[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

Consideration of the Hydrodynamic Properties of Proteins[1,2]

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A treatment of the hydrodynamic properties of proteins is presented wherein the molecule is assumed to possess some degree of flexibility and solvation. The configuration of the protein molecule is represented in terms of an effective hydrodynamic ellipsoid whose axial ratio and size may be determined from accurate measurements of sedimentation constant, intrinsic viscosity, molecular weight and flow birefringence, all made in the same solvent.

Introduction

The configurations of protein molecules have been determined from hydrodynamic measurements by use of the theories of Simha[4] and Perrin[5,6] for rigid ellipsoids of revolution. In the past[7,8,9,10] the problem has been treated by considering a boundary to be assigned to the particle and approximating this molecular domain by an ellipsoid of revolution. The volume of this domain was regarded as the sum of $M\bar{v}/N$ and $Mw/N\rho$ where M is the anhydrous molecular weight, N is Avogadro's number, $\bar{v}$ is the partial specific volume of the anhydrous protein, ρ is the density of the solvent and w is the g. of solvent bound per g. of dry protein; w has been taken to be the amount of water associated with the protein molecule in solution.[7,10] It has been further assumed that the hydrodynamically effective volume, V_e, is equal to $M\bar{v}(1 + w/\bar{v}\rho)/N$, *i.e.*, the effective volume has been identified with the molecular domain of the protein molecule in solution. There is, however, no *a priori* reason to assume this equality, for it neglects possible flow of solvent through the domain, deviations of the shape of the domain from that of an ellipsoid of revolution, deformation of the domain by the hydrodynamic forces, selective adsorption from mixed solvent, electrostriction, and similar effects. Besides these problems, it does not, in general, appear reasonable to obtain the effective volume in the manner indicated, *i.e.*, by expressing hydration as an excess over $M\bar{v}/N$. The value of $\bar{v}$ may be positive, negative, or zero, as is the case, for example, in aqueous solutions of magnesium sulfate.[11] While such a behavior has not been observed for proteins, nevertheless, the value of $\bar{v}$ has to reflect the interaction with the solvent, making it impossible to identify $M\bar{v}/N$ with the volume of the anhydrous protein[12a]; in addition, electrostriction would render unknown the value of ρ in the correction term for the bound water. Thus, erroneous interpretations of experimental results are possible if the molecular domain is identified with the effective hydrodynamic volume and if the definition of w is retained. More property w should be given that value which allows the observations to be reconciled with rigid particle hydrodynamics. Since, in general, the value of w would have *no direct relation to the amount of water in the domain of the molecule,* the introduction of $\bar{v}$ in the expression for V_e becomes superfluous and also misleading.[12b]

This is most clearly seen in the graphical method[7,9] which requires that curves of axial ratio as a function of w for observed values of intrinsic viscosity and frictional coefficient intersect at the correct value of w and axial ratio, thus giving consistency between the two hydrodynamic measurements. However, in all cases cited[7,9] these curves could be made to intersect only by imposing *large experimental errors on the data.* In one case—pepsin—*the curves do not cross at all.* If one examines the data closely, it can be seen that these curves *can* be made to intersect at *negative* values of w without introducing large experimental errors in the data. The negative values of w, which are incompatible with the original definition of w, arise because of the arbitrary assignment of a portion of the effective volume to the term $M\bar{v}/N$.

Besides the difficulty involved in the interpretation of w (as originally defined), the tendency seems to have been to avoid determining it altogether. Instead, as indicated by numerous examples in the literature, arbitrary values such as zero hydration[13,15,17-21] or 20–30% hydration[14,16,17] have been assumed, thereby confusing the relative contribu-

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(4) R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

(5) F. Perrin, *J. phys. radium*, [7] **5**, 497 (1934).

(6) F. Perrin, *ibid.*, [7] **7**, 1 (1936).

(7) J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941).

(8) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, London, 1940.

(9) J. W. Mehl, J. L. Oncley and R. Simha, *Science*, **92**, 132 (1940).

(10) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943.

(11) G. N. Lewis and M. Randall, "Thermodynamics," McGraw-Hill Book Co., Inc., New York, 1923, p. 37.

(12a) Ref. 10, p. 516.

(12b) The degree of hydration of proteins, obtained from hydrodynamic measurements, has been compared to the hydration of protein crystals. It has even been suggested "that the degree of hydration of a protein is essentially the same in solution as in the crystal."[12c,d] This conclusion is based on the interpretation of w cited above. See also H. K. Schachman and M. A. Lauffer, THIS JOURNAL, **71**, 536 (1949).

(12c) T. L. McMeekin, M. L. Groves and N. J. Hipp, *ibid.*, **72**, 3662 (1950).

(12d) A. D. McLaren and J. W. Rowen, *J. Polymer Sci.*, **7**, 314 (1951).

See for example:

(13) H. Neurath and A. M. Saum, *J. Biol. Chem.*, **126**, 347 (1939).

(14) G. R. Cooper and H. Neurath, *J. Phys. Chem.*, **47**, 383 (1943).

(15) S. Brohult, *J. Phys. Colloid Chem.*, **51**, 206 (1947).

(16) J. L. Oncley, G. Scatchard and A. Brown, *ibid.*, **51**, 184 (1947).

(17) J. T. Edsall, J. F. Foster and H. Scheinberg, THIS JOURNAL, **69**, 2731 (1947).

(18) H. Kahler, *J. Phys. Colloid Chem.*, **52**, 676 (1948).

(19) J. F. Foster, *ibid.*, **53**, 175 (1949).

(20) E. H. Mercer and B. Olofsson, *J. Polymer Sci.*, **6**, 671 (1951).

(21) C. S. Hocking, M. Laskowski, Jr., and H. A. Scheraga, THIS JOURNAL, **74**, 775 (1952).

tions of both the solvation and the asymmetry to the frictional behavior of the protein molecule. In fact this procedure is equivalent to assuming a volume and then explaining the observations on the basis of asymmetry.

A different procedure is considered here in which the configurations of globular type protein molecules in solution are represented in terms of an *effective* hydrodynamic ellipsoid. The size and shape of this ellipsoid are defined as those which allow the experimental hydrodynamic observations to be treated by the hydrodynamic equations developed by Simha and Perrin for rigid ellipsoids of revolution even though the actual molecular configuration may not be a rigid one; *i.e.*, there is a rigid ellipsoid (the effective or equivalent hydrodynamic ellipsoid) which exhibits the same hydrodynamic behavior as the solvated protein molecule in solution. The size and shape of this rigid ellipsoid are such as will take into account possible flexibility of the molecule, permeation of the molecule by the solvent, etc. This procedure is analogous to the use of an effective hydrodynamic sphere[22] for flexible, solvated polymers in consideration of the Einstein–Stokes relations for spheres. In general, the relationship between the effective ellipsoid and the actual protein configuration in solution is not known, nor need it be specified. In the very special case, where the molecules are completely rigid and impermeable to solvent, the effective ellipsoid and the actual configuration could coincide.

It is worth noting that neither the configuration nor the effective hydrodynamic ellipsoid of protein molecules in the anhydrous state can be determined by measurements of intrinsic viscosity, sedimentation and diffusion, flow birefringence, etc., in protein solutions because it has not yet been possible to calculate the solute–solvent and intramolecular interactions, as has been done for randomly coiled chain polymers.[22] As a consequence of these interactions, it is reasonable to expect that a protein molecule should not be completely rigid but flexible enough to swell to expanded configurations in various solvents[23] and exhibit a hydrodynamic behavior similar to that of randomly coiled chain molecules. Thus if an unhydrated protein were placed in an aqueous solution a swelling effect should occur. Evidence for such a phenomenon has been reported on the basis of low angle X-ray scattering from protein solutions.[24a,b] For the case of southern bean mosaic virus almost a two-fold increase in volume has been found for the virus in solution compared to that in the dry state.[24a] Similarly, there is an indication that serum albumin swells in solution.[24b]

In the present treatment it is, first of all, recognized that only the dimensions of the effective ellipsoid (for whatever degree of flexibility or permeation by the solvent the molecule may possess) may be obtained from the hydrodynamic measurements in dilute solution and not those of the molecular domain. In addition, relationships between axial ratio, sedimentation constant, intrinsic viscosity and molecular weight are developed in a more concise and systematic manner than heretofore. The basis for determining the size of the effective ellipsoid and for distinguishing between a prolate and oblate one is explicitly given in terms of two independent hydrodynamic measurements. It is also shown that there is a similarity in hydrodynamic behavior between globular type proteins and flexible chain molecules.

Theory

Intrinsic viscosity-translational frictional coefficient: Whatever the actual configuration of a protein molecule may be in a particular solvent at a given *p*H and ionic strength, there is associated with it an effective hydrodynamic ellipsoid of volume V_e and axial ratio p, which will account for the frictional effects arising from the presence of the molecule in the solvent. V_e and p, of course, depend on the solvent, temperature, *p*H and ionic strength.

In the absence of solute–solute interaction, *i.e.*, at infinite dilution, the specific viscosity, η_{sp}, can be written as a product of three factors, (1) the number of particles per cc., (2) the effective volume of an individual particle, and (3) a shape factor. The number of particles per cc. is equal to $Nc/100M$ where N is the Avogadro number, c is the *dry-weight* concentration in g./100 cc., and M is the *unhydrated* molecular weight. The intrinsic viscosity, $[\eta]$, may then be written as

$$[\eta] = (N\nu/100)(V_e/M) \qquad (1)$$

where ν is a shape factor which depends on the axial ratio, p, of the effective hydrodynamic ellipsoid which is assumed to be an ellipsoid of revolution. The quantity ν has been calculated by Simha[4] for the condition of prevalent Brownian motion. Values of ν as a function of p for prolate and oblate ellipsoids have been tabulated by Mehl, Oncley and Simha.[9]

According to Perrin,[6] the frictional coefficient of the effective hydrodynamic ellipsoid is given by the equation

$$f/f_0 = 1/F \qquad (2)$$

where

$$F = \frac{p^{2/3}}{\sqrt{1-p^2}} \ln \frac{1+\sqrt{1-p^2}}{p} \quad \text{for prolate ellipsoids } (p < 1)$$

$$= \frac{p^{2/3}}{\sqrt{p^2-1}} \arctan \sqrt{p^2-1} \quad \text{for oblate ellipsoids } (p > 1)$$

f is the mean frictional coefficient at infinite dilution, f_0 is the frictional coefficient of a sphere of radius a_0 having the same volume as the equivalent hydrodynamic ellipsoid and obeying Stokes' law, and $p = b/a$ where a and b are the semi-axes of the equivalent ellipsoid, a being the semi-axis of revolution and b the equatorial radius. It should be noted that our use of f_0 for a hydrated sphere differs from the usual interpretation of f_0 in terms of an unhydrated sphere.[7]

Since $f_0 = 6\pi\eta a_0$, where η is the viscosity of the solvent

$$f = 6\pi\eta a_0/F \qquad (3)$$

The value of a_0 is $(3/4\pi)^{1/3}(V_e)^{1/3}$. Therefore

$$f = (162\pi^2)^{1/3}(V_e)^{1/3}\eta/F \qquad (4)$$

In other words $[\eta]$ and f both depend on p and V_e. A solution of the simultaneous equations (1) and (4) would then give both p and V_e.

The Svedberg equation for the sedimentation constant at infinite dilution is[8]

$$s = M_h(1 - \bar{v}_h\rho)/Nf \qquad (5)$$

where ρ is the density of the solution and $\bar{v}_h$ is the partial

(22) P. J. Flory and T. G. Fox, Jr., THIS JOURNAL, **73**, 1904 (1951).

(23) The type of flexibility envisaged for proteins is that which allows them to imbibe solvent and deform from the configuration in which they exist in the anhydrous state. In general, the globular type protein molecule is not necessarily to be thought of in terms of the random flight configuration of a flexible chain polymer. However, as in the case of polymers, the swelling can involve immobilization of solvent within the molecule, in excess of stoichiometric quantities.

(24a) B. R. Leonard, Jr., J. W. Anderegg, P. Kaesberg, S. Shulman, and W. W. Beeman, *J. Chem. Phys.*, **19**, 793 (1951).

(24b) J. W. Anderegg, W. W. Beeman, and S. Shulman, *Phys. Rev.*, **87**, 186 (1952).

specific volume of the hydrated particle. Since eqn. (5) is being considered at infinite dilution $M_h(1 - \bar{v}_h\rho)$ may be replaced by $M(1 - \bar{v}\rho)$, where $\bar{v}$ refers to the unhydrated particle. This relation is true for a binary system and holds approximately for three component systems.[7,25,26] Therefore eq. (5) becomes

$$s = M(1 - \bar{v}\rho)/Nf \qquad (5')$$

Combination of eqs. (1), (4) and (5') gives

$$\beta \equiv Ns[\eta]^{1/3}\eta/M^{2/3}(1 - \bar{v}\rho) = \gamma F\nu^{1/3} \qquad (6)$$

where $\gamma = N^{1/3}/(16200\,\pi^2)^{1/3}$ and β corresponds to the function $\Phi^{1/3}P^{-1}$ used in a similar discussion of flexible chain molecules.[27,28] β is determinable from the sedimentation constant at infinite dilution, the intrinsic viscosity, the molecular weight, the partial specific volume, the solution density, and the solvent viscosity. It should be emphasized that all measurements must be made in the same solvent in order that p and V_e shall not vary.

For flexible chain polymers $\Phi^{1/3}P^{-1}$ should be a universal constant independent of the nature of the polymer or of the solvent and temperature. This conclusion is reached on the assumption that, as far as frictional effects are concerned, the polymer molecule may be replaced by an effective hydrodynamic sphere. The constancy of $\Phi^{1/3}P^{-1}$ for flexible chain polymers has been confirmed by experiment.[27,29]

For proteins, where an effective hydrodynamic ellipsoid is used, β should depend only on p according to eq. (6). This dependence can be calculated from the functions of Perrin[6] and Simha[4] and the results are shown in Table I. For oblate ellipsoids β is essentially independent of axial ratio whereas for prolate ellipsoids it varies considerably with axial ratio, providing a criterion to distinguish between prolate and oblate ellipsoids. If *accurate* data are available to calculate β it is thus possible to determine the axial ratio and dimensions of the effective hydrodynamic ellipsoid as discussed below.

TABLE I

DEPENDENCE OF β ON AXIAL RATIO FOR PROLATE AND OBLATE ELLIPSOIDS[a]

Prolate		Oblate	
$1/p = a/b$	$\beta \times 10^{-6}$	$p = b/a$	$\beta \times 10^{-6}$
1	2.12	1	2.12
2	2.13	2	2.12
3	2.16	3	2.13
4	2.20	4	2.13
5	2.23	5	2.13
6	2.28	6	2.14
8	2.35	8	2.14
10	2.41	10	2.14
12	2.47	12	2.14
15	2.54	15	2.14
20	2.64	20	2.15
25	2.72	25	2.15
30	2.78	30	2.15
40	2.89	40	2.15
50	2.97	50	2.15
60	3.04	60	2.15
80	3.14	80	2.15
100	3.22	100	2.15
200	3.48	200	2.15
300	3.60	300	2.15

[a] ν was obtained from Mehl, Oncley and Simha,[8] and Perrin's function F from Svedberg and Pedersen,[3] p. 41.

As can be seen in eq. (1) and eq. (4), $[\eta]$ and f depend on *both* p and V_e. Therefore, p cannot be determined from $[\eta]$ or f alone but only by a *combined* measurement of $[\eta]$ and f. The pair measurement, $[\eta]$-f, then gives both p and V_e for the *solvated* protein. In other words, high viscosity and high frictional coefficient have usually been attributed to high asymmetry, whereas, as can be seen from eq. (1) and (4), increased effective volume, rather than (or in addition to) increased asymmetry, could be just as important a factor.

Intrinsic viscosity-rotary frictional coefficient: The rotary diffusion constant of protein molecules can be treated in a similar manner.

The rotary frictional coefficient, ζ, for ellipsoidal particles at infinite dilution has been considered by Perrin[5] and is given by the equation

$$\frac{\zeta}{\zeta_0} = \frac{1}{J} \qquad (7)$$

where

$$J = \frac{3}{2}\frac{\dfrac{p^2(2-p^2)}{\sqrt{1-p^2}}\ln\dfrac{1+\sqrt{1-p^2}}{p} - p^2}{(1-p^4)} \quad \text{for prolate ellipsoids}$$

$$= \frac{3}{2}\frac{\dfrac{p^2(p^2-2)}{\sqrt{p^2-1}}\arctan\sqrt{p^2-1} + p^2}{(p^4-1)} \quad \text{for oblate ellipsoids}$$

Again, ζ_0 refers to a sphere of the same volume as the equivalent hydrodynamic ellipsoid.[30] Values of ζ/ζ_0 are given in Table II for various values of p. Since

$$\zeta_0 = 8\pi\eta a_0^3 = 6\eta V_e \qquad (8)$$

the rotary frictional coefficient may be written

$$\zeta = 6\eta V_e/J \qquad (9)$$

The rotary diffusion constant,[31] Θ, is related to ζ.

$$\Theta = kT/\zeta = kTJ/6\eta V_e \qquad (10)$$

TABLE II

DEPENDENCE OF ζ/ζ_0 AND δ ON AXIAL RATIO FOR PROLATE AND OBLATE ELLIPSOIDS

Prolate			Oblate		
$1/p = a/b$	ζ/ζ_0	δ	$p = b/a$	ζ/ζ_0	δ
1	1	2.50	1	1	2.50
2	1.505	1.93	2	1.132	2.52
3	2.340	1.57	3	1.464	2.34
4	3.395	1.37	4	1.843	2.20
5	4.638	1.25	5	2.240	2.10
6	6.061	1.17	6	2.645	2.03
8	9.401	1.07	8	3.471	1.93
10	13.37	1.02	10	4.305	1.87
12	17.94	0.990	12	5.143	1.83
15	25.86	.959	15	6.407	1.78
20	41.80	.923	20	8.519	1.74
25	61.05	.904	25	10.64	1.71
30	83.45	.893	30	12.75	1.69
40	137.3	.880	40	16.99	1.67
50	202.9	.870	50	21.24	1.65
60	279.9	.865	60	25.48	1.64
80	465.9	.859	80	33.96	1.62
100	694.5	.854	100	42.44	1.62
200	2428	.845	200	84.86	1.60
300	5085	.841	300	127.3	1.60

Combination of eq. (1) and (10) gives

$$\delta \equiv \frac{600}{Nk}\left(\frac{\eta\Theta}{T}\right)[\eta]M = J\nu \qquad (11)$$

(25) W. D. Lansing and E. O. Kraemer, THIS JOURNAL, **58**, 1471 (1936); see also reference 8, pp. 62–66.

(26) H. K. Schachman and M. A. Lauffer, *ibid.*, **72**, 4266 (1950).

(27) L. Mandelkern and P. J. Flory, *J. Chem. Phys.*, **20**, 212 (1952).

(28) A relation analogous to eq. (6) is obtainable from the translational diffusion constant at infinite dilution, D, using $D = kT/f$ and eq. (1) and (4) giving $\beta \equiv D[\eta]^{1/3}M^{1/3}\eta/kT = \gamma F\nu^{1/3}$.

(29) L. Mandelkern, W. R. Krigbaum, H. A. Scheraga and P. J. Flory, *J. Chem. Phys.*, **20**, 1392 (1952).

(30) For rotational diffusion of ellipsoids of revolution there are two rotary frictional coefficients corresponding to rotation about the a- and b-axes, respectively. However, rotation about the a-axis does not affect the orientation of the particle. Therefore, since only rotation about the b-axis is observable in a flow birefringence experiment, only the rotary frictional coefficient for this case is considered here.

(31) The relaxation time, τ, determined from dielectric dispersion measurements[32] is related to Θ by the equation $\tau = 1/2\Theta$.

(32) J. L. Oncley, *Chem. Revs.*, **30**, 433 (1942).

δ, like β, should depend only on p for proteins whose configuration is represented in terms of an effective hydrodynamic ellipsoid. δ is determinable from the rotary diffusion constant at infinite dilution (by means of flow birefringence or dielectric dispersion measurements), the intrinsic viscosity, the molecular weight, and the solvent viscosity. Values of δ, calculated from J and ν are given in Table II for various values of p.

The identical form (except for numerical coefficients) of eq. (11) for several models for macromolecules has been discussed by several authors.[33-36] For effective ellipsoids the explicit dependence of δ on axial ratio has not been given, heretofore, as it is by the factor $J\nu$ tabulated in Table II.

Discussion

From the foregoing it is apparent that it is possible to calculate the dimensions of the effective hydrodynamic ellipsoid from the types of experiments considered here. Equation (6) provides a basis for considering the determination of the axial ratio.

For $\beta > 2.15 \times 10^6$ it is possible to rule out an oblate ellipsoid from consideration and find the axial ratio of the prolate ellipsoid from the data of Table I. This value of p determines ν which together with the experimental values of intrinsic viscosity and molecular weight determines V_e according to eq. (1). Alternatively, V_e can be obtained from F and the sedimentation constant according to eq. (4) and (5'). a and b are then determinable from p and V_e. An independent determination of a can be made by means of flow birefringence measurements which give the rotary diffusion constant Θ. For the known value of p (and, therefore, J) V_e is determinable from eq. (10). It may be noted that a δ value $\leqq 1.57$ would also rule out an oblate ellipsoid from consideration.

For $\beta \leqq 2.15 \times 10^6$ the shape may be either a prolate ellipsoid of axial ratio $\geqq \frac{1}{3}$ or an oblate ellipsoid of any axial ratio. In such a situation flow birefringence measurements can be helpful in determining the dimensions especially since p, for an oblate ellipsoid, is not determinable with accuracy from β. Even though β is independent of p for oblate ellipsoids, δ does vary sufficiently with p so that the axial ratio is determinable from δ. Alternatively, use may be made of the fact that the rotary diffusion constant of an oblate ellipsoid[5] depends only on b for $b \gg a$. b is thus determinable from Θ. Once b is determined a can be calculated from the intrinsic viscosity according to eq. (1) or from the sedimentation constant according to eq. (4) and (5'). The experimental value of Θ can also be examined from the point of view of a prolate ellipsoid of axial ratio $\geqq \frac{1}{3}$ to decide which type of ellipsoid is consistent with the observed Θ and also the various experimental data used in the original calculation of β. For molecules which are not large enough it may be difficult to determine Θ. β is more amenable to accurate determination, especially since δ is not a very sensitive function of p for $1/p > 15$.

Thus eq. (6) and the auxiliary eq. (11) provide a basis for correlating the hydrodynamic behavior of proteins with an effective hydrodynamic ellipsoid whose dimensions are calculable. Of course, if the protein *is* anhydrous in solution then the effective ellipsoid applies to the anhydrous particle.[37] In general, though, the protein will be considerably hydrated. In such cases only the effective ellipsoid for the hydrated and not the anhydrous particle can be determined.

Table III gives the values of β for several proteins to show that the numerical values of β fall in the range indicated in Table I.

TABLE III

VALUES OF β FOR SEVERAL PROTEINS AND POLYMERS[a]

Substance	$[\eta]$	$s_{20} \times 10^{13}$	M	$\beta \times 10^{-6}$
Egg albumin	0.043	3.55	44,000	2.40
Horse serum albumin	.049	4.46	70,000	2.33
Hemoglobin	.040	4.48	63,000	2.34
Amandin	.052	12.5	330,000	2.30
Octopus hemocyanin	.067	49.3	2,800,000	2.36
Gliadin	.105	2.1	27,500	2.39
Homarus hemocyanin	.047	22.6	760,000	2.28
Helix pomatia hemocyanin	.047	98.9	6,600,000	2.36
Serum globulin	.067	7.1	167,000	2.27
Thyroglobulin	.071	19.2	630,000	2.35
Lactoglobulin	.045	3.12	41,500	2.28
Pepsin	.039	3.3	35,500	2.52
Tobacco mosaic virus	.285	185	33,200,000	2.63
Polystyrene in toluene				2.3
Polystyrene in methyl ethyl ketone				2.6
Cellulose acetate in acetone				2.7
Polyisobutylene in cyclohexane				2.5
Polysarcosine in water				2.3

[a] For the first 12 proteins, intrinsic viscosities are from Polson,[39] and sedimentation constants and sedimentation velocity–diffusion molecular weights are from Svedberg and Pedersen.[8] As pointed out by Cohn and Edsall[10] other values have been reported for these proteins. Data for tobacco mosaic virus are from Lauffer.[38] See reference 29 for the polymer data.

It should be kept in mind that all the quantities appearing in β must be determined in the same solvent. This may not be true for all the data listed and, therefore, no further calculations of

TABLE IV

CALCULATION OF DIMENSIONS OF EFFECTIVE ELLIPSOIDS IN UREA SOLUTIONS OF HORSE SERUM ALBUMIN USING DATA OF NEURATH AND SAUM[13] AT 25°

Urea concn., M	$[\eta]$	$D \times 10^7$	$\beta \times 10^{-6}$	r,[a] Å.
0	0.049	6.85	2.23	$a = 82, b = 16.4$
0.5	.050	6.20	2.04	38
1.5	.056	6.08	2.07	39
3.0	.065	5.69	2.04	41
4.5	.123	4.45	1.98	51
6.0	.147	4.27	2.01	54
6.66	.170	4.15	2.05	56

[a] For the native albumin the axes of the effective ellipsoid are listed. All other values in this column are the radii of the respective effective hydrodynamic spheres.

(33) R. Simha, "High Polymer Physics," Chemical Publ. Co., New York, N. Y., 1948, p. 398.

(34) J. Riseman and J. G. Kirkwood, *J. Chem. Phys.*, **17**, 442 (1949); **18**, 512 (1950). Also see footnote 5 in the 1949 paper for quotation of a personal communication from Simha about a similar conclusion.

(35) J. G. Kirkwood and P. L. Auer, *ibid.*, **19**, 281 (1951).

(36) N. Saito, *J. Phys. Soc. Japan*, **6**, 302 (1951).

(37) For example, tobacco mosaic virus has negligible hydration.[38]

(38) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

(39) A. Polson, *Kolloid Z.*, **88**, 51 (1939).

dimensions have been made with these values.[40] Data for several chain type polymers are also included[41] in Table III. The similar values of β for proteins and polymers seem to indicate that the same kind of hydrodynamic treatment is applicable to both kinds of molecules and that the point of view presented here appears reasonable.

Several examples, where the effects discussed here are large and rather striking, will serve to emphasize the need for considering the effective hydrodynamic ellipsoid.

Neurath and Saum[18] have investigated the denaturation of horse serum albumin by performing parallel diffusion and viscosity measurements in solvents containing various amounts of urea. The intrinsic viscosity increased and diffusion coefficient decreased with increasing urea concentration. This was interpreted in terms of increasing asymmetry (from about 5:1 to 20:1) arising from the uncoiling of polypeptide chains, the axial ratios being obtained from the intrinsic viscosity or diffusion measurements *alone*. If they are combined, and β computed as indicated in footnote 28, significantly different results are obtained as shown in Table IV.

Within the experimental error, all values of β, except for the native albumin, are 2.12 indicating that $p \geqq 1/2$. Thus, an asymmetrical prolate ellipsoid is completely ruled out. With these data alone, a further distinction cannot be made between a sphere or an oblate ellipsoid. Flow birefringence measurements on the same systems, making use of δ, would help. However, interpreting the data in terms of a sphere, for the present, the radii of these effective spheres are listed in the last column of Table IV. The denaturation process thus appears to involve an increased effective volume due to swelling instead of uncoiling of polypeptide chains. It is of interest to point out that Doty and Katz[43] concluded from light scattering studies of urea–water mixtures of bovine serum albumin that the "principal change undergone by the serum albumin molecule in concentrated urea solutions is that of approximately isotropic swelling" with preferential adsorption of either urea or water depending upon the *p*H.

As another example we may cite the recent flow birefringence studies of Foster and Samsa[44] on the denaturation of ovalbumin in the presence of urea. Here again, interpretations were based on rotary diffusion constants alone instead of a pair measurement of $[\eta]$–Θ to obtain δ. It was concluded that, in those cases where no aggregation occurred, the denaturation involved essentially an intramolecular unfolding. Foster and Samsa reported their data in terms of lengths which are presumed to have been calculated from observed Θ-values. As can be seen in eq. (10), decreased values of Θ upon denaturation could arise *either* from increased asymmetry *or* increased effective volume *or* both. Thus the interpretation that decreased Θ means chain unfolding is not necessarily correct. A pair measurement of $[\eta]$–Θ in the same solvent would be required to answer this question. As an illustration, particles whose effective ellipsoids have dimensions of a = 300Å., b = 10Å. and a = 228Å., b = 57Å., respectively, would both have the same rotary diffusion constants. Therefore, since the larger effective volume of the second particle could be the result of increased solvation, the flow birefringence measurements on urea-denaturated ovalbumin cannot be unambiguously interpreted in terms of chain unfolding.

The use of an effective hydrodynamic ellipsoidal model thus permits the protein molecule to be considered as a partially flexible, solvated one with its hydrodynamic properties related to the axial ratio of the effective ellipsoid by eq. (6) or (11). It is therefore possible in conjunction with eq. (1), (4) and (5′), or (10), to decide between an oblate and prolate shape and determine the dimensions of the effective ellipsoid. It remains to be seen whether suitable variation of the solvent medium can give rise to changes in the solute–solvent and intramolecular interactions which will be reflected in variations in a/b and V_e. This would be analogous to the variation of the root-mean-square end-to-end distance of a flexible polymer chain caused by such interactions and would provide a deeper insight into the problem of the configuration of protein molecules in solution. Also, this approach will be helpful for denaturation studies. For example, as can be seen from eq. (1) and as illustrated in the examples cited above, the increased viscosity usually observed in denaturation need not necessarily imply increased axial ratio but possibly increased effective volume of denaturated proteins. Thus, correct values of p and V_e in such cases can be obtained by the method indicated here, and a decision made as to the relative importance of increased asymmetry, on the one hand, and increased effective volume or swelling on the other, in protein denaturation.

(40) For bovine fibrinogen[21] a δ value of 0.99 is obtained from $[\eta]$ = 0.25, η_0/T = 1.34, and M = 407,000.

(41) It should not be inferred from the values of β for polymers that the chain molecule must be considered in terms of an effective ellipsoid. In the treatments previously given for the intrinsic viscosity[22] and for the frictional coefficient[27] the assumption was made in each case that the radius of the effective hydrodynamic sphere is proportional to an average linear dimension of the chain molecule in solution, and that the constants of proportionality applying to the viscosity and to the frictional coefficient, respectively, are the same for all high polymer chains. These constants were not assumed to be identical, however. On this basis, β should be a universal constant as is confirmed by experiment.[27,29] If it is further assumed that the equivalent spheres for the intrinsic viscosity and for the frictional coefficient are identical in size then β should be equal to 2.12×10^6 in disagreement with experiment. The hydrodynamic theory of Kirkwood and Riseman,[42] on the other hand, gives $\beta = 2.5 \times 10^6$. It would appear that the assumption of the identity of the equivalent spheres for chain molecules is not valid. This is not an unexpected result since the average distribution of segments for a chain polymer molecule is approximately Gaussian and, presumably, the linear parameter of the Gaussian distribution required for the intrinsic viscosity is not identical with that required for the frictional coefficient. For proteins, on the other hand, it is reasonable to assume that the distribution of segments along the axes of the molecule is rectangular so that one would expect the equivalent ellipsoids for the intrinsic viscosity and for the frictional coefficient to be identical. However, for flow birefringence studies in protein solutions, it is possible that the effective ellipsoid for the rotary diffusion constant may not be identical with those for the intrinsic viscosity and frictional coefficient. This arises because of the relatively high rates of shear used in flow birefringence measurements.

(42) See ref. (27) for the revisions introduced into the Kirkwood–Riseman theory and for further discussion of this point.

(43) P. Doty and S. Katz, Abstracts of A.C.S. Meeting, p. 14C, Chicago, Ill., September, 1950.

(44) J. F. Foster and E. G. Samsa, THIS JOURNAL, **73**, 5388 (1951).

The method developed here furnishes values of p and V_e by an analytical solution of two simultaneous equations. Such solutions, of course, should also be obtainable by graphical methods[7,9] without the necessity of introducing large experimental errors in the data. The effective volume in many cases cited,[7,9] calculated according to the procedure developed here, is less than $M\bar{v}/N$. Since, in the previous treatment,[7,9] $M\bar{v}/N$ has been interpreted as a part of the effective volume (*i.e.*, using the assumption that $V_e = M\bar{v}(1 + w/\bar{v}\rho)/N$), negative w values are, therefore, required to obtain consistency between intrinsic viscosity and frictional coefficient measurements in that procedure. This shows clearly that the interpretation of w and the procedure used formerly[7,9,10] are incorrect.

Accurate measurements of sedimentation constants, intrinsic viscosities, molecular weights, partial specific volumes and rotary diffusion constants for monodispersed native and denatured proteins in various solvents are required to explore the implications of the point of view presented here.

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The Preparation of Anhydrous Perchloric Acid

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An improved method is described for the preparation of anhydrous perchloric acid. The procedure involves the use of 72% perchloric acid and 20% fuming sulfuric acid. Mixtures of these acids in the proportions of 1 to 4, in the order given, serve as the reaction medium. The anhydrous perchloric acid is evolved from this mixture at pressures of 1 mm. or less and at temperatures from 27–75° in 75% yield. The product is completely recovered by chilling to Dry Ice temperatures. A discussion of the hazards involved is given. The process is favored also for the preparation of oxonium perchlorate OH_3ClO_4. The finished product is not contaminated by sulfuric acid.

Introduction

For many operations in the study of perchloric acid and its salts the use of anhydrous perchloric acid may be required. Because of the hazards involved, a procedure for the preparation of this product, to be suitable, should involve the use of starting materials which are readily available in pure form, an apparatus assembly of simple but effective design, and a procedure that involves the least hazardous manipulations. The objective in the present investigation was the study of operative procedures leading to this goal.

Applicable Reactions.—The most appropriate reactions serving as a preparative scheme are

$$KClO_4 + H_2SO_4 + \text{vac. distln. (50 mm).} \longrightarrow HClO_4\uparrow + KHSO_4 \quad (1)$$

$$x HClO_4\cdot 2H_2O + \text{vac. distiln. (18 mm., 120°)} \longrightarrow HClO_4\uparrow + HClO_4\cdot H_2O\uparrow + HClO_4\cdot 3H_2O\uparrow \quad (2)$$

$$HClO_4\cdot H_2O + \text{vac. distiln.} \longrightarrow HClO_4\uparrow + HClO_4\cdot 2H_2O \quad (3)$$

$$Cl_2O_7 + H_2O \longrightarrow 2HClO_4 \quad (4)$$

$$HClO_4\cdot 2H_2O + 2SO_3 + \text{vac. distiln.} \longrightarrow HClO_4\uparrow + 2H_2SO_4 \quad (5)$$

Reaction 1 has been employed frequently in studies involving the preparation of anhydrous perchloric acid for example by Roscoe[1] in one of the pioneer studies in this field. It is not a convenient process and was originally employed for the preparation of anhydrous perchloric acid to be at once diluted with water to 20 or 60% acid composition. This method was employed by van Wyk[2] and by van Emster[3] in important early studies of the physical constants of anhydrous perchloric acid.

Reaction 2 was employed by Goehler and Smith[4] in their study of the improved preparation of anhydrous perchloric acid and in the study of the dissociation of the concentrated acid at moderately low, (8–18 mm.), pressures. The yield of anhydrous acid by this procedure attained up to 10% of the starting material only.

Reaction 3 is convenient and effective but a supply of the monohydrated perchloric acid is dependent upon the preparation of anhydrous acid followed by dilution with water or the dihydrate of perchloric acid.

Reaction 4 involves the synthesis of the anhydride of perchloric acid, (Cl_2O_7), by the method of Michael and Cohn.[5] Aqueous perchloric acid is dehydrated by reaction with excess phosphoric anhydride, (P_2O_5), followed by distillation.

Reaction 5 is utilized in the present study. The reaction ingredients taken are commercially available in pure form. The apparatus employed is of simple design. The reaction temperature covers the range 25–80°. The yield ranges from 50–80% and the raw materials may be recovered.

General Description of the Process.—Fuming sulfuric acid (15–20%), is added in various proportions to 72% perchloric acid. The heat of reaction is moderate and the reaction mixture is chilled to 25°. This mixture is digested at gradually increasing temperatures, 25–80°, and at low pressure to volatilize anhydrous perchloric acid. The finished product is condensed using Dry Ice as coolant and collected as a colorless liquid, freezing point −112°. Anhydrous perchloric acid may be stored without explosive decomposition for 30–60 days at liquid air temperatures and without the accumulation of the least coloration from decomposition products. Pure samples do not explode when stored at ordinary temperatures for approximately 30 days.

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