necessary by the efficient photooxidation of stilbene and bromostilbene to phenanthrene or its derivative.[31]

Solutions of stilbenes in MCH/IP or MCH/IH were dried and deoxygenated by the usual Na–K alloy treatment.[13] Fluorescence spectra were measured at right angles to the exciting light, as described previously.[13] The exciting light was isolated by a Bausch & Lomb 500-mm grating monochromator from a 450-w Osram xenon lamp. No isomerization was observed during the recording of the fluorescence spectra. Fluorescence yields were determined by comparison with a solution of anthracene in ethanol, of absorbance 0.15 at 315 mμ, serving as a standard with $\phi_F = 0.27$.[32]

Photoisomerization quantum yields were measured and calculated by the method of Zimmerman, Chow, and Paick.[12,33] Quantum yields with 313-mμ light in glycerol triacetate were corrected for the absorption of light by this solvent ($D = 0.20$, independent of temperature). The quantum yields of isomerization and of fluorescence are probably accurate to better than ±10%, except the isomerization quantum yields below 0.1, which are much less accurate. Emission and absorption spectra, especially at low temperatures, did not show any evidence of dimerization, association, or aggregation of solute molecules, phase separation of components of the solvent mixtures, or cloudiness due to humidity.

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(32) C. A. Parker, *Advan. Photochem.*, 2, 305 (1964).
(33) G. Zimmerman, L. Chow, and U. Paick, *J. Am. Chem. Soc.*, 80, 3528 (1958).

Estimation of Particle Dimensions from the Relaxation of Transient Electric Birefringence of Suspensions

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Abstract: Analysis is continued of the relaxation of transient electric birefringence of suspensions of rigid particles. The relaxation curve of suspensions of a rigid particle of arbitrary shape is shown to be describable in general by a sum of two exponential terms, defining two relaxation constants. Apart from a physically unlikely class of exceptions, a relaxation curve consisting of a single exponential term is shown to occur only for an axisymmetric particle, and always in this case. For the asymmetric ellipsoid, combination of the relaxation constants with the particle volume is shown to lead to definition of two reduced relaxation constants which are functions only of the axial ratios of the particle. A procedure is presented for convenient estimation of particle dimensions from relaxation data in terms of these reduced quantities, and necessary computed values required in this procedure are tabulated for a wide range of axial ratios. Finally, the estimation procedure is applied to the protein hemocyanin from the snail, *Helix pomatia*.

Analysis of the relaxation of transient electric birefringence of protein solutions provides a convenient method for study of protein structure if suitable expressions are available to relate the relaxation constants inferred from the data to shape parameters of the individual particles. In an earlier paper[2] (designated in the following as RI), we have obtained a general equation for the birefringence of an oriented suspension of particles in terms of the angular probability density of the particles. Introducing results of the theory of rotational Brownian motion of Perrin,[3] we used this equation to write the specific form for the decay in birefringence of a suspension of asymmetric ellipsoidal particles in free rotation following initial orientation in terms of the optical and frictional properties of individual particles. The present investigation is an extension of the preceding work directed toward its generalization and application in estimation of particle shape.

Numerous problems in application are apparent from the theoretical analysis. The question of the actual amount of information in a relaxation curve gives rise to many of these. In the absence of knowledge about the general form of the relaxation curve, *i.e.*, for particles of arbitrary shape, uncertainty necessarily arises in connection with misinterpretations produced by assumption of an incorrect particle-shape class for the analysis. The lack of utility of definition of an equivalent-spheroid model in the study of protein structure has exemplified this problem in the past. The significance of a simple exponential decay of birefringence, the form observed for some proteins, is of particular interest because of the known result that it can arise in an ellipsoidal model for the monodisperse axisymmetric spheroid. Moreover, one concludes from the analysis of suspensions of the asymmetric ellipsoid that the relaxation curve does not itself contain enough information to specify uniquely the dimensions of an ellipsoid. The relaxation data therefore cannot be applied except in a confirmatory fashion to ellipsoids without determination of what additional observable parameters are required to obtain such specification. Finally, there are already described in the literature birefringence curves which do require the form of the relaxation equation derived for the asymmetric ellipsoid (as opposed to the spheroid) if one assumes the suspensions under study to be monodisperse. It is of interest to evaluate an example of such data in terms of the theory to establish

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(2) D. Ridgeway, *J. Am. Chem. Soc.*, 88, 1104 (1966).
(3) F. Perrin, *J. Phys. Radium*, [7] 5, 497 (1934); [7] 7, 1 (1936).

whether they are compatible with the asymmetric-ellipsoid model.

The present investigation consists of three sections. In the first, the form of the relaxation curve for suspensions of a rigid particle of arbitrary shape is obtained, and the condition under which it reduces to a simple exponential is given. The second section continues analysis of the asymmetric ellipsoid, the one class of completely asymmetric shape for which one can write the elements of the viscous drag tensor for the particle in terms of particle dimensions. A suitable procedure for estimation of axial lengths of asymmetric ellipsoids is presented, and values of derived relaxation times introduced in this procedure are tabulated for a wide range of axial lengths. Finally, in the third section, an example is given of application of the estimation procedure of section II to a particular protein, *Helix pomatia* hemocyanin.

I. Form of Birefringence Relaxation

We discuss first the generality of the relaxation equation for birefringence given in RI. The problem treated is that of a suspension of identical dielectric particles subjected to an orienting uniform electric field for some length of time and then removed suddenly. The typical particle may possess a permanent dipole moment of arbitrary fixed orientation, as well as an induced moment due to polarization. Of the assumptions made, we mention in particular the condition of applicability of Rayleigh–Gans light-scattering theory to the system. In RI a general expression for the time-dependent birefringence of an oriented suspension (eq RI22) is written in terms of the average values of functions of the angles formed by the instantaneous directions of axes fixed in the diffusing particles with their initial directions at the instant of removal of the field. The equation for relaxation of birefringence following sudden removal of the orienting field (eq RI23) is obtained from this expression by taking for the angular quantities results of theories of free-rotational Brownian motion (eq RI17). We give eq RI23 here for convenience of discussion

$$\Gamma = \frac{N}{2n_l}\left(A_+ e^{-6\Theta_- t} + A_- e^{-6\Theta_- t}\right) \tag{1}$$

where Γ is the birefringence, N the number density of particles in suspension, and n_l the index of refraction of the suspending medium; A_+ and A_- are complicated expressions, not to be displayed here, which depend on the initial orientation of particles and their dielectric and diffusion properties. The decay constants Θ_+ and Θ_- are given either by (RI19)

$$\Theta_\pm = \mathcal{R} \pm (\mathcal{R}^2 - \mathcal{P}^2)^{1/2} \tag{2}$$

in terms of the rotational diffusion constant R_i about the ith axis, with (RI18)

$$\mathcal{R} = \frac{1}{3}\Sigma\mathcal{R}_i \tag{3}$$

$$\mathcal{P}^2 = \frac{1}{3}\sum_{i>j}\sum\mathcal{R}_i\mathcal{R}_j$$

or by (RI19′)

$$\Theta_\pm = \frac{4\pi}{9}\left(\frac{kT}{\eta v}\right)\left[\sum_i \frac{1}{C_i'} \pm \left(\sum_i \frac{1}{C_i'^2} - \sum_{i>j}\sum \frac{1}{C_i' C_j'}\right)^{1/2}\right] \tag{4}$$

in terms of the frictional couple $C_i = (3\eta v/4\pi)C_i'$ encountered in the steady rotation of a particle at unit angular velocity about its ith axis (where η is the viscosity of the suspending medium, v the particle volume, and kT the thermal energy).

These equations are derived in RI with reference to the asymmetric ellipsoidal particle. The required solution to the rotational diffusion equation and a theory of Brownian motion relating the (macroscopic) diffusion constants to the frictional properties of individual ellipsoids and to the temperature were taken from Perrin.[3] It may be shown, however, that the equations apply directly to rigid particles of arbitrary shape. Those results which are taken from the Perrin theory for ellipsoids are algebraically identical with the analogous expressions given by Favro[4] in his general theory of rotational Brownian motion. Two series of equations are involved. The first concerns the averages of the angular quantities described above which appear in eq RI22. The identity of results of the two theories in this case is that of eq RI17 (from Perrin) and Favro's equation F6.10. This assures the generality of eq 2 here. The second concerns the relation between the rotational diffusion constants and viscous drag constants of individual particles. Favro's eq F2.7 defines a rotational diffusion tensor for particles in terms of elementary rotations which arise in his stochastic treatment. It is evident from eq F2.7 that the tensor is symmetric, so that it may always be diagonalized to define three orthogonal particle axes and the principal diffusion constants for rotation about them. In the case of the ellipsoid, these would coincide with the geometric axes, and the diffusion constants would be the C_i's employed by Perrin. The relation to elements of the viscous drag tensor is given in eq F7.10, which, for the diagonalized tensor, reduces to $\mathcal{R}_i = kT/C_i$. This is precisely the relation employed in going from eq 2 to 4 here. Equation 4 therefore applies, as does eq 2, without modification to rigid particles of arbitrary shape.

We find then that at most two relaxation constants may be observed for the relaxation curve of any monodisperse suspension (of rigid particles). Conversely, we conclude that it is not possible to infer the shape class of the suspended particle from the relaxation curve.

Let us now inquire into the origin of a simply exponential relaxation curve. It was pointed out in section IV of RI that the coefficient A_-, that of the more slowly decaying exponential in eq 1, vanishes identically in the case of the spheroid possessing optical as well as geometric symmetry about its axis. Since the expressions for the $A_\pm$'s are unchanged in going from the spheroid to the axisymmetric particle of arbitrary shape class, the same result obtains for the latter as well. Thus, axial symmetry is a sufficient condition for simply exponential relaxation behavior. We may demonstrate, however, that it is not a necessary condition, although the exception is probably rare. The singly exponential curve can arise either through the vanishing of one of the coefficients $A_\pm$ or through the equality $\Theta_+ = \Theta_-$. We investigate each of these possibilities in turn.

Consider first the vanishing of A_+ or A_-, a situation which arises at least in the case of the spheroid, as has been pointed out. The expressions (RI24) for $A_\pm$ con-

(4) L. D. Favro, *Phys. Rev.*, **119**, 53 (1960).

sist of sums of three terms, each weighted by a difference, $\langle a_{3j}^2 - a_{1j}^2 \rangle$, of direction cosines mentioned earlier which describe the initial positions of the particle axes of each particle relative to directions fixed in space. If the initial orientation of the particles is to be kept arbitrary in the treatment of the relaxation phenomena, it is required that the terms weighted by each of these differences vanish identically for the relaxation coefficient A_+ or A_- itself to vanish. This leads to three conditions

$$\begin{aligned} &2\alpha_1(1 \mp \Theta_1) - \alpha_2(1 \pm 2\Theta_3) - \alpha_3(1 \pm 2\Theta_2) = 0 \\ &-\alpha_1(1 \pm 2\Theta_3) + 2\alpha_2(1 \mp \Theta_2) - \alpha_3(1 \pm 2\Theta_1) = 0 \\ &-\alpha_1(1 \pm 2\Theta_2) - \alpha_2(1 \pm 2\Theta_1) + 2\alpha_3(1 \mp \Theta_3) = 0 \end{aligned} \quad (5)$$

where (RI18)

$$\Theta_i = \frac{\Re_i - \Re}{2(\Re^2 - \Im^2)^{1/2}} \quad (6)$$

Equations 5 are formally three simultaneous equations for three unknowns, *i.e.*, the α_i's. The associated matrix is found, upon introduction of (6) into (5) and observation that the Θ_i's are of mean zero and mean-square $1/2$, to be singular and of order one (that is, the complementary minor of each element is singular, whereas the elements themselves are in general nonzero). The row space of the matrix is therefore of dimension one, so that the three equations in (5) are in fact equivalent, and any solution to one of the three is a solution to the other two as well. It follows that any particle for which the principal polarizabilities α_i and the Θ_i, derivable from its principal frictional constants, are related in a way which satisfies one of the equations in (5) leads to a vanishing of one (or both) of the coefficients $A_\pm$ and to a one-term exponential relaxation curve (or to zero birefringence). We mention a particular solution required from physical considerations, that of the completely isotropic case, in which the three α_i's are equal, for which the distinction between upper and lower signs is lost and both coefficients vanish simultaneously.

The only other way in which a simply exponential relaxation curve could arise from eq 1 is that in which the two relaxation constants Θ_+ and Θ_- are equal. It is evident from eq 4 that this equality arises if and only if

$$\sum \frac{1}{C_i^2} = \sum_{i>j} \frac{1}{C_i C_j} \quad (7)$$

Since this is true only in the completely degenerate case, $C_1 = C_2 = C_3$ (*i.e.*, for the sphere), condition 7 is never fulfilled by an ellipsoid and cannot lead to $\Theta_+ = \Theta_-$.

Let us summarize the additional information provided by a one-term exponential relaxation curve. First, this relaxation behavior always implies that the optical and frictional properties of the suspended particle are related in a way which satisfies one of the three equivalent equations in (5). Since these properties are related in an asymmetric particle only in a very weak fashion, through their common dependence on the axial ratios, the coincidental agreement with (5) in the asymmetric case must be very unlikely. In contrast, the degenerate case in which the particle possesses optical and geometric rotational symmetry about one of its axes always satisfies eq 5 for the lower sign to lead to a zero A_- in the relaxation equations. The singly exponential relaxation curve thus provides strong evidence that the suspended particle is axisymmetric, although a class of exceptions does exist.

II. Ellipsoidal Dimensions

Equation 4 relates the observable relaxation constants to the shapes of individual suspended particles directly. However, it is useful in determining particle shape only if expressions are available for the viscous drag constants C_i in terms of particle dimensions for the shape class of the particle. The most general shape class for which the C_i's are known is that of the asymmetric ellipsoid. Since proteins are frequently represented as ellipsoids, it is worthwhile to develop a method of interpreting measured relaxation constants in terms of this shape class. This is the purpose of the present section.

Evaluation of relaxation constants presents two problems. First, the $\Theta_\pm$'s do not themselves contain enough information to permit estimation of shape, as was pointed out in RI. We must, therefore, determine what additional knowledge about the particle is necessary to specify its dimensions uniquely. The second problem is associated with the nature of the decay constants themselves. It is physically evident that the relaxation properties of the ellipsoid must depend on its actual dimensions and not simply on its axial ratios. However, it is found that the expressions for the decay constants of the ellipsoid cannot be written in closed form except in the degenerate case of the spheroid, so that it is desirable to tabulate their computed values once and for all. Unless one defines a quantity independent of particle size scale from which the values of the decay constants can be inferred, given the additional knowledge about the particle selected in connection with the first problem here, it is necessary to retabulate the values of the decay constants for each new size scale. It is found that these problems are closely related and may be removed simultaneously.

We select the particle volume as an additional parameter of the particle because methods of its determination are open to less question than are those for any other measure of size scale. We then combine the volume with the particle frictional constants to derive a dimensionless decay parameter of the type described. For the asymmetric ellipsoid, the derived frictional constants C_i' (in eq 4) may be obtained directly from the expression for the principal frictional couples given by Edwardes[5]

$$C_i' = \frac{16\pi}{3} \frac{a_j'^2 + a_k'^2}{a_j'^2 P_j' + a_k'^2 P_k'} \quad (8)$$

where i, j, and k are all different, $a_i' = a_i/a_3$, a_i being the length of the ith semiaxis (we shall refer to the a_i' as the ratio lengths in the following) and, for definiteness, $a_1 > a_2 > a_3$, and where

$$P_i' = \frac{3v}{4\pi} \int_0^\infty \frac{d\lambda}{(a_i^2 + \lambda)K} \quad (9)$$

with $K^2 = (a_1^2 + \lambda)(a_2^2 + \lambda)(a_3^2 + \lambda)$ and v being the particle volume as before. Suitable expressions for computation of the elliptic integrals here are given in RI from Osborn.[6] Since the P_i''s, and therefore the C_i''s,

(5) D. Edwardes, *Quart. J. Math.*, 26, 70 (1893).
(6) J. A. Osborn, *Phys. Rev.*, 67, 351 (1945).

Table I. Tabulation of $100\Theta_{\pm}^{red} = 100\,(\eta v/kT)\Theta_{\pm}$ for Paired Values of Ratio Lengths a_1 and a_2 of Rigid Ellipsoids[a]

	1	2	3	4	6	8	10	15	25	50	100
1	*16.746*	17.467	17.227	17.045	16.856	16.672	16.744	16.702	16.680	16.669	16.657
2	11.101	*13.569*	14.912	13.996	13.717	13.569	13.488	13.397	13.346	13.223	11.652
3	7.1496	9.7074	*11.411*	10.857	10.567	10.380	10.265	10.125	10.041	10.001	9.9911
4	4.9336	7.1977	8.0550	*9.0652*	8.5163	8.3294	8.1985	8.0246	7.9106	7.8552	7.8403
6	2.7668	4.3374	5.2255	5.6688	*6.3149*	5.9890	5.8710	5.6744	5.5182	5.4337	5.4099
8	1.7851	2.8992	3.6301	4.0878	4.4878	*4.8134*	4.6025	4.4273	4.2520	4.1441	4.1119
10	1.2558	2.0830	2.6692	3.0780	3.5294	3.6833	*3.8804*	3.6532	3.4789	3.3535	3.3136
15	0.64951	1.1087	1.4644	1.7434	2.1311	2.3552	2.4712	*2.5161*	2.4340	2.2900	2.2339
25	0.27519	0.48158	0.65191	0.79580	1.0249	1.1944	1.3182	1.4884	*1.5706*	1.4405	1.3662
50	0.082814	0.14826	0.20489	0.25510	0.34159	0.41417	0.47600	0.59464	0.72595	*0.78362*	0.72285
100	0.024205	0.037832	0.061657	0.077704	0.10638	0.13169	0.15442	0.20295	0.27484	0.36652	*0.39255*

[a] Above the diagonal, elements $c_{ij} = 100\Theta_{+}^{red}$ for ellipsoids of axial ratios $j:1:1$. Below the diagonal, $c_{ij} = 100\Theta_{-}^{red}$ for ellipsoids of axial ratios $i:j:1$. Additional explanation is given in the text.

are dimensionless and as such independent of the particle size scale, the same is true of the quantity inside the square brackets on the right side of eq 4. We satisfy the above considerations, then, by defining a reduced decay constant

$$\Theta_{\pm}^{red} = \left(\frac{\eta}{kT}\right) v\Theta_{\pm} \tag{10}$$

It depends entirely on experimentally determined quantities and requires knowledge only of the solution conditions η and T and of the parameters we wish to combine, *i.e.*, the decay constants $\Theta_{\pm}$ and the volume v. It is seen from eq 4 that the reduced decay constants are dimensionless and therefore in properly normalized form for computation and tabulation. We shall find that the reduced decay constants in eq 10 do specify the particle dimensions uniquely, at least in the completely asymmetric case, so that they do remove the two problems discussed.

Computed values of $\Theta_{\pm}^{red}$ over a wide range of ratio lengths are compiled in Table I. In order to conserve space, we have brought values for both relaxation constants together in a single table, the value of Θ_{+}^{red} being given above the diagonal and those of Θ_{-}^{red} below the diagonal. The diagonal terms are printed in italics to facilitate separation of the table. The elements a_{1j} of row 1 in the table are the values for prolate spheroids of axial ratio j, and the diagonal elements a_{jj} are the values for oblate spheroids of axial ratio $1/j$.

The method of analysis of birefringence and volume data employing such tabulated values is best described in terms of its geometric significance. Let us consider the three-dimensional plot of the reduced decay constant Θ_{+}^{red} against the ratio lengths a_1' and a_2' (we recall that $a_3' = 1$ for all ellipsoids). The function $\Theta_{+}^{red}(a_1', a_2')$ is a surface in this space, onto which any given ellipsoid may be mapped as a point. The projection onto the $a_1'a_2'$ plane of the intersection of this surface and the plane Θ_{+}^{red} = constant is the locus of points (a_1', a_2') compatible with that value of Θ_{+}^{red}. An analogous projection exists for a given value of Θ_{-}^{red}. The intersections of these two projected curves in the $a_1'a_2'$ plane correspond to the only paired values of ratio lengths simultaneously compatible with the assumed values of the reduced relaxation constants. Two sets of plots are required, the permanent one of the values in Table I and the individual plot of projections corresponding to a particular protein. For the permanent plots, it is apparent from perspective drawings of the three-dimensional surfaces that Θ_{+}^{red} should be presented as a family of curves of Θ_{+}^{red} against a_2' for constant values of a_1', and Θ_{-}^{red} as a family of curves of Θ_{-}^{red} against a_1' for constant values of a_2'. For a particular relaxation curve, then, paired points (a_1', a_2') corresponding to intersections of each of the members of these families in the permanent plots with the appropriate values of Θ_{+}^{red} and Θ_{-}^{red} are obtained by inspection from the permanent plots. (The decision to present the computational results here by means of Table I rather than in graphical form is based on the greater precision provided by the tabulated values.) We note, finally, that the proper scale factor for a given ellipsoid for conversion from ratio lengths to physical lengths is provided by the relation $a_3^3 = 3v/4\pi a_1'a_2'$, where a_3 is, again, the shortest semiaxial length in the same system of units as the volume.

In working with this graphical method, we have not found a situation in which the projected curves intersected in more than one point for an asymmetric ellipsoid. Except in cases in which there are multiple intersections, determination of the axial lengths is, of course, unique.

III. Application

It is useful to illustrate the methods described here by application to a specific set of data. We select for discussion the hemocyanin of the snail, *Helix pomatia*, because of the availability of data on it. It is to be emphasized, however, that there is no reason to reject the original interpretation of Pytkowicz and O'Konski[7] of their relaxation data as reflecting the presence of aggregated material found in freshly prepared solutions which disappears upon long standing.

In the pH 4.6–7.4 region, hemocyanin, the copper-carrying respiratory pigment of mollusks and other lower invertebrates, has been shown from its sedimentation behavior to exist as a single species. Outside this pH range to either the acid or alkaline side, or at high ionic strengths, the protein dissociates in a manner which suggests that the molecule at neutral pH is an aggregate of several subunits. These observations are confirmed by electron micrographs.[8]

Pytkowicz and O'Konski have studied the relaxation of electric birefringence of snail hemocyanin at neutral pH following sudden removal of the external field. They found that the relaxation curve can be fitted very well

(7) R. M. Pytkowicz, and C. T. O'Konski, *Biochem. Biophys. Acta*, **36**, 466 (1959).

(8) E. F. J. Van Bruggen, E. H. Wiebenga, and M. Gruber, *J. Mol. Biol.*, **4**, 1 (1962).

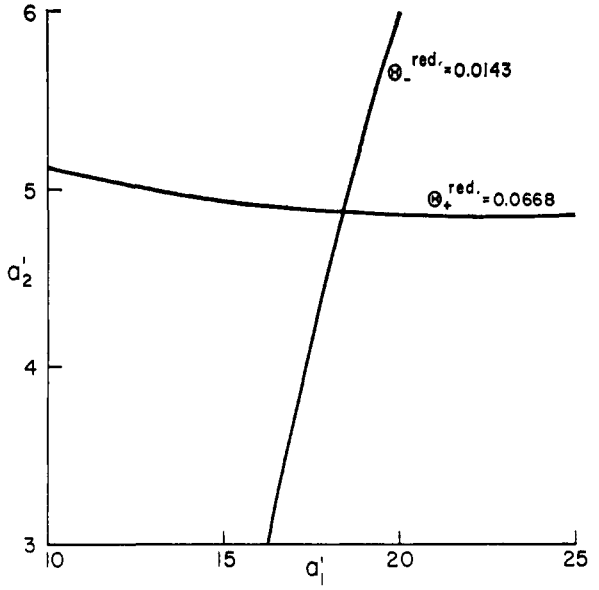

Figure 1. Plots of ratio lengths compatible with values of the reduced decay constants $\Theta_+^{red} = 0.0668$ and $\Theta_-^{red} = 0.01443$ as estimated by the method described in the text and data in Table I.

with a relation of the form of eq 1. A mixed decay curve such as this is characteristic of three types of suspensions of rigid ellipsoids.[9] These are, respectively, mixed suspensions of two different kinds of spheroids, a suspension of a single kind of spheroid consisting of a material which in bulk is itself anisotropic, and a monodisperse suspension of an asymmetric ellipsoid. Although the ratio of the two relaxation times is predictable for the second case, so that one might demonstrate it as such, the first and third cases cannot be distinguished from the observed relaxation times alone. Pytkowicz and O'Konski interpreted the two relaxation times in terms of a mixed suspension of a circular cylinder and its end-to-end dimer. Let us, in contrast, assume a monodisperse suspension and determine the axial lengths of an asymmetric ellipsoid of the volume of the undissociated hemocyanin molecule which would lead to the observed relaxation times.

In estimating the dimensions of the molecule, we shall take the molecular weight of the dry protein to be 8.9×10^6 (from sedimentation velocity)[10] the partial specific volume in water 0.738,[10] the hydration 0.5 g of water/g of dry protein (a typical value), and the birefringence decay constants[8] $\Theta_+ = 15 \times 10^3$ and $\Theta_- = 3.2 \times 10^3$ sec^{-1}. If the density of water of hydration is assumed to be that of pure water, then at 25° the hydrated volume of the protein, as inferred from partial specific volume data, is 2.75×10^{-17} cm^3, and the reduced decay constants are $\Theta_+^{red} = 0.0668$ and $\Theta_-^{red} = 0.01443$, respectively. The projected a_1a_2-locus plots described in section II for these values are shown in Figure 1.

The intersection of the two curves corresponds to the ratio lengths $1 \times 4.89 \times 18.5$. The axial lengths of an ellipsoid of the assumed volume with these ratio lengths are $83.4 \times 408.3 \times 1540$ A. If the assumption is made that the hydration layer is of the uniform thickness d over the surface of the anhydrous molecule, then the value of d may be estimated from the equation

$$\frac{4\pi}{3}(a - d)(b - d)(c - d) = v_{anh} \qquad (11)$$

where v_{anh} is the volume of the anhydrous protein molecule. Substituting the estimated values of the hydrated molecule for a, b, and c, and 1.091×10^{-17} cm^3 for v_{anh}, one finds that $d = 22.5$ A. The dimensions of the anhydrous molecule are thus $38.4 \times 363.4 \times 1495$ A. A monodisperse suspension of a ribbon-shaped molecule of these dimensions would produce the birefringence relaxation curve observed by Pytkowicz and O'Konski.

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(9) D. Ridgeway, *Virginia J. Sci.*, 17, 194 (1966).

(10) S. Brohult, *J. Phys. Colloid Chem.*, 51, 206 (1951).