, and vacuum applied with a liquid nitrogen trap in place. Measurements were made of the spiral extension until constant extension was achieved. Forepump and diffusion pump were then stopped and nitrogen gas was slowly drawn into the system through the trap. A sample of about 150 to 200 mgm. was then put into the basket and vacuum again applied until constant extension was achieved. The pumps were then stopped and nitrogen gas slowly bled into the system. Capillary G, Figure 8, was then opened, with a slight pressure of nitrogen gas being maintained, and 0.5 to 1 ml. of specially distilled water was directed into the basket with a syringe needle inserted through the capillary. The basket was supported during this operation, and at other times when it carried excessive load, by the two magnets L. The capillary was capped,

the nitrogen gas inlet closed and the system left to allow wetting or dissolution of the sample to proceed. The liquid nitrogen trap was not removed during these operations. The basket was lowered gently by means of the supporting magnets until it was within about 25 cm. of the bottom of the balance case. A dewar flask of liquid nitrogen was then brought up about the case to a level slightly above the basket. The sample froze in about 15 minutes, after which the dewar was set aside and the water removed from the sample by lyophilization. Evacuation was continued until the basket and contents had reached constant weight. A second addition of water and lyophilization was carried out in instances when the sample or system had been exposed to deuterium oxide. The trap was then removed and dried and the sequence of operations was repeated with at least two applications of deuterium oxide. Equilibrium periods of 15 to 20 hours were allowed to insure isotopic exchange. The deuterium oxide sequence was then followed of two more applications of water.

As a final step, the heater M was slid into place so that the basket was centrally located within the heater. A seal was made with plasticine between the heater and the glass balance case. The heater was brought to selected temperature and the new equilibrium weight of the basket and sample recorded while *in vacuo* and, of course, with the liquid nitrogen trap in place.

A magnetic float apparatus closely resembling that described by MacInnes *et al.* (11) had been assembled and tested by Charlwood (13). This apparatus with a few minor charges was used to determine the densities of the various solutes in aqueous

or deuterium oxide solution. The floats were similar in shape to inverted volumetric flasks and each stem contained a cylindrical magnet. The volume of the floats, which weighed about 70 grams, was adjusted so that the floats required the addition of a slight weight to sink in water at 25° C. The floats were operated in conical glass cells which required about 230 ml. of liquid for a measurement. A solenoid about the conical cell supplied a force on the magnet of the float, which imparted an accelerated motion to the float. Each float was calibrated in water by adding platinum weights to the top of the float and measuring the times, t, required for the bottom of the float to pass through a region of the cell defined by graduations on a telescope scale, as the current, i, through the solenoid was varied. For each weight, w, of platinum, the current at which the float would neither rise nor fall was found by plotting $^1/t$ versus i. The relation between this limiting current and the weight of platinum on the float was linear and the slope of this line, multiplied by $(1 - d_o/d_1)$, where d_o and d_1 are the densities of water and platinum, related milliamperes to milligrams for each float. This corrected slope, designated, f, was a constant for each float at 25.000°C.

It can be shown (11) that:

$$d_2 - d_o = \frac{(w - w_o)(1 - d_o/d_1) - f\,(i-i_o)}{W + w_o + \left[(w-w_o)d_o/d\right] - f\,i_o} \quad .....(55)$$

where W is the weight of the float and w is the weight of the platinum weights. Equation (55) was used extensively in conjunction with equation (17) for the calculations of ϕ of the solutes. Corrections to ,f, for variation in temperature or barometric

pressure were found from the relation between $^{1}/t$ and i when the bath temperature was changed or when pressure in the cell was changed.

The float apparatus was operated in an insulated water bath of about 800 litres capacity which was controlled to $\pm$ 0.001°C. by a mercury thermoregulator operating an infra-red lamp through a thyratron circuit. For routine measurements a Beckman thermometer was used. However, the bath also contained a platinum resistance thermometer connected to a Mueller bridge and Leeds - Northrup galvanometer with which the absolute temperature could be obtained to 0.001°C.

Samples of 3 to 5% solute concentration in a volume of 100 mls. were dialysed against a minimum of about 250 ml. of solvent. To avoid bubble formation on the float the final ten hours of dialysis was at a temperature five to ten degrees higher than that of the bath. Cells and floats were cleaned by standing in cold chromic acid overnight and then dried in vacuo. Solvent was added from a large weight burette to the cell containing the float. Appropriate platinum weights were added to the float and the assembly was then put into the bath for one hour to attain temperature equilibrium. A special circuit was used in which a maximum of 500 ma. could be passed through the solenoid to get an estimate of the final platinum weights required. However, current through the solenoid during actual measurements never exceeded 100 ma. Small platinum weights could be added to the float while the cell assembly remained in the bath. The current passing through the solenoid was observed

as a function of the potential drop across a standard resistance in a potentiometer circuit (Leeds & Northrup) and observed readings were therefore in millivolts rather than milliamperes. Readings of t and mv. were taken at different currents through the solenoid and a plot of $^{1}/_{t}$ against mv. was made. Solution was added to the cell from a weight burette and the measuring procedure repeated after temperature equilibrium was reached. Further additions of solution were made as desired.

Concentrations of the aqueous solutions were determined by drying weighed 5 ml. volumes of stock solution and solvent to constant weight at 105°C. in vacuo. However, for deuterium oxide solutions and solvents, weighed 5 ml. volumes were brought to dryness, two 5 ml. portions of distilled water added to each residue with intervening drying and then each residue was dried to constant weight at 105°C. in vacuo. In several experiments the residues were weighed before adding the distilled water to permit estimation of the extent of deuteration.

Special precautions were taken with solutions containing deuterium oxide. Dialysis was performed in flasks fitted with glass stoppers which were lightly greased with silicone grease. Transfers of solutions for density and dry weight measurements were made with pipettes which were protected from atmospheric moisture by drying tubes filled with silica gel. Owing to their small molecular weights, glycine and TGG could not be dialysed and therefore these substances were dissolved in the aqueous solvents and the densities of their various

solutions were determined. For operations in deuterium oxide, the dissolved solutes were allowed to equilibrate overnight at room temperature. The solutions were then frozen and the deuterium oxide removed completely by lyophilization. The deuterated samples were kept in sealed bottles and solutions made as required by the addition of D_2O that had been removed previously by lyophilization.

A typical experiment will now be described in which the system was glycine dissolved in deuterium oxide. Concentration will be expressed as weight fraction n, based on dry weights at 70°C. in vacuo.

grams of solution	5.5871	5.6016	
grams of dry residue	0.2095	0.2101	
corr.for residue in solvent	0.0001	0.0001	
g.deuterated glycine	0.2094	0.2100	
g. deuterated glycine / g. solution	0.03748	0.03749	av.0.03749

After two 5 ml. applications of distilled water:

g. protonated glycine	0.2020	0.2026	
g. protonated glycine / g. solution	0.03616	0.03617	av.0.03616

av. increment in weight due to deuteration = 0.0074 g.

av. % increase " " " " " = 3.65

However, since losses of deuterium must occur during the drying

process this value is minimal and the theoretical increase was derived and used to calculate the concentration of deuterated glycine.

% D_2O in solvent = (d solvent - d_{H_2O})/(d_{D_2O} - d_{H_2O}) x 100

= (0.10484/0.10744) x 100 = 97.6

Theoretical % increase in weight in 100% D_2O

= (labile H atoms/MW) x 100

= (3/75.07) x 100 = 4.00

Theoretical % increase in weight in 97.6% D_2O = 4.00 x 97.6

= 3.90

∴ conc. of deuterated stock glycine solution

= 0.03616 + (0.03616 x 0.039) = 0.03757 g./g.

grams of solvent in cell	268.48
" of stock glycine added	18.3402
" of solution in cell	286.8202

n_1 = (18.3402/286.8202) x 0.03757 = 0.002402

grams of stock glycine added	12.0491
" " " " in cell	30.3893
" " solution " "	298.8693

n_2 = (30.3893/298.8693) x 0.03757 = 0.003820

grams of stock glycine added	16.9588
" " " " in cell	47.3481
" " solution " "	315.8281

n_3 = (47.3481/315.8281) x 0.03757 = 0.005634

grams of stock glycine added 13.8666

" " " " in cell 61.2147

" " solution " " 329.6947

n_4 = (61.2147/329.6947) x 0.03757 = 0.006975

Factors for the float in special distilled water:

w_o/d = 0.0290, $w_o(1 - d_o/d)$ = 0.59274, i_o = 32.0$_{mv.}$

$(W + w_o - fi_o)/d_o$ = 69.0433, f = 0.000300 g./mv. ,

pressure factor = 0.045 mv./mm.Hg., temperature factor = 0.042 mv./0.001°C.

Beckman temperature 3500 = 25.000°C.

Standard pressure = 750 mm.Hg.

Table II A shows the recorded measurements from this experiment.

In the above experiment a shift occurred in the bath temperature after the measurements had been made on the 3rd weight fraction. Measurements were made on the 4th weight fraction at the higher temperature and then the temperature of the bath was decreased and the measurements repeated as a check on the temperature correction factor. Figure 9 shows the $^1/t$ against mv. relation for this experiment.

TABLE II A

Illustrative Float Apparatus Measurements

	Solvent	n_1	n_2	n_3		n_4
t° Beckman	3501	3501	3501	3501	3507	3497
press.mm.Hg.	759	759	759	759	768	768
i exp.	16.8	37.1	44.6	37.3	24.4	23.8
i corr.	16.4	36.7	44.2	36.9	23.3	23.1
$i - i_o$	-15.6	4.7	12.2	4.9	-8.7	-8.9
$(w - w_o)(1 - d_o/d)$	7.27135	7.35120	7.39736	7.45043	7.48688	7.48688
$f (i - i_o)$	-.00468	.00141	.00366	.00147	-.00261	-.00267
N	7.27603	7.34979	7.39370	7.44896	7.48949	7.48955
$(w - w_o)/d$	0.3559	0.3599	0.3622	0.3648	0.3665	0.3665
$(W + w_o - fi_o)/d_o$	69.0433	69.0433	69.0433	69.0433	69.0433	69.0433
D	69.3992	69.4032	69.4055	69.4081	69.4098	69.4098
$(d_{1,2,3,} - d_o) = {}^{N}/_{D}$	0.104843	0.105900	0.106529	0.107321	0.107903	0.107903
$d_{1,2,3,...}$	1.101917	1.102974	1.103603	1.104395	1.104977	1.104977
$d_{2,3,..} - d_1$	-	0.001057	0.001686	0.002478	0.003059	0.003059
$(d_{2,3,..} - d_1)/d_{2,3,..}$	-	0.000958	0.001528	0.002244	0.002769	0.002769
n = wt. fraction	-	0.00240_2	0.00382_0	0.00563_4	0.00697_5	0.00697_5
$(d_{2,3,} - d_1)/(d_{2,3,..})n$	-	0.398_0	0.399_1	0.397_6	0.396_1	0.396_1
ϕd_1	-	0.602_0	0.600_9	0.602_4	0.603_9	0.603_9
ϕ	-	0.545_6	0.544_6	0.546_0	0.547_3	0.547_3

FIGURE 9

Millivolt : Reciprocal Time Relation

△ Solvent

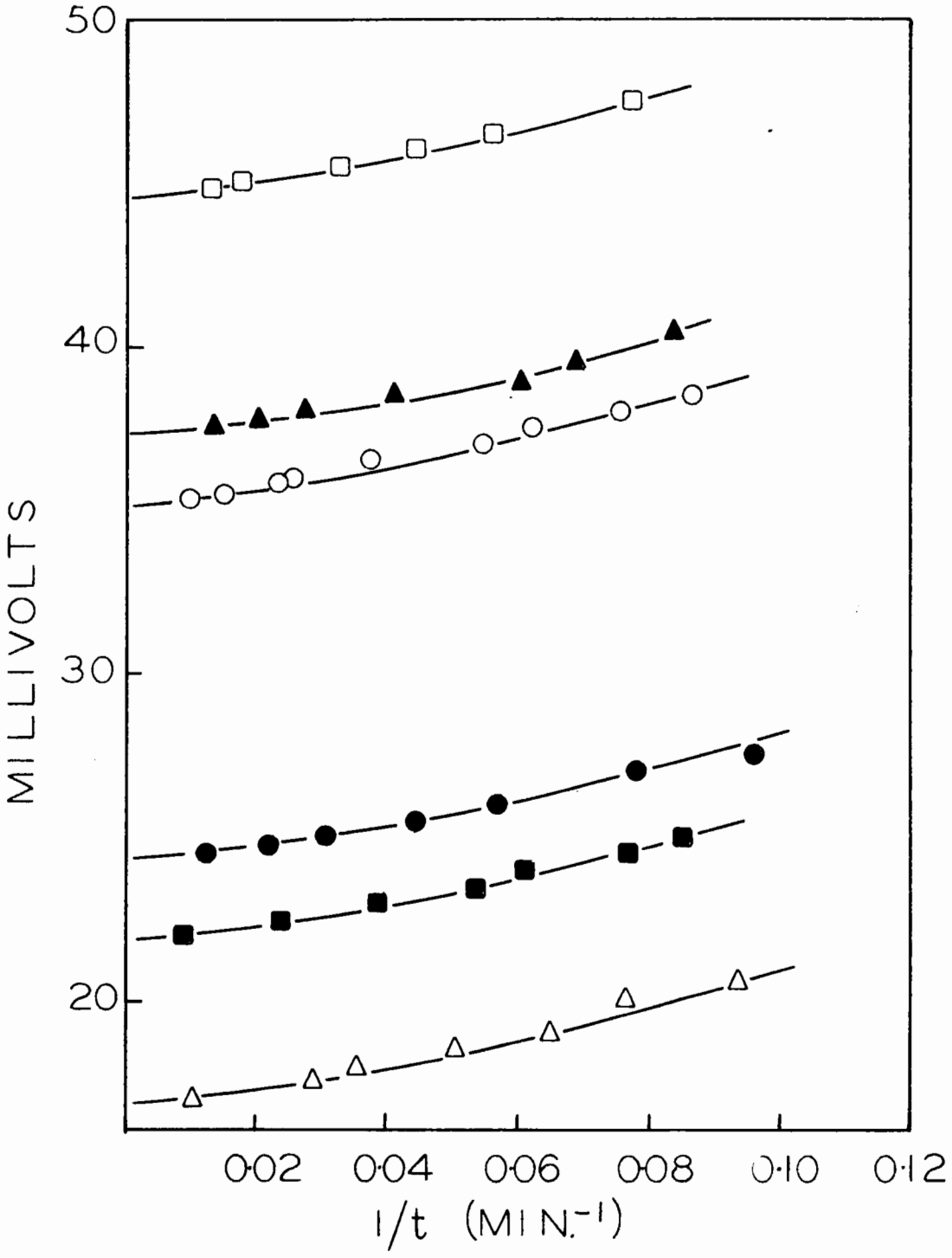
○ Weight Fraction n_1, 25.001°C, add 2 mv. to ordinates

□ " " n_2, " ,

▲ " " n_3, " ,

● " " n_4, 25.007°C,

■ " " n_4, 24.997°C, add 2 mv. to ordinates


50
40
30
20
MILLIVOLTS
0·02
0·04
0·06
0·08
0·10
0·12
1/t (MIN.⁻¹)

RESULTS

SPIRAL BALANCE MEASUREMENTS

The results of the calibration of the quartz spiral were as follows:

Load (mgm.)	Sensitivity (mgm./mm.extension)
24	1.00 ± 0.02
38	1.00 "
72	1.00 "
108	1.00 "
145	1.00 "
174	1.01 "
183	1.01 "
198	1.01 "

The sensitivities were determined from the slopes of the weight-extension relations at various loads. The error was based upon the reproducibility of the cathetometer readings. From these results it can be seen that mm. of extension are readily convertible to mgms. of load.

A stock of distilled water and deuterium oxide was stored in sealed bottles for experiments with the spiral balance. The concentration of D_2O was (0.10469/0.10744) x 100 or 97.4%, as calculated from measurements made with the float apparatus. There was no detectable residue remaining when 0.5 ml. volumes of the stock liquids were lyophilized from the spiral baskets. Loss in weight on heating the empty basket from room temperature to 100°C. was within the error of measurement. All spiral measurements were made at $< 10^{-4}$ mm.Hg., usually about 5×10^{-5} mm.Hg. with the apparatus in a room at 25°C.

TABLE III

BPA Isotopic Exchange Sequence

Exchange Medium† 25°C.	Extension by the Sample (mm.) 25°	68°	% Weight* Increase 68°
0.95 ml, 97.4% D_2O	159.13	159.07	
0.50 ml, " "	159.41	159.35	1.56
0.50 ml, H_2O	157.32	-	
0.50 ml, "	157.09	157.03	
0.50 ml, "	157.06	156.95	
0.60 ml, 48.7% D_2O	158.20	-	
0.60 ml, " "	158.13	158.14	0.79
0.80 ml, 73.1% D_2O	158.99	-	
0.80 ml, " "	159.15	158.73	1.17
0.60 ml, 97.4% D_2O	-	159.16	
0.60 ml, " "	-	159.16	1.44
0.80 ml, H_2O	-	156.94	
0.60 ml, "	-	156.82	

† added in the order set forth in the table

* based on a protonated extension of 156.90 mm.

Bovine plasma albumin was most intensively investigated with the spiral balance. The data are shown in Table III in the order in which exchange liquids were added to the sample. The relation between % increase in weight, based on dry weight at 68°, and volume % D_2O, is shown in Figure 10. This % was calculated from the extensions applicable to:

(deuterated sample - protonated sample) x 100/protonated sample.

The increase in weight, 1.55%, calculated on the basis of the amino acid constitution (20) and 100% D_2O is also shown in Figure 10.

In Table IV, the results of spiral measurements on PVA, lipovitellin + γ-livetin, α - + β -livetin, and glycine are given. The theoretical % weight increase for PVA is based on 88 monomer units per 100 monomer units of the polymer being in the hydroxyl form. Only the hydrogen atom of the hydroxyl group is labile. Glycine (aminoacetic acid) has three labile hydrogen atoms and a molecular weight of 75.07, and therefore a theoretical % weight increase of 3.89 for 97.4% D_2O. Calculation of the % weight increase based on the dry weights at the elevated temperatures makes no significant changes.

Difficulties in achieving constant dry weight values for glycine samples at 105° <u>in vacuo</u>, led to investigation of this material with the spiral balance, at several temperatures. The results are tabulated in Table V and are shown graphically in Figure 11. At 95°C. a white sublimate formed an increasingly heavy coat on the inner surface of the glass balance case,

FIGURE 10

Relation Between D_2O Concentration and Weight Increment of Bovine Plasma Albumin

● Experimental

■ Calculated

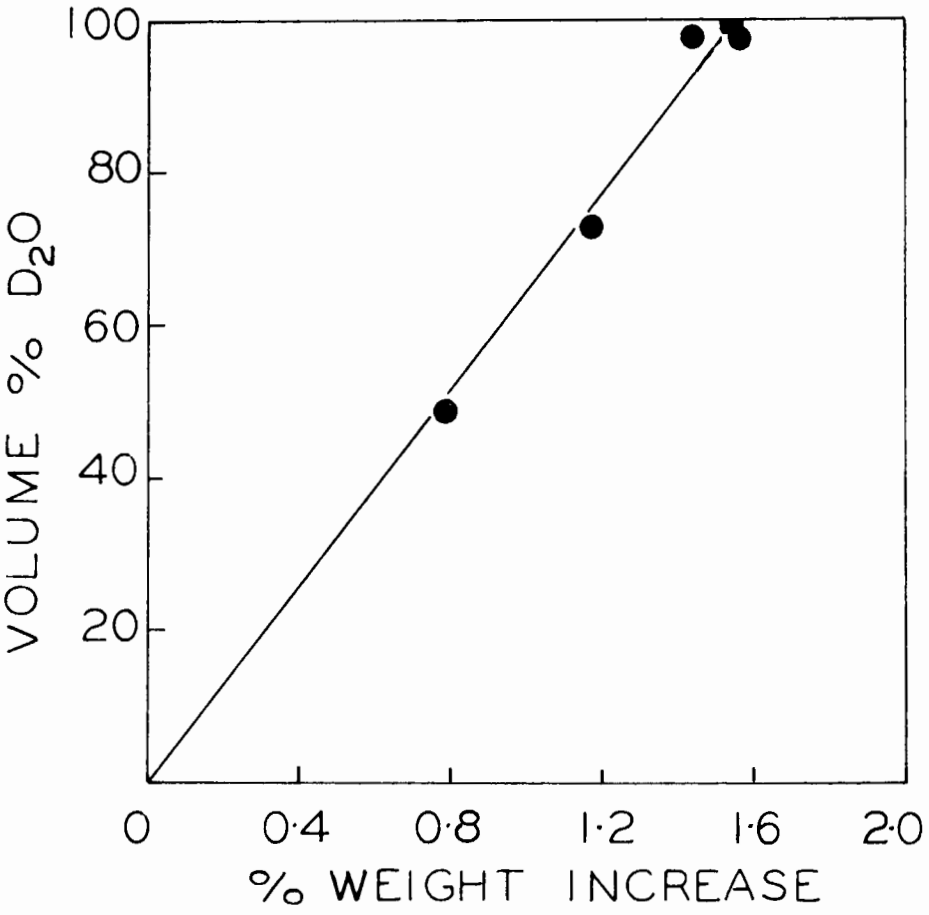

100
80
60
40
20
VOLUME % D_2O
0
0·4
0·8
1·2
1·6
2·0
% WEIGHT INCREASE

TABLE V.

Weight of Glycine as a Function of Time and Temperature

ELAPSED TIME (HRS.)	TEMP. (°C.)	Extension by the Sample (mm.)
0.0	25	-
4.5	"	189.39
5.5	"	189.15
6.0	"	189.10
7.0	"	189.03
25.0	"	188.70
35.5	"	188.63
36.1	47	188.58
36.3	"	188.54
37.0	"	188.46
50.2	"	187.87
61.8	"	187.66
71.1	"	187.56
73.3	"	187.56
74.1	"	187.53
74.6	"	187.51
75.5	"	187.50
77.1	"	187.48
78.1	"	187.54
79.1	"	187.51
96.0	56	187.14
98.5	"	187.12
99.5	"	187.07
101.9	"	187.02
104.7	"	187.01
110.4	74	186.30
111.1	"	186.29
112.5	"	186.28
113.6	"	186.27
116.7	"	186.20
117.1	"	186.17
134.3	"	185.81
134.8	"	185.62
158.8	"	185.46
170.7	95	184.24
181.1	"	182.71
182.0	"	182.62
183.5	"	182.46
184.2	"	182.34
185.4	"	182.24
186.5	"	182.00
187.8	"	181.93
189.5	"	181.73

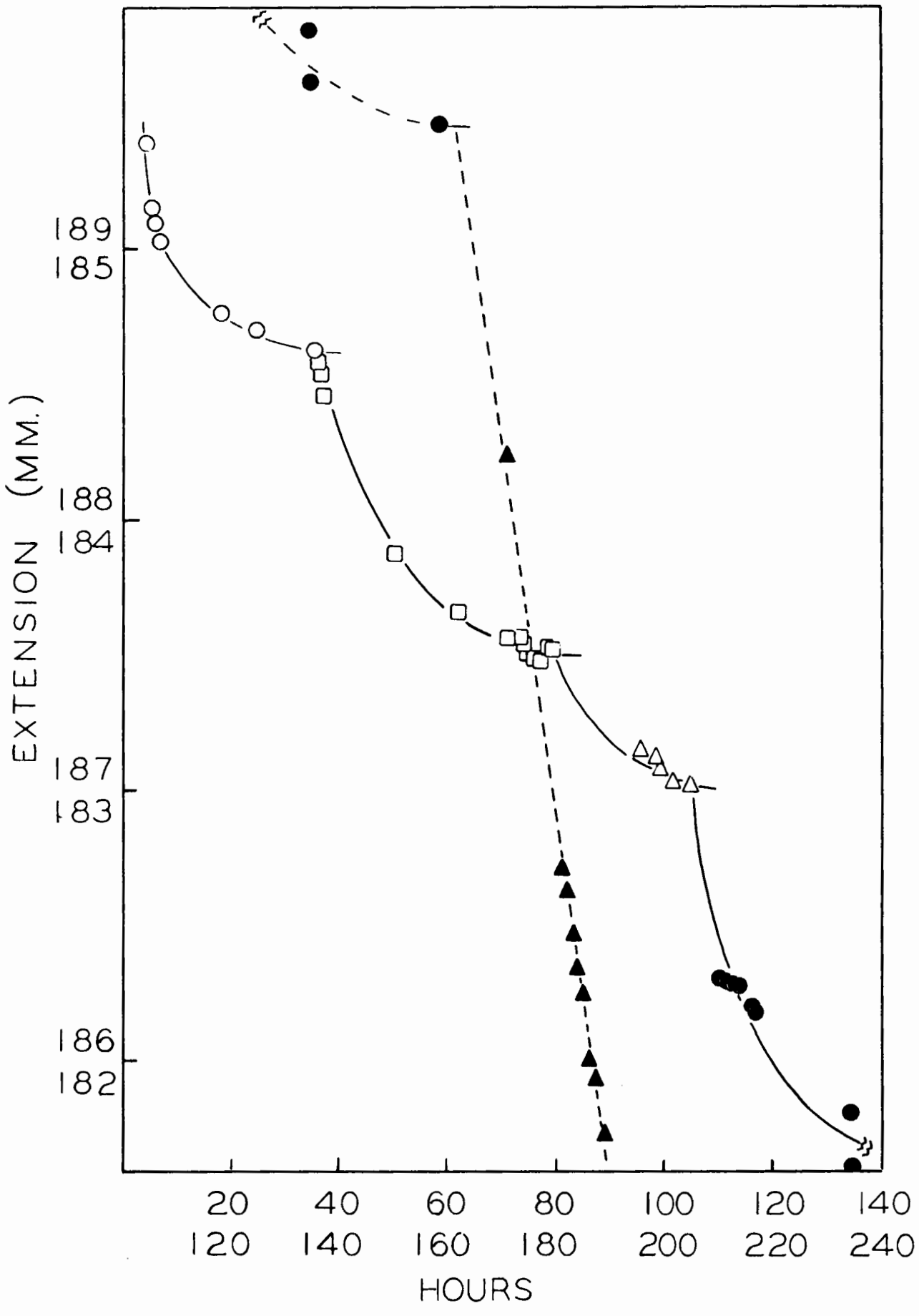
FIGURE 11

Weight (Extension) of Glycine as a Function of Temperature and Heating Time

Upper Scales _____

Lower Scales - - - - - -

○	25°C.	in vacuo
□	47° "	" "
△	56° "	" "
●	74° "	" "
▲	95° "	" "


EXTENSION (MM.)
189
185
188
184
187
183
186
182
20
120
40
140
60
160
80
180
100
200
120
220
140
240
HOURS

commencing about three inches above and below the ends of the heater, in zones about two inches in width. As some of this material also coated part of the spiral, the extensions applicable to the sample at this temperature should probably be less than those recorded. The sublimate was not apparent at 75°C., nor with the other materials at elevated temperatures.

VISCOSITY AND DENSITY RESULTS

Koenig (68) gives $\log \eta = -0.0391 + 0.0190$ C. for the viscosity of BPA in 0.2 ionic strength NaCl solution at 25°C. The value of 0.923 centipoises for BPA computed from the data in Table VI agrees with this equation. In 0.19 ionic strength D_2O solution the viscosity of BPA, at a concentration of 0.42 gm./dl., was 1.129 centipoises. The viscosity of D_2O stock solution was computed from the data in Table VI to be 1.099 centipoises, which may be compared with the value of 1.100 centipoises (8) for the viscosity of 100% D_2O at 25°C.

The intrinsic viscosity of PVA from the curve in Figure 12, plotted from the data in Table VI was 0.77. Flory (49) gives the relation between molecular weight and the intrinsic viscosity of PVA as $\log M = 4.865 + 1.32 \log [\eta]$. A molecular weight of 52,000 for the PVA was calculated from this relation. However, this molecular weight is at the extreme end of the range for which the relation is applicable. Dialer (50) relates molecular weight and $[\eta]$ in a curve which extends to molecular weights over 150,000. From this curve the molecular weight of PVA with $[\eta]$ of 0.77 would be about 68,000.

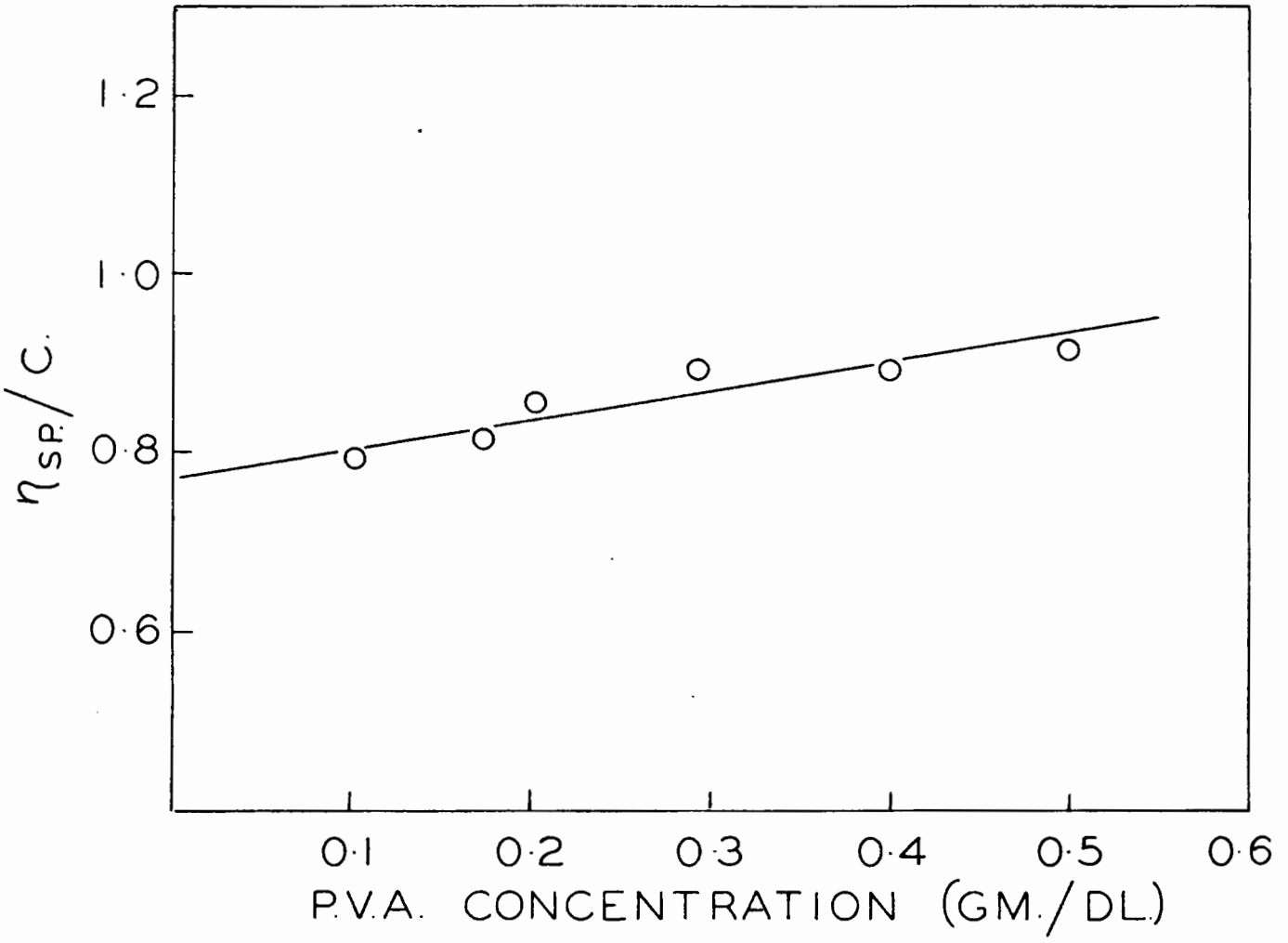
TABLE VI

Viscosity Measurements at 25° C.

Sample	Solvent	C. Gm./dl.	Density gm./ml.	Flow-time sec.	η cp.	$\eta_r - 1$	$\eta_{sp}/C.$ dl./gm.
H_2O			0.9971	249.7	0.894		
BPA	0.2μ NaCl (H_2O)	0.398	1.0066	254.4	0.920		
H_2O			0.9971	248.2	0.894		
BPA	0.2μ NaCl (H_2O)	0.398	1.0066	254.4	0.925		
H_2O			0.9971	252.7	0.894		
BPA	0.19μ NaCl(D_2O)	0.425	1.1131	285.8	1.129		
H_2O			0.9971	253.5	0.894		
D_2O			1.1043	281.5	1.099		
PVA	D_2O	0.382	1.1051	370.6	1.448		
H_2O			0.9971	252.5	0.894		
PVA	H_2O	0.102	0.9973	273.0	0.967	0.0812	0.793
PVA	H_2O	0.203	0.9976	296.2	1.049	0.1736	0.856
PVA	H_2O	0.294	0.9978	318.5	1.129	0.2623	0.891
PVA	H_2O	0.400	0.9981	341.9	1.213	0.3568	0.892
H_2O			0.9971	253.8	0.984		
PVA	H_2O	0.175	0.9975	289.5	1.0203	0.1413	0.806
PVA	H_2O	0.500	0.9983	370.0	1.3049	0.4595	0.918

FIGURE 12

The Intrinsic Viscosity of Polyvinyl Alcohol


1·2
1·0
0·8
0·6
$\eta_{SP.}/C.$
0·1
0·2
0·3
0·4
0·5
0·6
P.V.A. CONCENTRATION (GM./DL.)

The above relations would not be completely applicable since the sample of PVA contained some acetate groups.

Viscosity and density measurements made at 20°C. on mixtures of proteins are included in Table VII. These measurements were made in conjunction with sedimentation experiments at 20°C.

SEDIMENTATION DETERMINATION OF $\bar{V}$

The data compiled from the sedimentation experiments at fixed concentrations of BPA and PVA and at several densities of the medium are shown in Tables VIII and IX. Since PVA has a marked concentration dependence and the concentrations were not exactly the same for each experiment, it was necessary to find the relation between sedimentation rate and concentration. Experiments were made in the ultracentrifuge for a series of concentrations under the conditions and with the results set forth in Table X. Graphical representation of the relation between concentration and 1/s is shown in Figure 13. The slope was 0.330 for concentrations from 0.2 to 0.4, with an intercept of 0.501 from which the sedimentation coefficient, $S_{20,w}$ $C \longrightarrow 0$, was computed to be 2.00. From this data it was possible to correct the sedimentation coefficients of PVA for small deviations from the selected concentration. The concentration of 0.40% was selected to obtain peaks of a convenient height for measurement. The values of $\eta s/k$ versus d/k for the media are plotted in Figure 14. The lines were drawn by

TABLE VII

Viscosities and Densities of Solvents and Solutions of Macromolecules at 20°C.

Sample	Solvent	Conc.	Density g./ml.	Flow-Time sec.	η cp.
H_2O	-	-	0.9982	325.8	1.0087
0.2 μ NaCl	H_2O	-	1.0060	328.7	1.025_6
BPA + β-LG	0.2 μ NaCl(H_2O)	I*	1.0105	352.1	1.103_5
"	"	I x 3/5	1.0086	339.9	1.063_3
"	"	I x 9/25	1.0075	334.4	1.045_0
"	"	I x 27/125	1.0068	331.8	1.036_1
0.2 μ NaCl	D_2O	-	1.1078	366.3	1.258_6
BPA + β-LG	0.2 μ NaCl(D_2O)	I -	1.1117	389.4	1.342_7
"	"	I x 3/5	1.1101	379.8	1.307_7
"	"	I x 9/25	1.1093	371.9	1.279_6
"	"	I x 27/125	1.1089	369.6	1.269_9
H_2O	-	-	0.9982	325.8	1.0087
H_2O	-	-	0.9982	325.3	1.0087
Buffer†	H_2O	-	1.0185	348.1	1.101_3
Lipovitellin	buffer (H_2O)	I	1.0204	357.8	1.134_1
+	"	I x 3/5	1.0196	354.0	1.121_2
γ-livetin	"	I x 9/25	1.0192	351.3	1.112_2

TABLE VII (cont'd)

Sample	Solvent	Conc.	Density g./ml.	Flow-Time sec.	η cp.
Buffer	D_2O	-	1.1219	389.5	1.357_4
Lipovitellin	buffer (D_2O)	I	1.1235	397.5	1.387_3
+	"	I x 3/5	1.1229	394.0	1.374_3
γ-livetin	"	I x 9/25	1.1225	392.1	1.367_2
H_2O	-	-	0.9982	325.3	1.0087
0.2μ NaCl	H_2O	-	1.0076	329.0	1.0298
α- + β-	0.2μ NaCl(H_2O)	I	1.0103	354.4	1.1122
Livetin	"	I x 3/5	1.0092	344.3	1.0794
"	"	I x 9/25	1.0085	337.8	1.0582
"	"	I x 27/125	1.0082	334.5	1.0476
"	"	I x 27/250	1.0079	331.4	1.0376
0.2μ NaCl	D_2O	-	1.1117	367.5	1.2691
α- + β-	0.2μ NaCl(D_2O)	I	1.1141	392.7	1.3591
Livetin	"	I x 3/5	1.1131	382.4	1.3222
"	"	I x 9/25	1.1125	376.3	1.3005
"	"	I x 27/125	1.1122	373.1	1.2890
"	"	I x 27/250	1.1119	370.3	1.2790

* I = initial concentration

† Buffer = μ 0.3, pH or pD 9.0, plus 0.05M Mg SO_4

TABLE VIII

Sedimentation Experiments on Polyvinyl Alcohol Based on 4.00% Concentration

R.P.M.	CONC. g./100 ml.	Av. Temp. °C.	$s \times 10^{13}$ sec.	Viscosity of medium cp.	$\eta s \times 10^{13}$ corr. for conc. cp. sec.	Density of medium g./ml.	k	$\frac{\eta s}{k} \times 10^{13}$ cp. sec.	d/k g./ml.
52640	0.400	25.8	1.860	0.877	1.63	0.997	1.000	1.63	0.997
"	0.200	25.8	2.037	0.877	1.59	0.997	"	1.59	0.997
"	0.408	25.0	1.776	0.893	1.59	0.997	"	1.59	0.997
"	0.379	25.0	1.103	1.100	1.22	1.104	1.018	1.20	1.086
"	0.250	25.4	1.921	0.895	1.59	0.997	1.000	1.59	0.997
"	0.399	23.0	1.715	0.937	1.61	0.998	"	1.61	0.998
"	0.406	23.0	1.048	1.162	1.24	1.105	1.018	1.22	1.086
"	0.403	23.0	1.206	1.101	1.34	1.076	1.013	1.32	1.061
"	0.404	23.0	1.419	1.042	1.49	1.048	1.008	1.48	1.039
"	0.391	21.5	1.214	1.149	1.41	1.076	1.013	1.39	1.061
"	0.381	21.5	1.391	1.086	1.51	1.048	1.008	1.50	1.039
59780	0.369	24.5	1.092	1.114	1.22	1.104	1.018	1.20	1.085
"	0.381	24.5	1.727	0.904	1.55	0.997	1.000	1.55	0.997

TABLE IX

Sedimentation Experiments on Bovine Plasma Albumin
Based on 0.40% Concentration

R.P.M.	CONC. g./100 ml.	Av. Temp. °C.	s X 10^{13} sec.	Density of medium g./ml.	k	Viscosity of medium cp.	$\frac{\eta s}{k}$ x 10^{13} cp. sec.	d/k g./ml.
52640	0.400	26.4	4.87	1.005	1.000	0.881	4.29	1.005
"	0.400	26.4	4.96	1.005	"	0.881	4.37	"
"	0.397	25.4	4.71	1.005	"	0.902	4.25	"
"	0.397	25.4	4.75	1.005	"	0.902	4.29	"
"	0.411	22.0	2.63	1.113	1.015	1.218	3.15	1.098
"	0.408	24.8	4.70	1.006	1.000	0.915	4.30	1.006
"	0.400	24.8	2.86	1.112	1.015	1.129	3.18	1.097
"	0.384	24.0	3.25	1.084	1.011	1.086	3.49	1.072
"	0.377	24.0	3.68	1.057	1.008	1.032	3.78	1.049
59780	0.40	22.8	4.49	1.006	1.000	0.956	4.29	1.006
"	0.40	23.2	2.78	1.113	1.015	1.178	3.22	1.098
"	0.40	25.1	2.89	1.113	1.015	1.120	3.19	1.098

TABLE X

Calculations for Evaluating the Concentration Dependence of Polyvinyl Alcohol

Exp. No.	C. gm./100 cc.	Av. Temp. °C.	$S \times 10^{13}$ sec.	η of Medium cp.	$\eta S \times 10^{13}$ cp.sec.	$\frac{\eta S \times 10^{13}}{\eta_{20,w}}$	$\frac{1}{s_{20,w}} \times 10^{-13}$ sec.$^{-1}$
6	0.200	25.8	2.037	0.877	1.786	1.776	0.563
8	0.250	25.4	1.921	0.895	1.719	1.710	0.585
9	0.399	23.0	1.715	0.937	1.607	1.598	0.622
7	0.408	25.0	1.776	0.893	1.586	1.577	0.634

FIGURE 13

The Concentration Dependence of Polyvinyl Alcohol

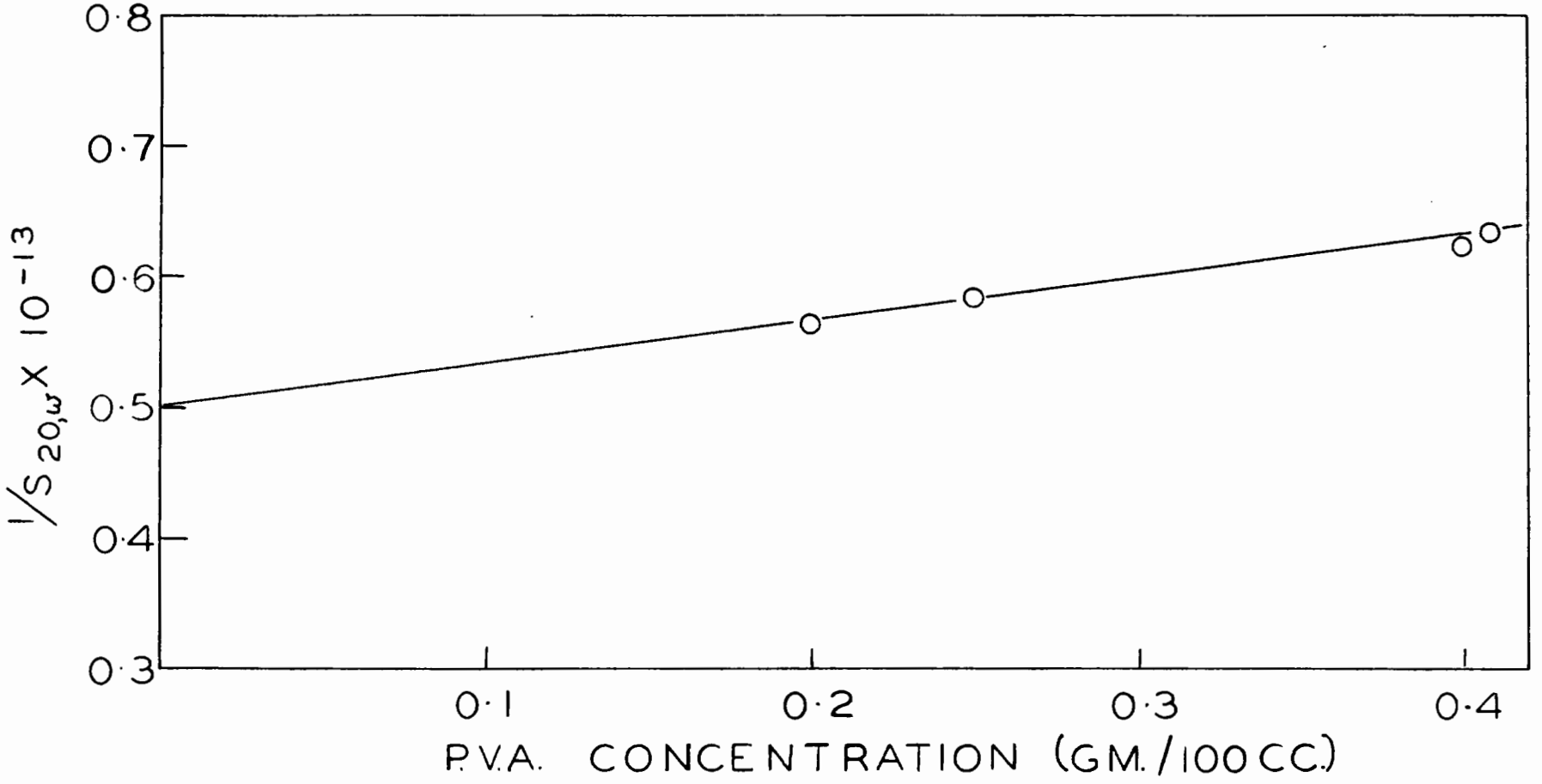
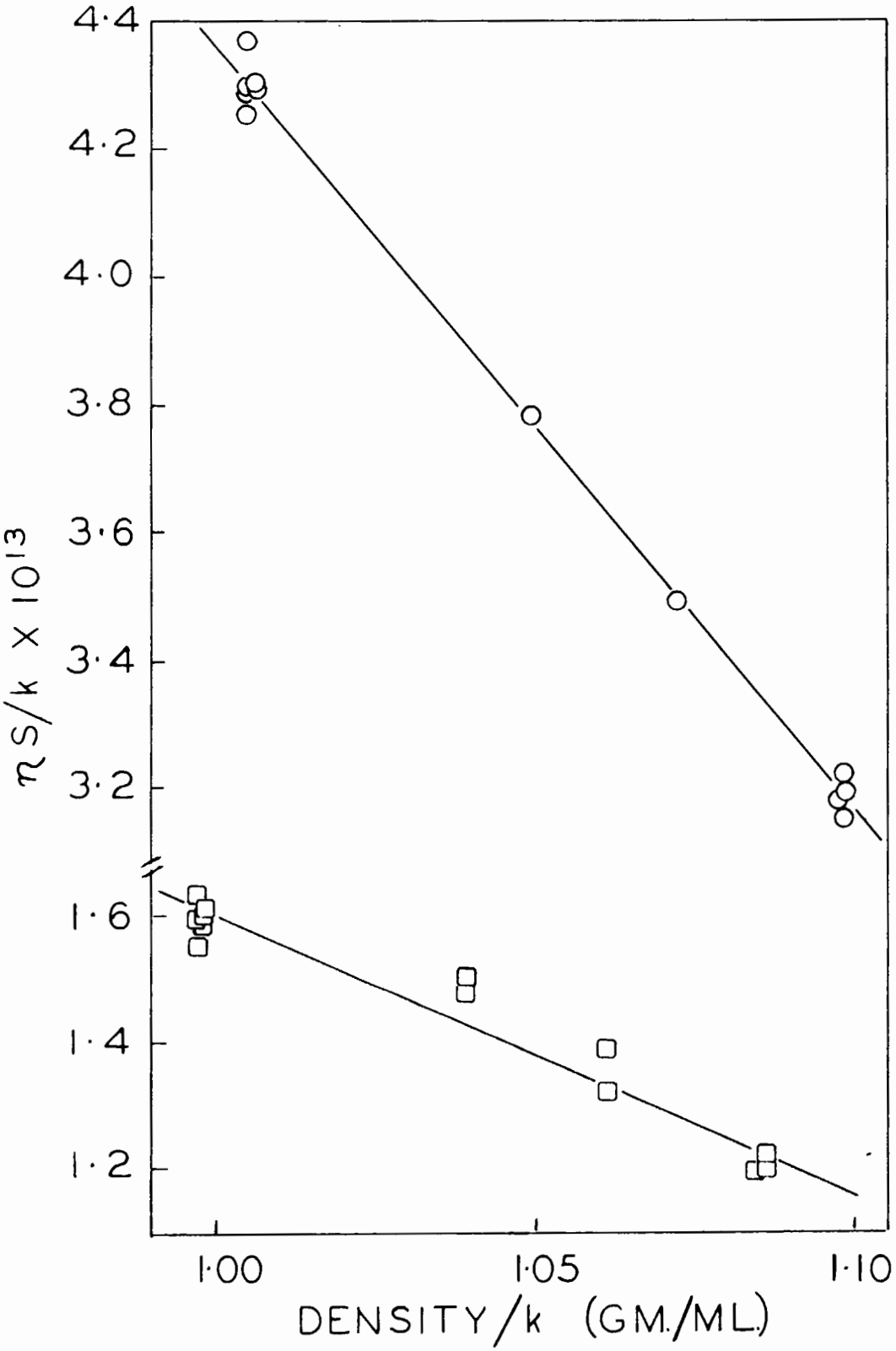

0·8
0·7
0·6
0·5
0·4
0·3
$1/S_{20,w} \times 10^{-13}$
0·1
0·2
0·3
0·4
P.V.A. CONCENTRATION (GM./100 CC.)

FIGURE 14

Relation Between $\eta s/k$ (Solvent) and d/k (Solvent) for Bovine Plasma Albumin and Polyvinyl Alcohol.

○ BPA

□ PVA


4·4
4·2
4·0
3·8
3·6
3·4
3·2
1·6
1·4
1·2
1·00
1·05
1·10
ηS/k X 10¹³
DENSITY/k (GM./ML.)

the method of least squares. The value of k was calculated from the % weight increase of BPA at 100% D_2O concentration from Figure 10 and the use of equation (46). The value of κ for PVA was calculated similarly from the spiral measurements. A linear relation is evident although with PVA the deviations of the points from the straight line are greater than for BPA. From the intercepts at the values of $\eta s/k$ equal to zero, where $\bar{V}$ equals the reciprocal of the intercept d/κ, $\bar{V}$ of BPA was 0.735 ± 0.003 and $\bar{V}$ of PVA was 0.735 ± 0.013.

Following the sedimentation experiments at fixed concentration of macromolecules and several densities of media, experiments were made to obtain sedimentation coefficients extrapolated to zero concentration at two values for the densities of the medium, so that equation (49) might be applied to calculate $\bar{V}$ values. The data for the experiments on PVA in 0.2μNaCl solution, AG_1,(sodium alginate), in 0.15μsodium phosphate - sodium chloride buffer at pH or pD 6.6, and BPA in 0.2μ NaCl are contained in Table XI. The results are presented by the curves of Figure 15 following least square analyses. The intercepts at zero concentration in aqueous medium for AG_I and BPA were calculated from published data (53, 69, 70). The data were corrected for the drop in rotor temperature with acceleration unless, as in some experiments, the temperature was controlled by a rotor temperature indicator and control unit. The experiments were made at approximately 25°C. and the

TABLE XI.

Calculation and Data for Sedimentation Coefficients Extrapolated to zero Concentration.

Substance	Conc. g./dl.	Temp. °C.	$s_{t,m}$ x 10^{13} sec.	η_t^m cp.	η_{25}^m cp.	$s_{25,m}$ x 10^{13} sec.	$\frac{1}{s_{25,m}}$ x 10^{-13} $sec.^{-1}$	d_{25}^m g./ml.
PVA	0.500	24.9	0.986	1.119	1.117	0.987	1.013	1.1126
"	0.417	24.4	0.988	1.133	"	1.003	0.997	"
"	0.240	24.1	1.082	1.143	"	1.108	0.904	"
"	0.209	25.3	1.102	1.109	"	1.093	0.915	"
"	0.166	25.2	1.136	1.111	"	1.131	0.884	"
"	0.427	25.0	1.607	0.912	0.912	1.607	0.622	1.0053
"	0.327	"	1.719	"	"	1.719	0.582	"
"	0.303	"	1.716	"	"	1.716	0.582	"
"	0.213	"	1.846	"	"	1.846	0.542	"
"	0.121	24.8	1.947	0.915	"	1.952	0.512	"
AC_1	0.299	25.0	1.282	1.124	1.124	1.282	0.779	1.1107
"	0.299	24.5	1.288	1.139	"	1.303	0.778	1.1107
"	0.222	24.8	1.452	1.130	"	1.462	0.684	1.1107
"	0.222	25.9	1.467	1.098	"	1.432	0.698	1.1107
"	0.199	26.6	1.552	1.079	"	1.488	0.673	1.1101
"	0.100	25.8	1.728	1.100	"	1.693	0.592	1.1103
"	0.100	23.1	1.603	1.182	"	1.686	0.593	1.1103
"	0.20	23.9	1.832	0.942	0.919	1.88	0.532	1.0041
"	"	23.4	1.836	0.944	0.919	1.88	0.532	1.0041
"	"	24.8	1.941	0.913	0.919	1.93	0.518	1.0041

TABLE XI. (contd.)

Calculation and Data for Sedimentation Coefficients Extrapoled to zero Concentration.

Substance	Conc. g./dl.	Temp. °C.	$s_{t,m}$ x 10^{13} sec.	η_t^m cp.	η_{25}^m cp.	$s_{25,m}$ x 10^{13} sec.	$\frac{1}{s_{25,m}}$ x 10^{-13} $sec.^{-1}$	d_{25}^m g./ml.
BPA	0.458	22.0	2.63	1.207	1.117	2.85	-	1.1126
"	0.445	23.2	2.78	1.170	"	2.91	-	"
"	0.445	25.1	2.89	1.114	"	2.89	-	"
"	0.445	24.8	2.86	1.123	"	2.87	-	"
"	0.256	25.0	2.92	1.117	"	2.92	-	"
"	0.185	25.0	2.92	1.117	"	2.92	-	"
"	0.108	25.2	2.96	1.109	"	2.94	-	"
"	0.400	26.4	4.87	0.881	0.912	4.71	-	1.0053
"	0.400	26.4	4.96	0.881	"	4.79	-	"
"	0.397	25.4	4.71	0.902	"	4.65	-	"
"	0.397	25.4	4.75	0.902	"	4.70	-	"
"	0.408	24.8	4.71	0.915	"	4.71	-	"
"	0.400	22.8	4.49	0.960	"	4.73	-	"
"	0.400	22.6	4.47	0.959	"	4.69	-	"

* m, and t denote experimental medium and temperature respectively.

FIGURE 15

Concentration Dependence of the Sedimentation Coefficients of Polyvinyl Alcohol, Sodium Alginate, and Bovine Plasma Albumin, in Aqueous and Heavy Water Media at 25°C.

■ PVA (0.2μ NaCl - D_2O)

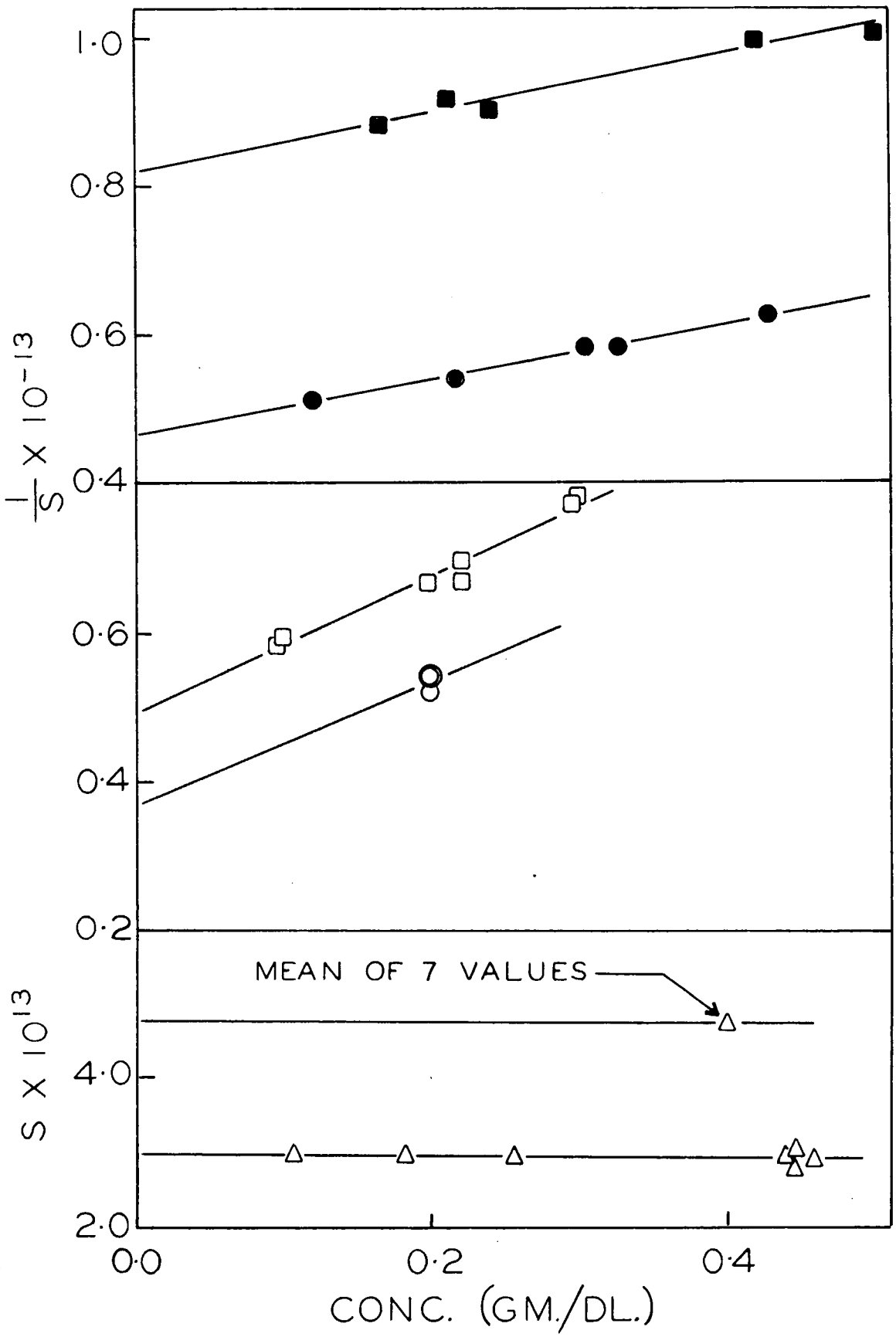
● PVA (0.2μ NaCl - H_2O)

□ AC_1 (0.15μ, pH 6.6, sodium phosphate - sodium chloride buffer - D_2O)

○ AC_1 (0.15μ, pD 6.6, sodium phosphate - sodium chloride buffer - H_2O)

▲ BPA (0.2μ NaCl - H_2O)

△ BPA (0.2μ NaCl - D_2O)


1·0
0·8
0·6
0·4
$\frac{1}{S} \times 10^{-13}$
0·6
0·4
0·2
MEAN OF 7 VALUES
S X 10^13
4·0
2·0
0·0
0·2
0·4
CONC. (GM./DL.)

sedimentation coefficients were corrected to 25°C. by multiplying by η_t/η_{25}, where η refers to the viscosity of the sedimentation medium (4).

The results of the above experiments are summarized in Table XII. The $\bar{V}$ values were computed by equation (49). The value of k for BPA and PVA were calculated from the measurements with the spiral balance. However, AC_1 was not susceptible to measurement under the vacuum conditions of the spiral balance and therefore k was calculated from the number of labile hydrogen atoms of each repeating unit of AC_1 (52) which is the sodium salt of mannuronic acid.

The viscosity and density data for protein mixtures are given in Table VII. The initial solutions of BPA + β-LG mixtures were 1.00% in concentration of each component, while with α- β- livetin, the concentration of the two components totalled 1.60% in the initial solution. The concentrations of the initial solutions of lipovitellin plus γ – livetin were not known because some precipitation occurred during the dialysis. However with the sedimentation method of estimating $\bar{V}$ only relative concentrations are necessary.

Sedimentation coefficients of the unresolved BPA + β-LG peaks, the resolved BPA and β-LG peaks, the resolved lipovitellin and γ-livetin peaks, and the unresolved peaks of and livetin are given in Table XIII. The data and calculation of $\bar{V}$ for each of these sedimenting species are also given in the table. The plots for sedimentation

TABLE XII

Data for Calculation of $\bar{V}_{25}$

		BPA (0.2 μ NaCl)		AC_1 (0.15 μ buffer)		PVA (0.2 μ NaCl)	
		H_2O	D_2O	H_2O	D_2O	H_2O	D_2O
$s^o_{25,m} \times 10^{13}$	sec.	4.78 ± 0.04	2.97 ± 0.03	2.76 ± 0.06	2.02 ± 0.05	2.14 ± 0.03	1.22 ± 0.03
$\eta_{25,m}$	cp.	0.912	1.117	0.919	1.124	0.912	1.117
$d_{25,m}$	g./ml.	1.005	1.113	1.004	1.111	1.005	1.113
k			1.016		1.010		1.018
$\bar{V}$ (equation (49)) ml./g.		0.734 ± 0.008		0.546 ± 0.063		0.778 ± 0.011	

TABLE XIII

$\bar{V}$ Calculations from Protein Mixtures

Substance	BPA +β-LG	BPA	β-LG	Lipovitellin	γ-Livetin	γ- + β-Livetin
s_{20},m, H_2O, I*	---	4.10	2.64	9.59	6.05	3.14
s_{20},m, H_2O, I x $\frac{3}{5}$	3.70	4.26	2.73	9.63	6.07	3.32
s_{20},m, H_2O, I x $\frac{9}{25}$	3.69	4.22	2.78	9.67	6.29	3.49
s_{20},m, H_2O, I x $\frac{27}{125}$	3.67	4.32	2.65	---	---	3.70
s_{20},m, H_2O, I x $\frac{27}{250}$	---	---	---	---	---	3.70
s_{20},m, H_2O	3.66 ± 0.01	4.36 ± 0.05	2.74 ± 0.08	9.71 ± 0.01	6.36 ± 0.16	3.77 ± 0.05
s_{20},m, D_2O, I	2.09	2.65	1.66	5.18	3.28	2.12
s_{20},m, D_2O, I x $\frac{3}{5}$	2.17	2.62	1.60	5.24	3.12	2.22
s_{20},m, D_2O, I x $\frac{9}{25}$	2.14	2.75	1.63	5.26	3.39	2.28
s_{20},m, D_2O, I x $\frac{27}{125}$	2.25	2.73	1.69	---	---	2.30
s_{20},m, D_2O, I x $\frac{27}{250}$	---	---	---	---	---	2.34
s_{20},m, D_2O	2.25 ± 0.05	2.76 ± 0.06	1.66 ± 0.05	5.31 ± 0.01	3.34 ± 0.29	2.36 ± 0.01
η_{20},m, H_2O, cp.	1.026	1.026	1.026	1.101	1.101	1.030
η_{20},m, D_2O, cp.	1.259	1.259	1.259	1.357	1.357	1.269
d_{20},m, H_2O, g./ml.	1.006	1.006	1.006	1.019	1.019	1.008
d_{20},m, D_2O, g./ml.	1.112	1.112	1.112	1.122	1.122	1.112
k	1.015	1.016	1.015	1.013	1.013	1.013
$\bar{V}_{20}$ ml./g.	0.738 ± 0.013	0.724 ± 0.019	0.746 ± 0.033	0.779 ± 0.003	0.791 ± 0.029	0.722 ± 0.016

*I = initial concentration, assumed = 1.000
s is in Svedberg units (10^{-13} secs.)

coefficients against relative concentrations are shown in Figure 16; the lines are drawn by the least squares method. The value of k for β-LG was calculated from the amino acid analysis of the protein (20), while k was computed for the other materials on the basis of the measurements with the spiral balance (Table IV). $\bar{V}_{20}$ was calculated with the aid of equation (49) and the errors by equation (51).

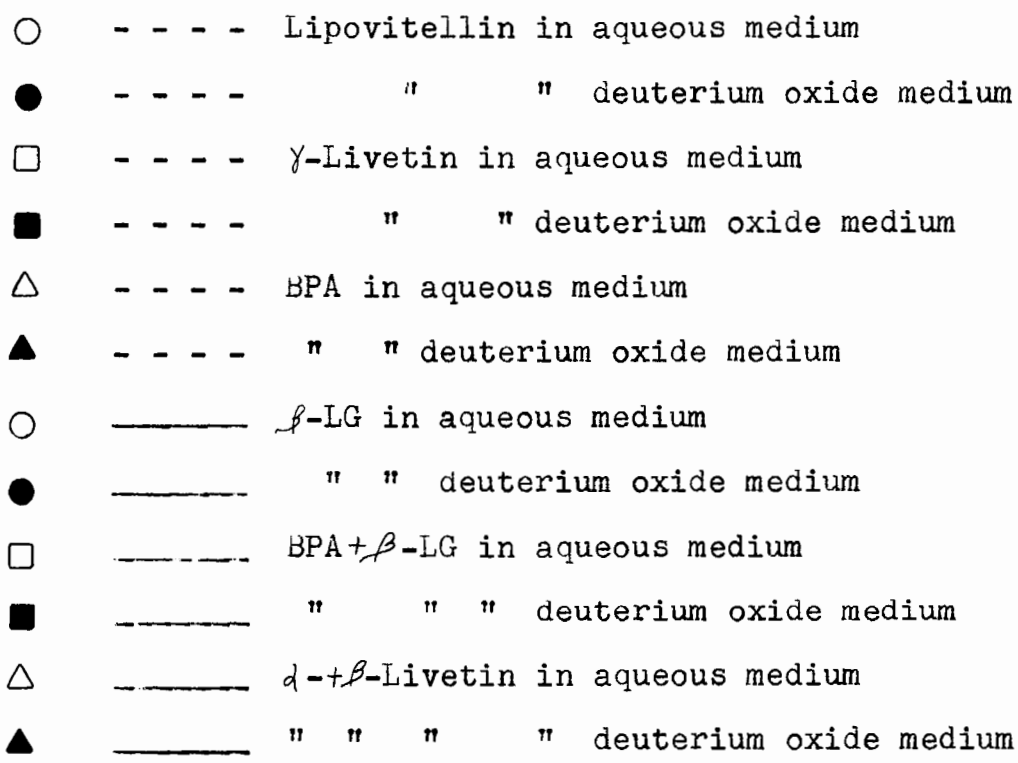
DETERMINATION OF $\bar{V}$ WITH THE MAGNETIC FLOAT.

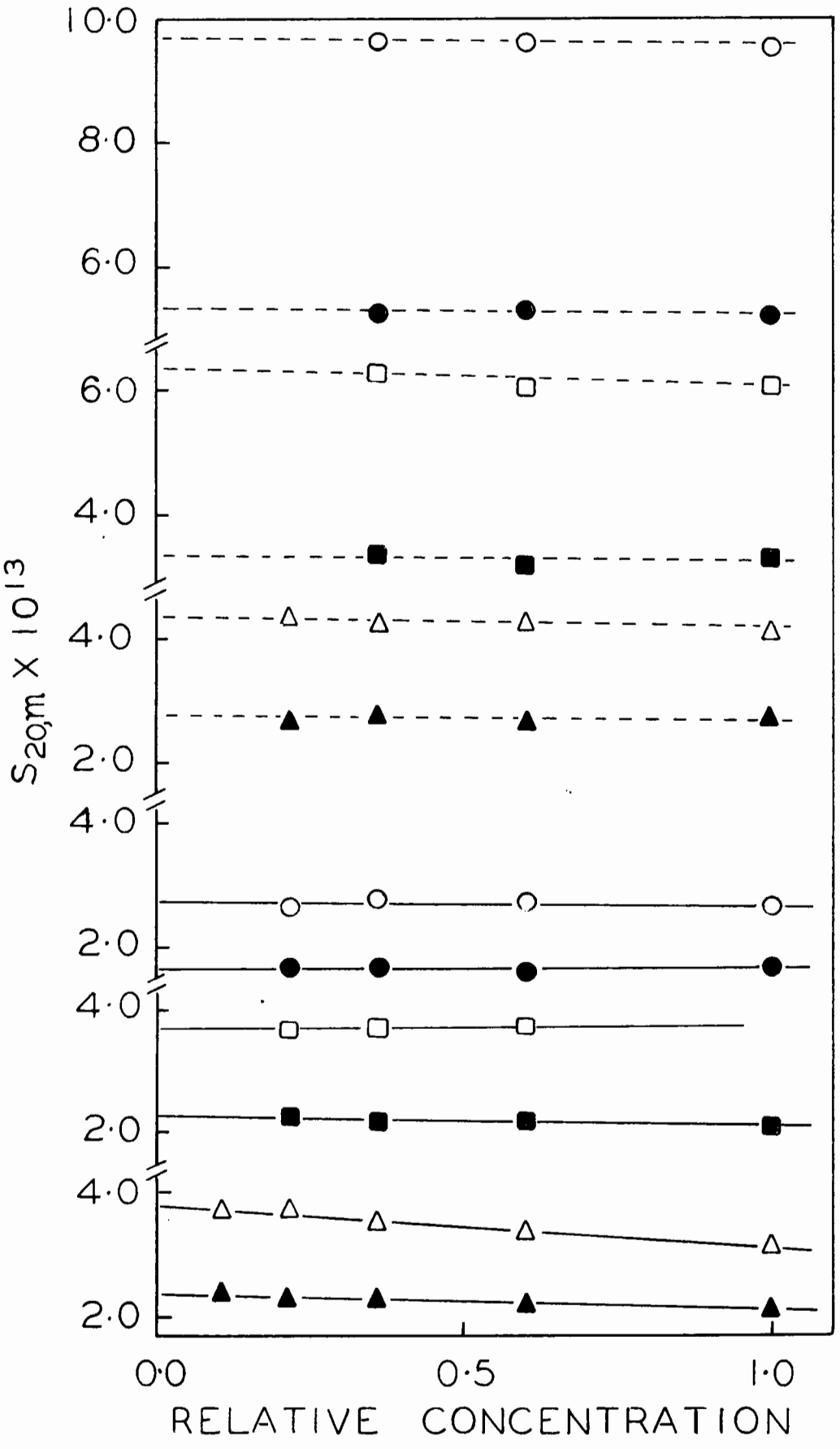
The performance of the float apparatus was tested with potassium chloride solutions made from specially distilled water and recrystallized potassium chloride. The density of the pure water was measured in the cell and then stock salt solution was added to the solvent. The concentrations of the potassium chloride solutions were calculated on the basis of the dry weight of samples of the stock solutions at 105°C. in vacuo. The densities of 0.4481 and 0.5965% K Cl solution at 25.000°C. were 0.999907 and 1.000845 g./ml. respectively. These values agree within 1 p.p.m. with the values calculated from the data of MacInnes and Dayhoff (71).

The procedure outlined in the experimental section was checked by an experiment in which deuterium oxide from the same stock was placed inside a dialysis membrane and in the outer vessel enclosing the membrane. The D_2O inside the dialysis membrane was treated in the same manner as other experimental solutions containing D_2O solvent. The D_2O

FIGURE 16

Relation Between Sedimentation Coefficients and Concentration at 20°C.




10·0
8·0
6·0
6·0
4·0
4·0
2·0
4·0
2·0
4·0
2·0
4·0
2·0
$S_{20,m} \times 10^{13}$
0·0
0·5
1·0
RELATIVE CONCENTRATION

external to the dialysis membrane was put into the float cell from a weight burette and then additions to the cell of the D_2O from inside the membrane were made from a smaller weight burette. Measurements made on these solutions failed to detect any change in the extrapolated value of $^1/t$ against mv. The D_2O was poured from the cell through a funnel into a large weight burette and then returned to the cell as in the regular procedure. Each time this process was repeated the extrapolated value rose by 4 mv. or the equivalent of about 15 p.p.m. This rise in mv. value is due to the decrease in the density of the D_2O due to the exposure of large liquid surfaces to the moisture of the atmosphere. This result showed the necessity of protecting solutions containing D_2O from exposure to the atmosphere. It may also be added that exposure of the float to solutions of D_2O for more than twenty hours had no effect on the mv. readings.

Before presenting the results of the float measurements, it is necessary to consider the data obtained from dry weight measurements. With the exception of glycine and AC_1, constant dry weights were obtained within 48 hours from aqueous solutions at 105°C. in vacuo. With AC_1, the sample weights approached an asymptotic value after 20 days at 105°C. in vacuo. Concentrations derived from these dry weights were within 2% of the values computed from differential refractometer measurements, employing 1.52×10^{-3} g./dl. (53) for the specific refractive index increment of sodium alginate.

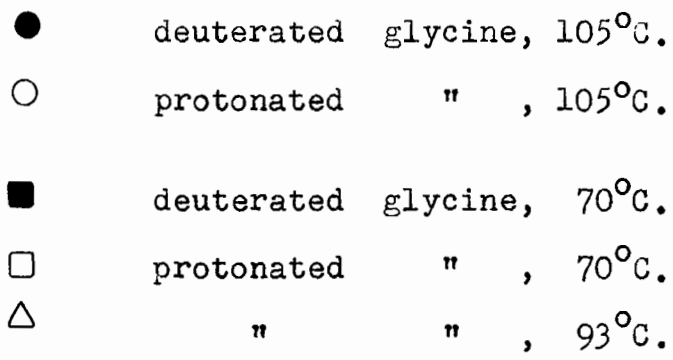
concentrations of AC_1 solutions were therefore determined from refractive index measurements.

In the first three experiments with glycine in the float apparatus, samples were dried at 105°C. in vacuo. From the results obtained with the spiral balance (Figure 11), it can be seen that constant weight was achieved before 48 hours at temperatures below 95°C., while constant weight was not realized at temperatures greater than 94°C. The upper curve of Figure 17 shows the mean decrease in weight of three glycine samples in the deuterated and protonated state at 105°C. in vacuo. The lower curve shows the mean decrease in weight of three glycine samples in which the first four points represent drying at 70°C., and the remaining four points 93°C., all in vacuo. Figure 18 shows the relation between the mean sample weight of three protonated glycine samples in three experiments, and drying time at 105°C. in vacuo. The dry sample weights applicable to the computation of glycine solution concentrations were obtained by extrapolation of the curves of sample weight against time to zero time. In those samples which had been dried in the deuterated state and then in the protonated state, a correction was applied to the protonated weights for the weight loss while drying in the deuterated state.

From the specific refractive index increment equation of Lyons (72) at 25°C. and 5462Å and the dispersion equation of Perlmann and Longsworth (73), the specific refractive index increment of glycine at 25°C. and 5780Å

FIGURE 17

Relation Between Sample Weight and Drying Time of Glycine in Vacuum



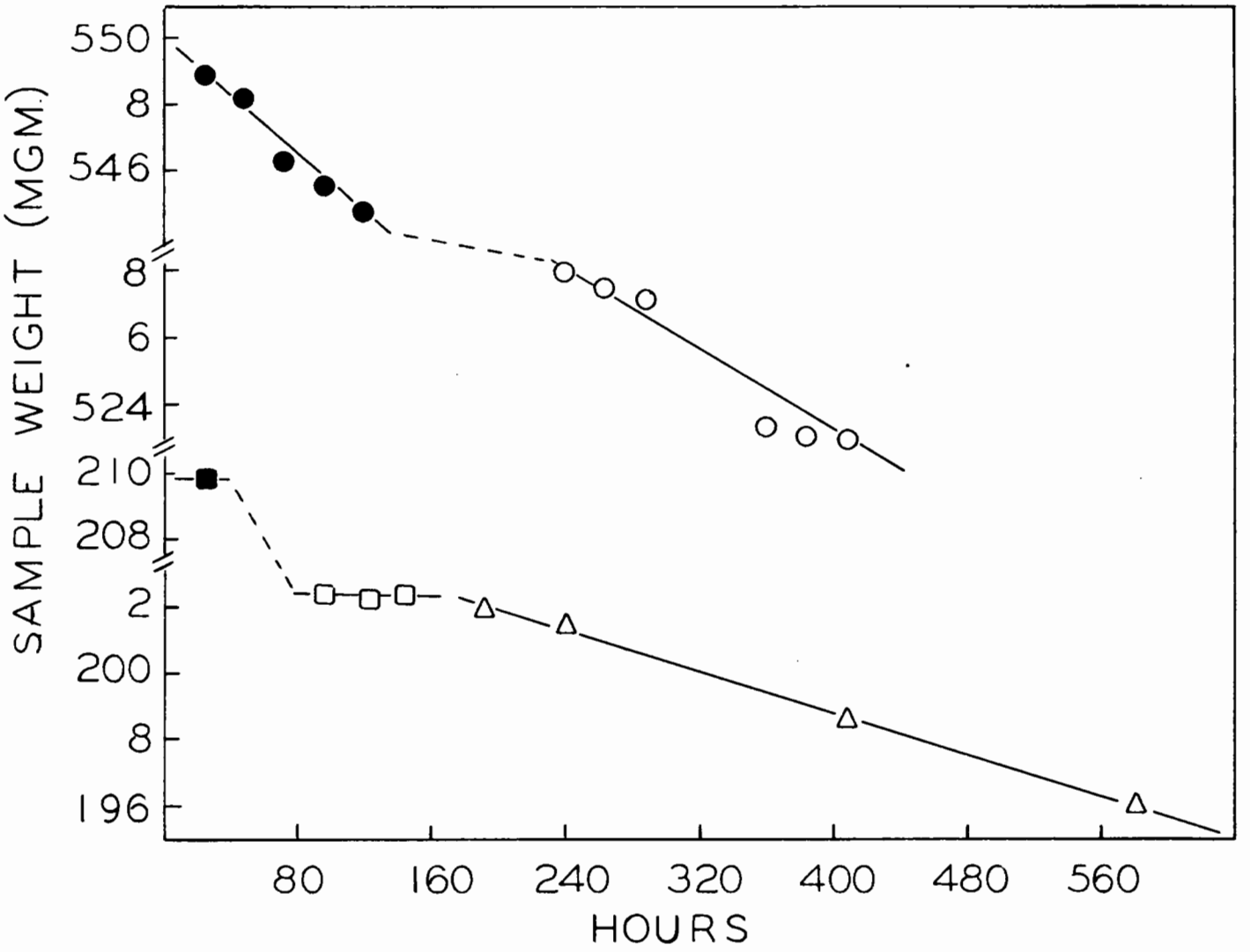
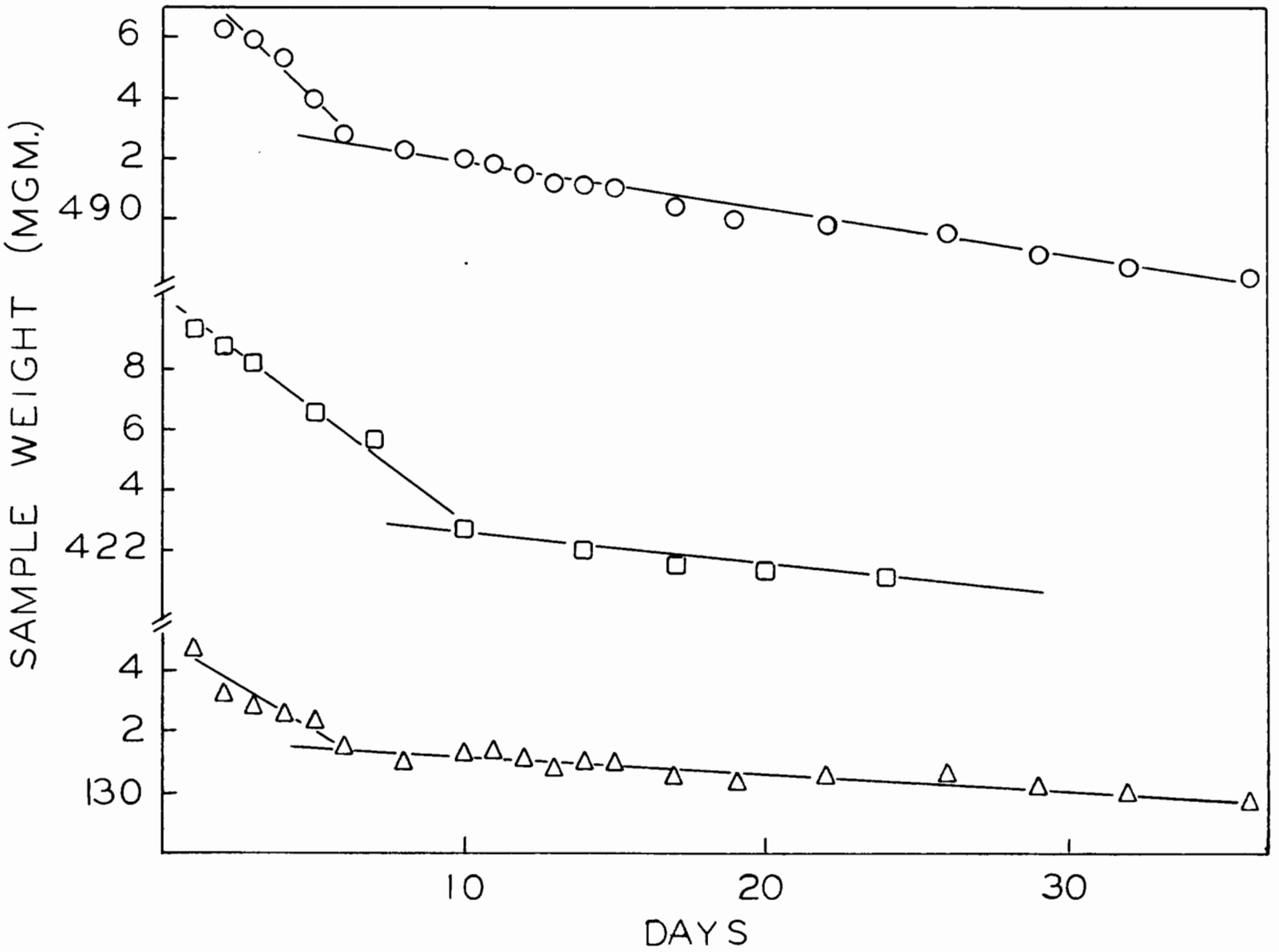

SAMPLE WEIGHT (MGM.)
550
8
546
8
6
524
210
208
2
200
8
196
80
160
240
320
400
480
560
HOURS

FIGURE 18

Relation Between Sample Weight and Drying Time of Protonated Glycine at 105°C. *in Vacuo*


SAMPLE WEIGHT (MGM.)
6
4
2
490
8
6
4
422
4
2
130
10
20
30
DAYS

may be expressed as:

$$(\Delta n/\Delta c)_{5780} = (1813.5 - 11.4c)(0.940 + 2.00 \times 10^{6}/5462^{2})^{-1} \times 10^{-6} \text{ g./dl.} \quad \ldots\ldots\ldots(56)$$

where c is the concentration in g./dl. The refractive index increments of the glycine solutions from experiments with the float apparatus were determined at 5780Å and 25°C. as a secondary check of the concentrations. The concentrations in g./dl. were easily converted to g./g. of solution from the densities of the solutions obtained from the $\bar{V}$ measurements. The good agreement of the two methods of determining the concentration is shown by the data of Table XIV. The concentrations based on dry weight were used in calculating $\bar{V}$.

The data obtained from measurements with the float apparatus are given in Tables XV to XIX. Since ϕ of amino acids and peptides are concentration dependent (19) the data on glycine and TGG were extrapolated to zero concentration. The curves of ϕ versus concentration, as shown in Figure 19, were drawn in accordance with least squares analysis. The value of ϕ of TGG at the lowest concentration in D_2O and H_2O were not used in the calculation because of the uncertainty of these low concentrations. The values of ϕ for BPA, PVA, and AC_1 do not show concentration dependence within the error of measurement. Perusal of equations (18) and (22) shows that $\phi = \bar{V}$ if ϕ does not change with concentration or if concentration is zero. The values of $\bar{V}$ are given in Table XX. The theoretical values are based upon complete exchange of labile hydrogen atoms in concentrated deuterium oxide media.

TABLE XIV

Glycine Concentration Data

Expt.	Stock Conc.g./g.of solution Dry Weight			Final Cell Conc.g./g.of solution Dry Weight		
	$\Delta n/\Delta c$	Measured	Calculated	$\Delta n/\Delta c$	Measured	Calculated
O	-	0.09644	0.09728	0.0272	-	0.02741
T	-	0.09636	0.09640	0.0270	0.02669	0.02666
U	-	0.07914	0.07930	0.0169	-	0.01694
X	0.0375	0.03748	0.03757	0.00693	-	0.00698

TABLE XV

Float Data and Calculations of Ø of Bovine Plasma Albumin at 25°C.

Expt.	Quantity		Conc. 0	Conc. 1	Conc. 2	Conc. 3
D	d	g./ml.	1.108314	1.108704	1.109111	1.109633
	Conc.	g./ml.	(99.3%D_2O	0.001832_2	0.003711_1	0.006132_2
	Ø	ml./g.	0.1NKCl)	0.729_0	0.727_5	0.727_4
E	d	g./ml.	1.108632	1.109016	1.109350	1.109889
	Conc.	g./ml.	(99.3%D_2O	0.001793	0.003323	0.005835
	Ø	ml./g.	0.1NKCl)	0.727_9	0.726_4	0.727_1
J	d	g./ml.	0.997090	0.998209	0.999004	0.999848
	Conc.	g./ml.	(0.0 %	0.004182	0.007146	0.010301
	Ø	ml./g.	D_2O)	0.734_1	0.734_1	0.734_4
K	d	g./ml.	1.101759	1.102304	1.102817	1.103828
	Conc.	g./ml.	(97.4 %	0.002530	0.005250	0.009505
	Ø	ml./g.	D_2O)	0.730_5	0.729_4	0.728_7
Q	d	g./ml.	1.075061	1.075466	1.075953	1.076729
	Conc.	g./ml.	(72.6 %	0.001783	0.003876	0.007247
	Ø	ml./g.	D_2O)	0.733_3	0.731_0	0.731_2

TABLE XVI

Float Data and Calculation of ϕ of Polyvinyl Alcohol at 25°C.

Expt.	Quantity	Conc. 0	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5	Conc. 6
H	d g./ml.	0.997074	0.997730	0.998092	0.998548	---	---	---
	conc. g./g.	(0.0% D_2O)	0.00277_4	0.00432_0	0.00626_0	---	---	---
	ϕ ml./g.		0.765_3	0.766_0	0.766_4	---	---	---
I	d g./ml.	1.102150	1.102389	1.102705	1.102993	---	---	---
	conc. g./g.	(97.8% D_2O)	0.001346	0.003056	0.004642	---	---	---
	ϕ ml./g.		0.760_8	0.757_7	0.757_9	---	---	---
L	d g./ml.	1.068490	1.068990	1.069478	1.070112	---	---	---
	conc. g./g.	(66.4% D_2O)	0.002420	0.004813	0.007878	---	---	---
	ϕ ml./g.		0.755_2	0.756_3	0.756_0	---	---	---
P	d g./ml.	1.102321	1.102623	1.102973	1.103531	---	---	---
	conc. g./g.	(98.0% D_2O)	0.001626	0.003559	0.006595	---	---	---
	ϕ ml./g.		0.754_2	0.756_3	0.756_3	---	---	---
W	d g./ml.	1.101285	1.101395	1.101588	1.101755	1.101938	1.102089	1.102215
	conc. g./g.	(97.0% D_2O)	0.000568	0.001590	0.002482	0.003466	0.004274	0.004937
	ϕ ml./g.		0.748_2	0.750_9	0.752_2	0.752_9	0.752_8	0.752_8

TABLE XVII

Float Data and Calculation of ø of Triglycylglycine at 25°C.

Expt.	Quantity		Conc. 0	Conc. 1	Conc. 2	Conc. 3	Conc. 4
R	d	g./ml.	1.106243	1.106464	1.106696	1.106926	1.107195
	Conc.	g./g.	(99.1% D_2O)	0.00055_5	0.00111_2	0.00167_1	0.00230_3
	ø	ml./g.		0.57_9	0.571_0	0.570_0	0.566_5
S	d	g./ml.	0.999814	1.000020	1.000250	1.000508	1.000769
	Conc.	g./g.	(0.0 % D_2O)	0.00050_2	0.00106_9	0.00171_2	0.00235_9
	ø	ml./g.		0.59_1	0.592_9	0.595_1	0.595_8

TABLE XVIII

Float Data and Calculation of ø of Sodium Alginate at 25°C.

Expt.	Quantity		Conc. 0	Conc. 1	Conc. 2	Conc. 3
A	d	g./ml.	1.004180	1.004447	1.004738	1.005005
	conc.	g./g.	(aqueous	0.00051_1	0.00108	0.00158
	ø	ml./g.	buffer)	0.478	0.483	0.480

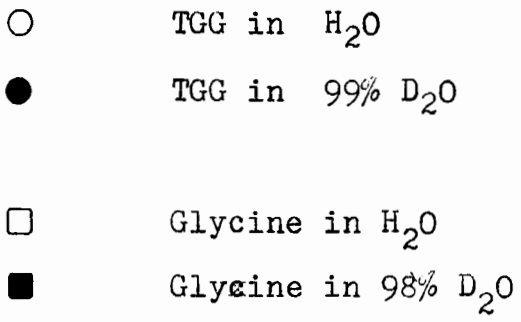
TABLE XIX

Float Data and Calculation of Ø of Glycine at 25°C.

Expt.	Quantity	Conc. 0	Conc. 1	Conc. 2	Conc. 3	Conc. 4	Conc. 5
0	d g./ml.	1.102936	1.106572	1.109122	1.111852	1.114941	---
	conc. g./g.	(98.5% D_2O)	0.008277	0.01413	0.02032	0.02741	---
	Ø ml./g.		0.5467	0.5488	0.5489	0.5505	---
T	d g./ml.	0.997084	0.998759	1.000845	1.003141	1.005730	1.008539
	conc. g./g.	(0.0% D_2O)	0.003942	0.008815	0.01408	0.02013	0.02666
	Ø ml./g.		0.5764	0.5754	0.5728	0.5746	0.5757
U	d g./ml.	1.103112	1.104348	1.05722	1.107375	1.108945	1.110603
	conc. g./g.	(98.7% D_2O)	0.002825	0.005932	0.009620	0.01318	0.01694
	Ø ml./g.		0.5473	0.5458	0.5437	0.5446	0.5456
X	d g./ml.	1.101917	1.102974	1.103603	1.104395	1.104977	---
	conc. g./g.	(97.6% D_2O)	0.002402	0.003820	0.005634	0.006975	---
	Ø ml./g.		0.5456	0.5446	0.5460	0.5472	---

FIGURE 19

The Relation Between ϕ and Concentration of Triglycylglycine and Glycine at 25°C.



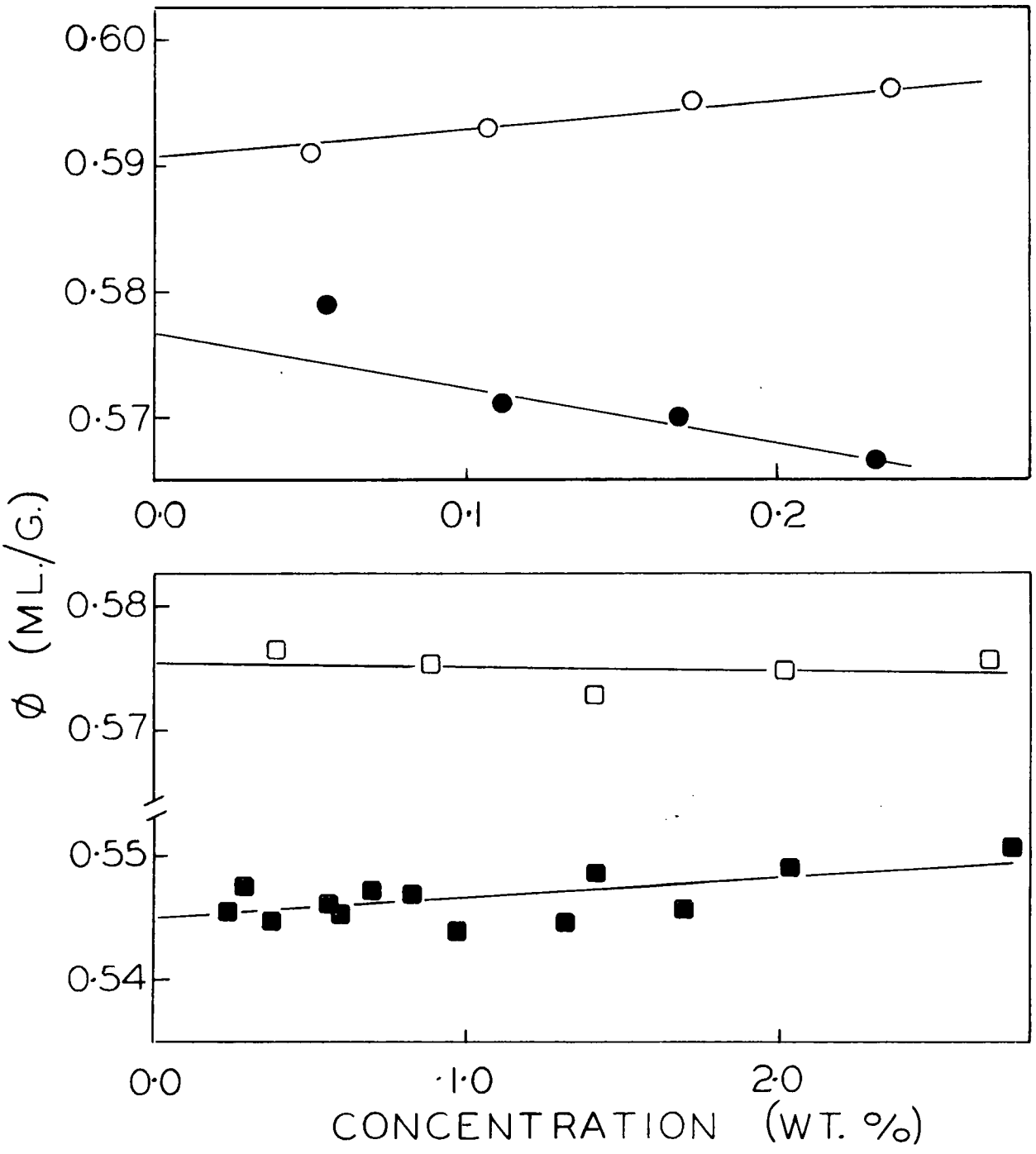

0·60
0·59
0·58
0·57
0·0
0·1
0·2
0·58
0·57
0·55
0·54
0·0
·1·0
2·0
Ø (ML./G.)
CONCENTRATION (WT. %)

TABLE XX

$\bar{V}_{25}$ Calculations from Float Apparatus Measurements

	BPA	PVA	AC_1
$\bar{V}_P \times 10^3$ ml./g.	734.2±0.2	765.9±0.6	481 ± 3
$\bar{V}_D \times 10^3$ "	728.2±1.3	754.4±3.5	----
$\bar{V}_P \times 10^3$ (literature)	734.9(13)	765 (51)* 748±5(50)*	440 ± 10(53)
$(-\Delta\bar{V}/\bar{V}_P) \times 10^3$ (measured)	8.2 ± 1.3	15.0±3.5	----
$(-\Delta\bar{V}/\bar{V}_P) \times 10^3$ (theory)	15.5	17.9	10.0

	Glycine	TGG
$\bar{V}_P \times 10^3$ ml./g.	575.4±1.5	590.7± 1.2
$\bar{V}_D \times 10^3$ "	544.9±0.8	576.6± 1.9
$\bar{V}_P \times 10^3$ (Literature)	575.5(74)	----
$(-\Delta\bar{V}/\bar{V}_P) \times 10^3$ (Measured)	53 ± 3	24 ± 3
$(-\Delta\bar{V}/\bar{V}_P) \times 10^3$ (Theory)	40	24

* 100% in the hydroxyl form

subscripts P and D refer to protonated and deuterated.

DISCUSSION

As shown in Figure 10 and Table IV, the measurements with the spiral balance on materials of known compositions and functional groups (BPA, PVA, glycine), indicated that all labile hydrogen atoms in such material would exchange with deuterium atoms in 100% D_2O solution at 25°C. within 20 hours. It was also shown that the % exchange of labile hydrogen atoms of BPA with deuterium atoms was a linear function of the volume % D_2O present. Lipovitellin, which has a lipid content of about 20% (58), and the livetins were shown to exchange labile hydrogen in deuterium oxide solution to about the same extent as proteins.

The weight increases that occur upon exchange of labile hydrogen atoms with deuterium atoms are accompanied by changes in $\bar{V}$ as shown by the data in Table XX. The experimentally determined decreases in $\bar{V}$ that accompany deuteration of PVA, glycine, and TGG were in reasonable agreement with the values calculated on the assumption that the volume occupied by deuterium atoms in a material is similar to that of the hydrogen atoms which they displaced. The experimental value for the decrease in $\bar{V}$ of BPA in D_2O solution was less than the theoretical value, although the spiral balance measurements indicated that complete exchange had occurred.

The errors in density measurements with a float apparatus similar to the one used in this work have been

discussed by MacInnes et al. (11). It should be possible to attain a precision of better than 1 p.p.m. in density measurements with dilute solutions. This was borne out by the density values for potassium chloride solutions which agreed within 1 p.p.m. with the measurements of MacInnes and Dayhoff. An additional error, that might have been involved in the present study, could arise from undetected splashing of stock solution on the inner cell walls of the float apparatus when additions of concentrated stock solutions are made with weight burettes.

To consider the effect on the calculation of $\overline{V}$ of errors in density measurements, equation (17) may be transformed and differentiated as follows:

$$\phi = \left[1 - \Delta d/n(d_1+\Delta d)\right] /d_1 \qquad \ldots\ldots\ldots(57)$$

where Δd is equal to $d_{1,2} - d_1$ and n is the weight fraction of the solute.

$$\partial\phi /\partial\Delta d = -1/n(d_1+\Delta d)^2 \approx -1/n$$

$$\Delta \phi \approx -\Delta d/n \qquad \ldots\ldots\ldots(58)$$

Thus, an error in Δd of 1 p.p.m. would cause changes in ϕ to the extent of 1 p.p. 10000 and 1 p.p. 1000 at solute concentrations of 1.0 and 0.1% respectively. Error from this source would be much more likely to occur with deuterium oxide solutions than with aqueous solutions since there is the possibility of exchange of deuterium atoms with hydrogen atoms present in the moisture of the atmosphere or on the glassware. Gross errors from this source were not observed in the measurements of ϕ,

but the possibility exists that the measurements in D_2O might be biased to some extent.

The effect of concentration errors on the evaluation of ϕ may also be estimated by the differentiation of equation (57):

$$\partial\phi / \partial n = -\Delta d/n^2 d_1 d_{1,2}$$

$$\Delta \phi = - (\phi - 1/d_1)\Delta n/n \qquad \text{.....(59)}$$

Therefore it is the relative error in the determination of n which is significant. Since errors in measurements with the weight burettes are negligible, errors in concentrations determined for the stock solutions are of major significance. To illustrate the errors that might enter, a relative error of 1% in concentration at a solution density of 1.1 g./ml. and $\phi \approx 0.75$ ml./g. would bring about a change in ϕ of 0.0016 ml./g. Similarly with ϕ 0.55, or 0.45 ml./g., ϕ would change 0.0036 and 0.0046 ml./g. respectively. An error in concentration of the stock solution might account for the experimental value of $-\Delta\bar{V}/\bar{V}_p$ of glycine being about 20% greater than the calculated value. The concentrations of the stock solutions were checked by evaporation of known weights of the solutions and drying the residues and the difficulty of drying glycine to constant weight was amply illustrated in Figures 11, 17, 18. When glycine was heated in vacuo on the spiral balance the equilibrium dry weight changed with each increment in temperature until at 95°C. a sublimate formed. Brown (75) has shown that glycine will sublime at 160°C. at about 15 mm. Hg. With the vacuum of

about 10^{-5} mm. Hg in the spiral balance it is very likely that the sublimation temperature of glycine was exceeded. The situation is probably complicated by factors other than sublimation, since Curtius and Benrath (76) have shown that glycine heated without a solvent may give rise to a mixture of glycine peptides and glycine anhydride.

Before discussing the evaluation of $\bar{V}$ by the sedimentation method, examination of Figure 14, shows that the relation of $\eta s/d$ (solvent) and d/k (solvent) is essentially linear over the range of solvent density which could be used. However, this range is very limited. Possibly the addition of salt or other additive to aqueous and deuterium oxide media to increase the density of the media would permit interpolation of the solvent density at zero sedimentation rate. However, this would probably give rise to preferential adsorption of portions of the media by, and result in change of, the sedimenting entity.

A compilation of $\bar{V}$ values has been made in Table XXI with a view to critical evaluation of the sedimentation method of obtaining $\bar{V}$. The major source of error in the evaluation of $\bar{V}$ by the sedimentation method lies in the determination of the sedimentation coefficients.

Errors due to measurement of solvent densities are negligible since densities are readily measured to better than 1 part per 1000. It is possible that the solvent viscosities might be in error to the extent of about 1 or 2 parts per 1000,

but calculations show that errors in viscosity of this magnitude have an insignificant effect on $\bar{V}$ for the material investigated in this work.

In the sedimentation experiments in which only one macromolecular species was present, the values obtained for $\bar{V}$ of BPA and PVA agreed within the experimental error with the values obtained from measurements with the float apparatus. However, the values recorded for $\bar{V}$ of BPA by the sedimentation method are calculated on the basis that the volumes occupied by deuterium and hydrogen atoms in the macromolecule are equal. In fact, this is probably not true and it might be that deuterium atoms contribute a slightly larger volume than the hydrogen atoms they displace in BPA. Correction for such an effect would slightly diminish the value of $\bar{V}$ obtained by sedimentation for this protein. $\bar{V}$ of AC_1, obtained by sedimentation, has a large error owing to the difficulty in measuring the sedimentation coefficients and also to the small difference in the rate of sedimentation in aqueous and deuterium oxide solution.

In the experiments in which two sedimenting macromolecular species were present, the values of $\bar{V}$ obtained for BPA and β-LG. agreed within the experimental error with the values obtained with the float apparatus, and also with published values (Table XXI). A mixture of macromolecules such as BPA+β-LG ($s^{o}_{20,w}$ 4.4 and 2.8 Svedbergs) probably represents the limit of the sedimentation method for evaluating

TABLE XXI

Compilation of $\bar{V}$ Values*

$\bar{V}$ ml./g. x 10^2 / Technique		BPA	PVA	AC_1
Float Apparatus,	25°C.	73.42±0.02	76.6±0.1	48.1±0.3
Sed.(c ⟶ o)	"	73.4 ±0.1	78 ± 1	55 ± 6
(2 macromolecules Sed. c ⟶ o)	20°C.	72 ± 2		
Calcd. from Composition,	20°C.	73.4(21)	75	40
Literature,	20-25°C.	73.4_9(13)	76.5 (51)† 74.8±0.5(50)†	44 ± 1(53)

		β-LG	Lipovitellin	γ-Livetin	α- +β-Livetin
Float Apparatus,	25°C.			72.6±0.1(56)	
Sed.(c ⟶ o)	"				
(2 macromolecules Sed. c ⟶ o)	20°C.	75 ± 3	77.9±0.3	79 ± 3	72 ± 2
Calcd. from Composition,	20°C.	74.6 (21)			
Literature,	20-25°C.	75.1 (21)			

*applicable to protonated macromolecules

†100% in the hydroxyl form

$\bar{V}$ with the ultracentrifuge available, since there is just enough resolution to separate molecules of their sedimentation rates. The α - and β - components of livetin ($s^{o}_{20,w}$ 4.4 and 3.0 Svedbergs) were not resolved sufficiently to calculate their individual partial specific volumes. Lipovitellin, on the other hand, was very well resolved from γ-livetin. $\bar{V}$ of lipovitellin by the sedimentation method had a small standard error and agreed very well with the value obtained with the float apparatus (58). The sedimentation method is much more accurate for macromolecules of high sedimentation rates than for ones of low sedimentation rates. Although the sedimentation results with γ- livetin have been included in Table XXI, inspection of Figure 2, shows that this component was present in very small amount, and large errors were introduced into the evaluation of its $\bar{V}$. It should be mentioned also that the partial specific volume of 0.726 ml./g. reported previously was obtained on samples that had been treated with organic solvents to remove lipid (56) while in the present study the macromolecules were probably associated with about 10% lipid. The effect whereby the rates of sedimentation of the slower components of a mixture are increased and those of the faster components decreased, is known as the Ogston-Johnston effect (77). No account of this effect has been taken in the sedimentation experiments on mixtures, since the concentrations of solutes were low and extrapolated to zero concentration. In any case, such effects should be similar in aqueous and deuterium oxide solutions.

It is worth mentioning, perhaps, that values of $\bar{V}$ are obtainable by sedimentation measurements without recourse to knowledge of the solute concentration. In fact, the evaluations of $\bar{V}$ from the experiments on mixed solutes were made on the basis of relative concentrations only. It might be noted also that $\bar{V}$ can be obtained by sedimentation with as little as 1 ml. of 1% solute concentration.

It was pointed out in the introduction to this thesis that the specific volume of organic substances with known composition and functional groups may be calculated from the atomic volume increments derived by Traube (18). This method has been used for proteins by Cohn and Edsall (19) and more recently by McMeekin and Marshall (21). The calculated value of 0.734 c.c./g. (Table XXI) agrees very well with the value of 0.734 ml./g. observed for BPA in the present work. However, as stated by Cohn and Edsall, the calculated value does not take into account the decrease in volume, (electrostriction) which should occur owing to the charged groups of the amino acids in the protein. Since the calculated value agrees with the observed value for the protein, the normal effect of electrostriction must be nullified by a compensating factor. There may be a steric effect due to the folding and packing of the peptide chains of BPA whereby a small volume (excluded volume) is rendered inaccessible to solvent (78). Charlwood (13) estimates this volume to be about 3 - 4% for BPA based upon a volume decrease of 18 ml. per charge pair per mole. There is some ambiguity in the concept of an

excluded volume, since the titration of the acid and basic groups (19) of the protein indicates a ready release or binding of protons which signifies access of solvent to all portions of the macromolecule.

That electrostriction occurs can be shown from the observed values of $\overline{V}$ (Table XX) of glycine and TGG. Since the molecular weights of glycine and TGG are 75.07 and 246.14, ϕ_o is 43.19 and 145.3 cc./mole for the respective molecules. The specific volume of these molecules may be calculated by Traube's method as follows:

Glycine groups	Groups per mole	Volume per group	Volume (cc./mole) increments
$-NH_2$	1	7.7	7.7
$-CH_2$	1	16.2	16.2
-COOH	1	18.9	18.9
		Covolume	13.0
		∴ Specific volume (Traube)	55.8
		Experimental value	43.2
		∴ Electrostriction	12.6 cc./mole

TGG groups	Groups per mole	Volume per group	Volume (cc./mole) increments
$-NH_2$	1	7.7	7.7
$-CH_2$	4	16.2	64.8
-CONH	3	20.0	60.0
-COOH	1	18.9	18.9
		Covolume	13.0

$\therefore$ Specific volume (Traube) 164.4
Experimental value 145.3

$\therefore$ Electrostriction 19.1 cc./mole

These values of electrostriction may be examined in relation to those of Cohn and Edsall (19).

Substance	Electrostriction cc./mole	
	Cohn & Edsall	Thesis
Glycine	13.5	12.6
Glycylglycine	16.1	--
Diglycylglycine	16.1	--
Triglycylglycine	--	19.1

Cohn and Edsall probably used 14 cc./mole for the covolume. It can therefore be seen that TGG exhibits the electrostriction effect attributed to charged groups, an effect which is not observed with BPA.

The specific volume of PVA may be calculated by Traube's method. The repeating units of this particular sample of PVA are (- CH_2COH-) and (- $CH_2COCOCH_3$-).

Volume of the OH unit		Volume of the $COCH_3$ unit	
2 x C	19.8	4 x C	39.6
3 x H	9.3	5 x H	15.5
1 x O	2.3	2 x O	11.0
Total	31.4 c.c./unit	Total	66.1 c.c./unit

In this polymer there are 88 -OH units and 12 -$COCH_3$ per 100 units of the polymer. The covolume is negligible owing to the high molecular weight. Therefore the volume of 100 units of the polymer is:

$$(88 \times 31.4) + (12 \times 66.1) = 35.5 \times 10^2 \text{ c.c.}$$

The weight of the 100 polymer units is:

$(88 \times 43) + (12 \times 85) = 48.0 \times 10^2$ g.

$\therefore$ the specific volume is:

$(35.5 / 48.0)$ or 0.741 c.c./g.

At 25°C. the volume would be 0.75 c.c./g. since the volume increments were based on values for 15°C. The calculated value is probably too low since the values observed experimentally (Table XXI) are both higher. Perhaps some interaction between the acetate and hydroxyl groups leads to an increase in volume.

The specific volume of AC_1 may be calculated by Traube's method, but the results are uncertain because of the presence of a ring structure and an ionizing group. From the repeating unit of the sodium salt of mannuronic acid the following calculation can be made:

6 carbon atoms	59.4
7 hydrogen atoms	21.7
2 oxygen (OH) atoms	0.8
3 oxygen atoms	16.5
1 oxygen (O Na) atoms	1.2
1 sodium atom	1.8
1 ionization constant	-13.5
1 six-membered ring	- 8.1
Total	79.8 c.c./repeating unit

$\therefore$ specific volume = 79.8/198 = 0.40 c.c./g.

Since AC_1 is a high molecular weight material the covolume is not significant. The above calculated value is lower than the value 0.44 (53) found by use of pycnometers, and very much lower than the values (Table XXI) found in the present work. However, $\bar{V}$ of the mannuronic acid polymer (alginic acid) has

been reported to be 0.59 and 0.605 (53). Traube (18) has given values for the volume contraction between acids and their sodium salts ranging from 10.6 to 15.0 c.c. per mole, the degree of dissociation of the salt having an effect on the volume contraction. On this basis the value of 0.481 ml./g. for $\bar{V}$ of AC_1 by the float apparatus is not unreasonable.

Upon examination of the measurements with the spiral balance and the float apparatus, there appears to be some evidence that the volume taken up by hydrogen atoms and by deuterium atoms with which they undergo exchange is not equal in some proteins. Berger and Linderstrøm-Lang (79) found that the exchange of labile hydrogen atoms of poly-DL-alanine in deuterium oxide solution was complete in less than three hours. The rate of exchange was believed to be retarded owing to stabilization of the α- helix structure by internal nonpolar bonds, thereby preventing ready access of the solvent. Haggis (46) reported that BPA exchanged labile hydrogen atoms of the back-bone N-H groups to the extent of 90% at 25°C. and that other protein molecules including insulin and ribonuclease might be expected to exchange to a smaller extent. Experiments reported by Linderstrøm-Lang (42) indicated that insulin and ribonuclease would exchange about 95% of their labile hydrogen atoms in heavy water at 25°C. Morowitz and Chapman (44) found experimentally that all the hydrogen atoms not involved in C-H bonds in several amino acids , peptides, hemoglobin, and ribonuclease, exchanged readily with deuterium atoms in heavy

water at room temperature in 20 minutes.

It would appear that the exchange of labile hydrogen, atoms of macromolecules of known composition and functional groups can be predicted. However, the direct determination on a spiral balance would be more certain for the macromolecules of known composition and functional groups, and necessary for those materials of unknown characteristics. The amount of material required may be reduced to a few milligrams with the use of sensitive spirals.

Since a virtually complete exchange of the labile hydrogen atoms should occur, the small decrease for $\overline{V}$ of BPA upon deuteration might be due to a biased error in the value of $\overline{V}$ determined with the float apparatus in heavy water. However, an alternative explanation is possible. Robertson (80) has reported that, for hydrogen-bonded structures, an expansion is likely to occur in the direction of the hydrogen bond upon deuteration. In substances such as proteins the effect would be unpredictable owing to the multiplicity of types of hydrogen bonding. The magnitude of the expansion is variable but could probably account for the small decrease in $\overline{V}$ obtained upon deuteration of BPA. Extremely precise experimental methods would be necessary to verify the reality of the effect with macromolecules in solution.

SUMMARY AND CONTRIBUTIONS TO KNOWLEDGE

1. A McBain-Bakr spiral balance , in conjunction with a high vacuum system, has been used to determine the degree of isotopic exchange of deuterium atoms for the labile hydrogen atoms of organic materials in solution.

2. Bovine plasma albumin, polyvinyl alcohol (88% hydrolysed), glycine, lipovitellin and α-β-livetin complex, were found to exchange labile hydrogen atoms to the extent of 1.56, 1.79, 3.84, 1.33, and 1.32 weight percent respectively. in 97% deuterium oxide solution.

3. The isotopic weight percent exchange of bovine plasma albumin was a linear function of the volume percent deuterium oxide of the solution.

4. A magnetic float apparatus was used to determine the apparent specific volumes of bovine plasma albumin, polyvinyl alcohol, sodium alginate, glycine, and triglycylglycine in aqueous solution and with the exception of sodium alginate, in heavy water solution.

5. By the above technique the partial specific volumes of bovine plasma albumin, polyvinyl alcohol, glycine, and triglycylglycine were, 0.7342 ± 0.0002, 0.766 ± 0.001, 0.575 ± 0.002, and 0.591 ± 0.001 in aqueous solution and 0.728 ± 0.001, 0.754 ± 0.004, 0.545 ± 0.001, and 0.577 ± 0.002 ml./g. in deuterium oxide solution respectively. The partial specific volume of sodium alginate in aqueous solution was 0.481 ± 0.003 ml./g.

6. Glycine and triglycylglycine showed electrostriction effects of 12.6 and 19.1 c.c. per mole, owing to their dipolar

nature.

7. Except for bovine plasma albumin, the increases in weight and decreases in partial specific volumes of the molecules studied, justified the assumption that deuterium atoms occupy a volume in a molecule similar to the volume of the labile hydrogen atoms with which they undergo exchange. In bovine plasma albumin, a hydrogen-bonded substance, the deuterium atoms contributed slightly more to the volume than the hydrogen atoms they displaced.

8. The sedimentation coefficients of bovine plasma albumin and polyvinyl alcohol, corrected for the viscosity of the solvent, were linearly related to the density of the solvent when sedimentation was brought about in mixtures of water and heavy water. Correction was made for isotopic exchange effects.

9. A method has been investigated, in which macromolecules are sedimented in aqueous and deuterium oxide media to obtain the partial specific volumes of the macromolecules, corrections being made for the isotopic exchange of labile hydrogen atoms. The method was extended to include systems in which two macromolecular species were present. The technique is applicable to as little as 10 milligrams of macromolecule in solution. Known concentrations of solutions of the macromolecules are not necessary.

10. With only one macromolecular species in solution, the partial specific volumes of bovine plasma albumin, sodium alginate, and polyvinyl alcohol, by sedimentation were 0.734 $\pm$0.008, 0.55 $\pm$ 0.06, and 0.78 $\pm$0.01 ml./g. respectively.

11. By application of the differential sedimentation technique to mixtures of bovine plasma albumin and β-lactoglobulin, α- and β-livetin, and lipovitellin and γ-livetin, the partial specific volumes of the first two mixtures were found to be 0.74 ± 0.01 and 0.72 ± 0.02 ml./g. respectively, and the partial specific volumes of the resolved components of the first and last mixtures were 0.72 ± 0.02, 0.75 ± 0.03, 0.779 ± 0.003, and 0.79 ± 0.03 ml./g. respectively.

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APPENDIX A

Papers published by W.G. Martin *et al.*

1. "Molecular Weight and Hydrodynamic Properties of Laminarin", Friedlaender, M.H.G., Cook,W.H. and Martin, W.G., Biochim. Biophys. Acta, 14, 136, (1954).

2. "The Determination of Partial Specific Volumes by Differential Sedimentation", Martin, W.G., Cook, W.H., and Winkler, C.A., Can.J. Chem., 34, 809 (1956).

3. "Fractionation of Livetin and the Molecular Weights of the α- and β-Components", Martin, W.G., Vandegaer, J.E., and Cook, W.H., Can. J. Biochem.Physiol., 35,241 (1957).

4. "Preparation and Molecular Weight of γ-Livetin from Egg Yolk", Martin, W,G, and Cook, W.H., Can.J. Biochem.Physiol., 36, 153 (1958).