This theoretical conclusion is easily confirmed experimentally. Apparent heats of ionization of carboxyl, imidazole and amino groups have been obtained for human serum albumin[22] and oxyhemoglobin.[37] The values in each case were found to agree, within experimental error, with the normal heats of ionization of the groups involved, even though the protein charge undoubtedly reached high values.[38]

Since electrostatic forces appear thus unable to account for the high heat of ionization, it is necessary to seek a chemical explanation, and hydrogen bonding is the logical choice. The difference observed, about 6 kcal., is very close to the average value of the heat required to break virtually all known O—H · · · O and O—H · · · N bonds.[39]

It is noteworthy that the heat of ionization of iodinated albumin is also higher than the normal heat of ionization of a di-iodophenolic group (Table IV), and again by about 6 kcal./mole. It is also of great interest that preliminary data with pepsin indicate that its phenolic groups have a "normal" heat of ionization.

The entropy values would also seem to support the existence of hydrogen bonding. The value in serum albumin (−8 e.u. at 25°) is much more positive than that in tyrosine (−26 e.u.) or in phenol (−24 e.u.), indicating the destruction of an element of order in the protein which is not present in tyrosine or phenol. (The negative value for the over-all process merely reflects the organization of water molecules around the newly-formed phenoxide ion.)

Hydrogen bonding is perhaps also indicated by the fact that the intrinsic pK_0 for ionization is 10.00 or greater, whereas a reasonable "normal" value is 9.60. Even more suggestive is the fact that the absorption peak for serum albumin in acid or neutral solution lies at 279–280 mμ, whereas that for tyrosine itself (and that for phenol) lies near 275 mμ. The peak in alkaline solution, on the other hand, occurs at 292.5 mμ both for serum albumin itself and for tyrosine.

Finally, however, it should be pointed out that even though the tentative conclusion has been reached here, that the phenolic groups of serum albumin are strongly hydrogen bonded to some other groups in the molecule, the evidence does *not* indicate that these bonds are concerned with maintaining the native structure of the molecule, for the ionization process occurs both instantaneously and reversibly. The difference between egg albumin and serum albumin in this respect has already been pointed out above. The kinetic studies on the alkaline denaturation of pepsin and ricin, made by Steinhardt[40] and Levy and Benaglia[41] are of interest in this connection, for they suggest that only a very small number of specific hydrogen bonds are involved in maintaining the native structure of the protein molecules. It would appear that in egg albumin some of the hydrogen bonds involving the phenolic groups are among these vital few; in serum albumin, however, they are not, and apparently can be broken without causing denaturation.

(37) J. Wyman, Jr., *J. Biol. Chem.*, **127**, 1 (1939).

(38) The observation that electrostatic charge has only a minor effect on the heat of ionization appears also to hold true for small molecules. The ionization constant of a carboxyl group (and therefore the free energy of ionization) can be changed considerably by electrostatic charge. In histidine, glycylglycine, acetic acid and hydrogen succinate, for example, we have, respectively, charges of +2, +1, 0 and −1 on the molecule prior to ionization of the carboxyl groups. The corresponding pK values are accordingly very different: being 1.82, 3.08, 4.76 and 5.55, respectively. The heats of ionization, however, have, within 1 kcal., the same value in all four cases (*cf.* E. J. Cohn and J. T. Edsall, "Proteins, Amino-Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943, chapters 4 and 5).

(39) See, for example, M. L. Huggins, *J. Org. Chem.*, **1**, 487 (1937).

(40) J. Steinhardt, *Kgl. Danske Videnskab. Selskab, Math.-fys. Medd.*, **14**, No. 11 (1937).

(41) M. Levy and A. E. Benaglia, *J. Biol. Chem.*, **186**, 829 (1950).

IOWA CITY, IOWA

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

The Partial Specific Volumes, in Aqueous Solution, of Three Proteins

BY MARGARET OAKLEY DAYHOFF, GERTRUDE E. PERLMANN AND D. A. MACINNES

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With the aid of the magnetic float method the densities at 25° of aqueous solutions of crystalline ovalbumin, crystalline bovine serum albumin and bovine γ-globulin have been determined. From these data the apparent specific volumes have been computed. Since these values are constant, in the concentration range studied, they are also the partial specific volumes of these proteins in aqueous solution.

In the sedimentation velocity method for the determination of the molecular weights, M, of proteins, with the ultracentrifuge, the familiar equation

$$M = \frac{sRT}{D(1 - \bar{V}\rho)}$$

is employed. Here s is the sedimentation constant, D, the diffusion coefficient, $\bar{V}$, the partial specific volume and ρ, the density of the solution. An inspection of this equation indicates that the accuracy of the determinations of M are particularly sensitive to errors in the product $\bar{V}\rho$. Since that product has the order of magnitude of 0.8, such errors are multiplied by four in their effects on M. Molecular weights obtained by the sedimentation equilibrium method are equally affected by these errors. In spite of this fact, comparatively little attention has been paid to improvement of the methods of determining the densities of protein solutions, from which the partial specific volumes are obtained. In many cases in which molecular weights have been reported, average or assumed values of V have been employed in the computations.

It has, therefore, appeared desirable to determine the densities of solutions of several proteins using the magnetic float method in the form developed in this Laboratory.[1,2]

In that procedure the float, in the shape of an inverted flask, has a permanent magnet mounted in its neck and its weight is such that it just rises to the surface of the solvent. Small platinum weights are added until it sinks slowly. The float is then made to rise by passing current through a solenoid surrounding the magnet. By observing the relative speeds of rise for different currents through the solenoid an extrapolation is made to a value of the current at which the float would remain motionless. Thus, with a coefficient relating current and weight, the final portion of the weighing is done electromagnetically. The process may be repeated for a solution, yielding data from which the specific gravity, or density, of the solution may be computed.

In obtaining the density measurements given below the following procedure was followed. About 250 g. of water was weighed into the vessel surrounding the float which weighed about 80 g. After bringing the system to equilibrium in a thermostat regulating to 0.001 at 25°, platinum weights were added and the limiting current measured as described above. A few ml. of a strong protein solution were then added from a weight buret.[3] After stirring, more platinum weights were added until the float sank and the limiting current once more determined. Further additions of the strong solution were made, yielding data for a series of densities of solutions with increasing protein concentration. That precision was attained, was shown in the case of two of the proteins, by repeated determinations in which the densities agreed to one in 10^6.

The question may be asked as to why the relatively complicated magnetic float method was used when pycnometers are available. In developing the float method, solutions of potassium chloride were studied.[1,2,3] The published density determinations on solutions of that salt made with pycnometers show surprisingly large deviations in the results from presumably careful work, tending to indicate that there are sources of error in that procedure, in spite of its apparent simplicity. The errors may arise from at least four sources: (a) obtaining reproducible external (air–glass) surfaces of the pycnometer, (b) adjusting the meniscus in capillaries, (c) evaporation at the meniscus, and (d) keeping bubbles out of the solution. Since the accuracy obtained by the magnetic float method is relatively little affected by inexactness in the determination of the dry weight of the float,[1] and no meniscus is involved, sources of error (a), (b) and (c) are eliminated. Bubbles are troublesome only if they are attached to the float or the weights, and can be completely avoided. Protein solutions are particularly difficult to investigate with pycnometers since they foam readily, especially when drawn through capillaries. With our apparatus no special difficulties were encountered in dealing with such solutions. In addition the determination of the densities of a series of solutions of increasing concentration with one sample of solvent is not possible with the pycnometer technique.

The objection that relatively large volumes of solutions are required with the float method is met, in part at least, by the fact that, due to the sensitiveness of the method, measurements yielding accurate partial specific volumes may be obtained with the use of quite dilute solutions, thus involving comparatively small amounts of the substances being studied.

Preparation and Analysis of the Proteins.—The three proteins chosen for study were crystalline ovalbumin, crystalline bovine serum albumin and bovine γ-globulin. Data concerning their preparation and analysis follow.

Crystalline Ovalbumin.—This material was prepared in this Laboratory by the method of Sørensen and Høyrup.[4] The purification procedure included three crystallizations from ammonium sulfate. The protein was stored as a paste in an 80% solution of that salt. Electrophoretic analysis in sodium phosphate buffer of *p*H 6.8 and 0.1 ionic strength indicated the two components which are normal for this material,[5] in their usual ratio. Salt free solutions of this material were prepared by dialysis against distilled water, and weighed samples of concentrated aqueous solution were analyzed by the Pregl micro-Kjeldahl method. The nitrogen factor used for the conversion of these results to a dry weight basis was 6.36.[6] The concentrated solution used for the additions from the weight buret contained about 5% of the protein.

Crystalline Bovine Serum Albumin.—This protein was obtained from the Armour Co., Chicago, as a dry powder and was stored at 2°. Electrophoresis in a sodium diethylbarbiturate buffer at *p*H 8.6 and 0.1 ionic strength indicated a single component. The solution was dialyzed against distilled water. Micro-Kjeldahl analysis was employed to determine the concentration of the solution, using a nitrogen factor of 6.23.[6] This was checked to 0.5% by evaporating a weighed sample of the salt free solution to dryness in air at 110° to a constant weight. The concentrated solution used for the additions from the weight buret contained about 7% of the protein.

Bovine γ-Globulin.—This material was also obtained from the Armour Co., Chicago, and is their "Fraction II." Electrophoresis in sodium diethylbarbiturate buffer of *p*H 8.6 and a 0.1 ionic strength indicated that this preparation contained 96% of the main component, the remainder of the protein being β-globulin. The ash content of the powder was 0.28%. A solution was prepared by dissolving a weighed sample of the protein in a known amount of 0.4726 molal potassium chloride solution. The moisture content of the powder was determined by drying 100-mg. samples at 110° in air to constant weight and a correction was made for this moisture in calculating concentration of the solution. The solution used in the weight buret contained about 1.6% of protein.

Results

The results of our measurements are given in Table I, in which the compositions of the protein solutions in per cent., the corresponding densities of these solutions at 25°, and the values of the apparent specific volume, ϕ, are given. This latter quantity may be defined by the relations

$$\phi = \frac{V - V_0}{m} = \frac{100/d - (100 - n)d_0}{n}$$

in which V is the volume of a solution, V_0 the volume of the solvent contained in it, and m the weight of the solute. In the equivalent expression to the right d is the density of the solution, d_0 that of the solvent and n the percentage of solute. It will be seen that the values of ϕ for the three proteins do not change appreciably with increasing concentration. The partial specific volume $\bar{V}$ of a component a is defined by $\bar{V} = (\partial V/\partial m_a)_{T.P.mb}$, in which the subscripts indicate that temperature, pressure and concentrations of the other compo-

(1) D. A. MacInnes, M. O. Dayhoff and B. R. Ray, *Rev. Sci. Instruments*, **22**, 642 (1951).

(2) D. A. MacInnes and M. O. Dayhoff, THIS JOURNAL, **74**, 1017 (1952).

(3) The type of weight buret used, which permits the addition of solution without exposure to stopcock grease, has been described by T. Shedlovsky and A. S. Brown, THIS JOURNAL, **56**, 1066 (1934).

(4) S. P. L. Sørensen and M. Høyrup, *Compt. rend. Lab. Carlsberg*, **12**, 12 (1917).

(5) L. G. Longsworth, R. K. Cannan and D. A. MacInnes, THIS JOURNAL, **62**, 2580 (1940).

(6) G. E. Perlmann and L. G. Longsworth, *ibid.*, **70**, 2719 (1948).

TABLE I

THE DENSITIES, AT 25°, OF SOLUTIONS OF THREE PROTEINS AND THEIR APPARENT OR PARTIAL SPECIFIC VOLUMES, $\phi = \bar{V}$

Protein	%	Density	$\phi = \bar{V}$
Ovalbumin	0	0.997074	0
	0.02945	0.997149	0.747
	.09707	.997319	.7493
	.14874	.997451	.7480
	.24300	.997691	.7478
	.24803	.997704	.7475
	.36045	.997990	.7477
	.50339	.998350	.7483
Bovine serum albumin	0	0.997074	0
	0.24543	0.997726	0.7346
	.42612	.998213	.7345
	.55264	.998586	.7335
	.55923	.998678	.7344
	.70535	.998969	.7334
	.82661	.999279	.7352
Bovine γ-globulin	0	1.018684	0
	0.06326	1.018855	0.7217
	.06818	1.018869	.7202
	.1390	1.019061	.7204
	.1544	1.019104	.7197
	.1762	1.019163	.7199
	.1992	1.019227	.7191
	.2609	1.019393	.7198
	.2949	1.019487	.7194

nents are to be kept constant. It can be readily shown that if ϕ does not vary with the solute concentration, as in the examples given above, $\bar{V}$ and ϕ have the same value. A comparison of our results with those of other workers is made in Table II. Pedersen's (1945) values are described as "tentative."

TABLE II

COMPARISON OF VALUES OF SPECIFIC VOLUMES OF PROTEINS OBTAINED BY DIFFERENT WORKERS

Protein	This paper (av.) 25°	Koenig[7] 20°	Pedersen[8] 20°	Svedberg and Pedersen[9] 20°
Ovalbumin (crystalline)	0.7479	...	...	0.749
Bovine serum albumin (crystalline)	.7343	0.730	0.736	...
Bovine γ-globulin	.7200	.725	.732	...

In addition to the work of Lamb and Lee[10] and of Geffcken, Beckmann and Kruis[11] on the magnetic float method for determining densities, previously referred to in this series of papers, mention should be made of the interesting use of the method by Hall and collaborators.[12]

The authors are indebted to Elaine Lackman, and Grace Peters for aid in making the density measurements.

(7) V. L. Koenig, *Arch. Biochem.*, **25**, 241 (1951).

(8) K. O. Pedersen, "Ultracentrifugal Studies on Serum and Serum Proteins," Almquist and Wiksells Boktryckeri A B, Uppsala, 1945.

(9) T. Svedberg and K. O. Pedersen, "Ultracentrifuge," Clarendon Press, Oxford, 1940.

(10) A. B. Lamb and F. E. Lee, THIS JOURNAL, **35**, 1666 (1913).

(11) W. Geffcken, C. Beckmann and A. Kruis, *Z. physik Chem.*, **B20**, 398 (1933).

(12) N. F. Hall and T. O. Jones, THIS JOURNAL, **58**, 1915 (1936); N. F. Hall and O. Alexander, *ibid.*, **62**, 3455 (1940).

NEW YORK, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

Molecular Dimensions of Cellulose Triesters[1]

BY LEO MANDELKERN AND PAUL J. FLORY

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The unperturbed end-to-end dimension ratios $(\overline{r_0^2}/M)^{1/2}$ for two cellulose triesters, cellulose tributyrate and cellulose tricaprylate have been determined from intrinsic viscosity measurements in γ-phenylpropyl alcohol, in dimethylformamide and in a 3:1 mixture of dodecane and tetralin. The measurements were carried out in each case at the characteristic temperature Θ for ideal behavior of the polymer–solvent system. Several fractions of each polymer were used and the molecular weights were determined osmotically. $K = [\eta]_\theta/M^{1/2}$ is independent of M in accordance with previously published results, but not independent of temperature; for cellulose tributyrate K decreases rather strongly with increasing temperature. The values of $(\overline{r_0^2}/M)^{1/2}$ calculated from K indicate that these esters are only two to three times more extended than they would be if rotation about the interunit ether linkages were completely unhindered. Thus, there appears to be no basis for the alleged rod-like extension of the cellulose chain.

Introduction

Deduction of characteristic dimensions of linear polymer molecules from appropriate solution viscosity measurements has been demonstrated by a number of recent investigations.[2-4] The following relations are employed to interpret the intrinsic viscosity results

$$[\eta] = KM^{1/2}\alpha^3 \quad (1)$$

$$K = \Phi(\overline{r_0^2}/M)^{3/2} \quad (2)$$

$$\alpha^5 - \alpha^3 = 2\psi_1 C_M (1 - \Theta/T)M^{1/2} \quad (3)$$

where $(\overline{r_0^2})^{1/2}$ is the root-mean-square distance from beginning to end of the chain *in the absence of perturbations due to interactions between remotely connected segments.* The actual root-mean-square distance $(\overline{r^2})^{1/2}$ exceeds this unperturbed distance in a particular solvent at a given temperature by the factor α. K is a parameter characteristic of the polymer and of the temperature but independent of the solvent; since Φ appears to be a universal constant, the same for all polymers in all solvents

(1) The work reported in this paper comprises a part of a program of research on the physical structure and properties of cellulose derivatives supported by the Allegany Ballistics Laboratory, Cumberland, Maryland, an establishment owned by the United States Navy and operated by the Hercules Powder Company under Contract NOrd 10431.

(2) T. G. Fox, Jr., and P. J. Flory, THIS JOURNAL, **73**, 1909 (1951).

(3) T. G. Fox, Jr., and P. J. Flory, *ibid.*, **73**, 1915 (1951).

(4) H. L. Wagner and P. J. Flory, *ibid.*, **74**, 195 (1952).