A NUMERICAL INVERSION OF THE PERRIN EQUATIONS FOR ROTATIONAL DIFFUSION CONSTANTS FOR ELLIPSOIDS OF REVOLUTION BY ITERATIVE TECHNIQUES

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ABSTRACT The rotational diffusion coefficients R_1 and R_3 for ellipsoids of revolution are shown to represent another pair of hydrodynamic data to obtain size and shape with theories by Sadron and Scheraga-Mandelkern. An iterative numerical technique is presented which allows the semiaxes to be determined from the Perrin equations for rotational diffusion constants. The use of this inversion technique is illustrated by application to literature data from dielectric dispersion studies.

INTRODUCTION

The study of hydrodynamic properties of macromolecular solutions is widely used to obtain information about the shape and dimensions of the macromolecule. Interpretation of the hydrodynamic data which leads to such information is based on an assumption that the macromolecule may be represented as a model particle. The model is restricted to those particles for which the theory exists relating the hydrodynamic property to the geometric parameters that characterize the particle.

Sadron (1) showed that all hydrodynamic parameters could be represented as a product of the volume and a shape factor for the particle. Scheraga and Mandelkern (2) developed a theory for interpreting hydrodynamic measurements in terms of an equivalent hydrodynamic ellipsoid. This theory is based on the simultaneous observation of two hydrodynamic properties of the protein solution, which allows both factors of shape and size to be determined, and is therefore consistent with that of Sadron.

Scheraga and Mandelkern derived two functions β and δ which are dependent on the axial ratio for both prolate and oblate ellipsoids. β is derived from a combination of the hydrodynamic properties; intrinsic viscosity and the translational frictional coefficient. δ is derived from a combination of intrinsic viscosity and the rotational diffusion coefficient. The rotational diffusion coefficient used is for rotation of the symmetry axis about a transverse axis of an ellipsoid of revolution. In a recent review, Bradbury (3) has listed other combinations of hydrodynamic properties which have been used to determine the dimensions of the equivalent ellipsoid. The purpose of this work is (*a*) to present the use of another pair of hydrodynamic parameters, the rotational diffusion coefficients R_1 and R_3, to obtain the size and shape consistent with the theory of Scheraga-Mandelkern; and (*b*) to present an iterative technique for inverting the Perrin equations, relating the rotational diffusion coefficients to the semiaxes of an equivalent rigid ellipsoid of revolution. Literature data are used to illustrate the application of this procedure.

THEORY

Several hydrodynamic techniques exist for which theory has been developed expressing two rotational diffusion coefficients for ellipsoids of revolution (4–6). It is therefore possible, in some cases, to obtain experimental measurements of the two distinct rotational diffusion coefficients for equivalent ellipsoids of revolution R_1 and R_3. The utility of determining the rotational diffusion coefficients is based upon the inverse functions of the Perrin equations. Numerical procedures are presented here which approximate these inverse functions for the prolate ellipsoid $a_1 < a_3$ and for the oblate ellipsoid $a_1 < a_3$.

It is convenient to remove the solution conditions η and T by defining reduced rotational diffusion coefficients,

$$R' = (16\pi\eta/3kT)R. \tag{1}$$

Case I: Prolate Ellipsoid

The Perrin equations for the reduced rotational diffusion coefficients are (7, 8).

$$\begin{aligned} R_1' &= f(\epsilon)/a_3^3, \\ R_3' &= g(\epsilon)/a_3^3, \end{aligned} \tag{2}$$

where

$$\begin{aligned} g(\epsilon) &= \frac{1}{2\epsilon^3}\left[\frac{2\epsilon}{1-\epsilon^2} - \ln\frac{1+\epsilon}{1-\epsilon}\right], \\ f(\epsilon) &= \frac{2}{(2-\epsilon^2)(1-\epsilon^2)} - \frac{1+\epsilon^2}{1-\epsilon^2}\,g(\epsilon). \end{aligned} \tag{3}$$

The argument was chosen to be the eccentricity $\epsilon = (1 - a_1^2/a_3^2)^{1/2}$.

The tabulated functions of Eqs. 3 are given as the column headings of Table I. The use of the table is based on the relationships which exist between the several columns, according to Eqs. 2. Interpolation procedures for the table are as follows:

TABLE I

THE ROTARY DIFFUSION COEFFICIENTS RATIO FUNCTIONS FOR PROLATE ELLIPSOIDS

ϵ	$f(\epsilon)$	$g(\epsilon)$	R_3/R_1
0.1	0.6727	0.6748	1.0030
0.2	0.6914	0.7001	1.0125
0.3	0.7248	0.7463	1.0297
0.4	0.7764	0.8210	1.0574
0.5	0.8532	0.9389	1.1005
0.6	0.9674	1.1313	1.1694
0.7	1.1435	1.4730	1.2881
0.8	1.4386	2.1946	1.5255
0.9	2.0342	4.4782	2.2014
0.91	2.1316	4.9979	2.3446
0.92	2.2420	5.6512	2.5206
0.93	2.3688	6.4964	2.7425
0.94	2.5169	7.6302	3.0317
0.95	2.6940	9.2279	3.4254
0.96	2.9130	11.6407	3.9962
0.97	3.1977	15.6908	4.9069
0.98	3.6017	23.8526	6.6226
0.99	4.2953	48.5439	11.3017
0.991	4.4008	54.0511	12.2821
0.992	4.5188	60.9412	13.4862
0.993	4.6525	69.8077	15.0042
0.994	4.8069	81.6402	16.9839
0.995	4.9895	98.2209	19.6854
0.996	5.2130	123.1147	23.6169
0.997	5.5010	164.6432	29.9296
0.998	5.9069	247.7801	41.9478
0.999	6.6004	497.4405	75.3648
0.9991	6.7058	552.9441	82.4571
0.9992	6.8237	622.3305	91.2018
0.9993	6.9572	711.5502	102.2747
0.9994	7.1114	830.5216	116.7867
0.9995	7.2938	997.0979	136.7047
0.9996	7.5170	1246.9872	165.8890
0.9997	7.8047	1663.5110	213.1414
0.9998	8.2102	2496.6425	304.0888
0.9999	8.9034	4996.2970	561.1649

(a) determine the bounds in the table of the ratio R_3/R_1, calculated from experiment, and the corresponding bounds of the eccentricity. (b) Determine $f(\epsilon)$ for the two values of ϵ and calculate a_3 from Eqs. 2 which is consistent with the reduced rotational diffusion coefficient R_1' and a_1 from the argument ϵ. The semiaxial lengths for the bounding values of ϵ determine a straight line in the a_3, a_1 plane. (c) Determine $g(\epsilon)$ as above, computing values of a_3 and a_1 for the bounds consistent with the reduced rotational diffusion coefficient R_3'. These values determine a straight line in the same plane. The intersection of these two straight lines corresponds to a first estimate of the semiaxial lengths of the prolate equivalent ellipsoid consistent with the experimental observations.

The straight lines discussed in the above interpolation procedure are actually the chords AB and CD of the contours $R_1'(a_1, a_3)$ = constant and $R_3'(a_1, a_3)$ = constant shown in Fig. 1. Lines of constant eccentricity appear as straight lines through the origin. The intersections of the lines corresponding to the upper and lower bounds of the eccentricity with the curves of constants R_1' and R_3' determine the points A, B and C, D, respectively. The intersection of the chords AB and CD determine a new eccentricity ϵ' which approximates the true value determined by the intersection of the curves R_1' and R_3'. ϵ' intersects these curves at points A′ and B′, respectively, as shown in Fig. 2. Pairs of coordinates a_3, a_1 are calculated for the

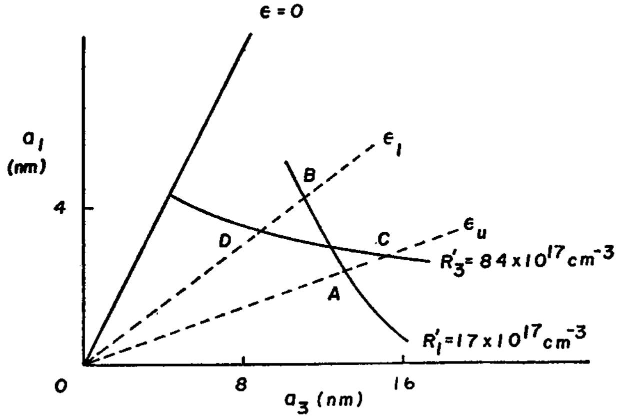

FIGURE 1 Contours of reduced rotary diffusion coefficients and lines of constant eccentricity for prolate ellipsoids.

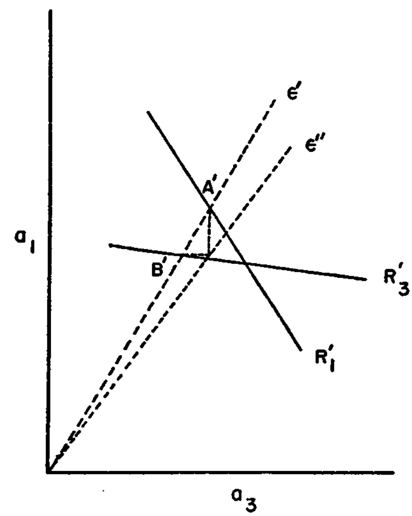

FIGURE 2 Diagram of an intermediate step in the iterative procedure for prolate case.

two points A′ and B′. Choosing a_3 from the point A′ and a_1 from the point B′ determines a new estimate of the true semiaxes. This procedure may be repeated until some fixed convergence criterion is satisfied.

Case II: Oblate Ellipsoid.

The method of analysis for the oblate ellipsoid is similar to that for the prolate ellipsoid, however interesting differences arise. The reduced rotary diffusion coefficients are (7, 8).

$$R_1' = k(\epsilon)/a_1^3\,,$$
$$R_3' = h(\epsilon)/a_1^3\,, \tag{4}$$

where

$$k(\epsilon) = \frac{1}{2-\epsilon^2}\,[2(1-\epsilon^2)^{1/2} - (1-2\epsilon^2)h(\epsilon)],$$

$$h(\epsilon) = \frac{1}{\epsilon^3}\,[\arcsin\epsilon - \epsilon(1-\epsilon^2)^{1/2}]. \tag{5}$$

The eccentricity characterizing the oblate ellipsoid is $\epsilon = (1 - a_3^2/a_1^2)^{1/2}$. The tabulated functions of Eqs. 5 are given as the column headings of Table II. The interpolation procedures for Table II are similar to those for Table I.

TABLE II
THE ROTARY DIFFUSION COEFFICIENTS RATIO FUNCTIONS FOR OBLATE ELLIPSOIDS

ϵ	$h(\epsilon)$	$k(\epsilon)$	R_1/R_3
0.0000	0.6667	0.6667	1.0000
0.3815	0.6983	0.7300	1.0454
0.5201	0.7303	0.7943	1.0870
0.6152	0.7629	0.8581	1.1247
0.6871	0.7962	0.9220	1.1580
0.7440	0.8302	0.9853	1.1868
0.7902	0.8651	1.0476	1.2109
0.8285	0.9011	1.1083	1.2300
0.8605	0.9383	1.1671	1.2439
0.8874	0.9768	1.2236	1.2527
0.9102	1.0168	1.2772	1.2561
0.9295	1.0586	1.3276	1.2541
0.9457	1.1025	1.3744	1.2467
0.9594	1.1486	1.4172	1.2339
0.9708	1.1973	1.4555	1.2157
0.9800	1.2490	1.4891	1.1922
0.9874	1.3041	1.5174	1.1635
0.9930	1.3632	1.5401	1.1298
0.9969	1.4268	1.5569	1.0912
0.9992	1.4957	1.5672	1.0478

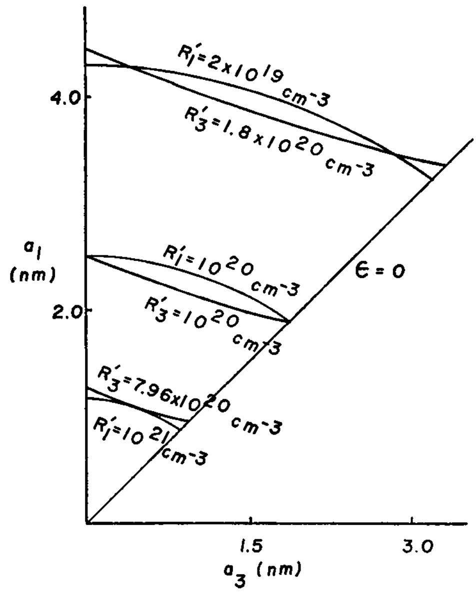

FIGURE 3 Three distinct types of contours for reduced rotary diffusion coefficients for oblate ellipsoids.

The interesting differences between cases I and II are made apparent by comparing the behavior of ratios of the rotational diffusion coefficients as functions of the eccentricity. In case I, R_3/R_1 increases monotonically as a function of the eccentricity, whereas R_1/R_3 in case II passes through a maximum. This maximum provides a constraint on the relative values of the rotational diffusion coefficients for oblate ellipsoids. Additionally, since this ratio passes through a maximum, there correspond two values of the argument for a given value of R_1/R_3. This implies that two different oblate ellipsoids yield identical rotational diffusion coefficients.

The interpolation procedure for Table II is divided into two steps, one for each oblate ellipsoid. The first step involves the determination of the semiaxial lengths corresponding to the smaller eccentricity. The iteration procedure is identical with case I. For the larger eccentricity iteration procedure involves choosing the complimentary coordinate as the succeeding pairs of coordinates a_3, a_1. This is because the roles of R_1' and R_3' in the iteration procedure have been interchanged since the relative magnitudes of their slopes changed. As in the prolate case, the iteration procedure is repeated until some convergence criterion is satisfied.

The contours $R_1'(a_3, a_1)$ = constant and $R_3'(a_3, a_1)$ = constant for several different values are shown in Fig. 3. The two points of intersection for the contours $R_1' = 2 \times 10^{19}$ cm^{-3} and $R_3' = 1.8 \times 10^{20}$ cm^{-3} show the desired two solutions for steps 1 and 2. The contours $R_1' = 10^{21}$ cm^{-3} and $R_3' = 7.96 \times 10^{20}$ cm^{-3} are tangential to each other at the point which corresponds to the maximum ratio $R_1/R_3 = 1.2561$.

The corresponding eccentricity, $\epsilon = 0.9129$ is a straight line which separates steps 1 and 2. The remaining contour shows the limiting properties of spheres. The semiaxial lengths for the two oblate ellipsoids of steps 1 and 2 in Fig. 3 satisfy the explicit forms of the Perrin ratios for oblate ellipsoids as given by Edsall (8).

In order to demonstrate the ability of the numerical procedure to provide estimates of the desired semiaxial lengths, rotational diffusion coefficients were calculated for several values of typical semiaxial lengths. These rotational diffusion coefficients were then used as data for the estimation procedure. All computations were carried out on an IBM 360/40 computer. The chosen convergence criterion was $|R_1/R_3 - (\hat{R}_1/\hat{R}_3)| < 10^{-4} R_1/R_3$ or 25 iterations, whichever came first, for prolate ellipsoids and for oblate ellipsoids the ratios appearing in the inequality are written as their inverses. $\hat{R}_1$ and $\hat{R}_3$ are the calculated constants corresponding to the last estimates calculated for a_1 and a_3. Table III shows the comparisons of the exact dimensions with the estimated dimensions obtained in the above manner. From the table it appears that the estimation procedure for the prolate case converges more rapidly than for the oblate case. By increasing the number of iterations allowed,

TABLE III

COMPARISON OF DIMENSIONS ELLIPSOIDS OF REVOLUTION WITH ESTIMATES FROM INVERSE FUNCTIONS OF ROTATIONAL DIFFUSION COEFFICIENTS

	Exact dimensions		*Estimated dimensions*	
	a_1	a_3	a_1	a_3
	nm		*nm*	
Prolate	1.5	2.0	1.50005	1.99984
	1.5	2.5	1.49999	2.49999
	1.5	3.0	1.50004	2.99987
	1.5	6.0	1.50004	5.99982
	1.5	15.0	1.50000	14.9999
	1.5	30.0	1.50005	29.9994
	3.0	3.8	3.00008	3.79971
	3.0	5.0	3.00000	4.99998
	3.0	6.0	3.00007	5.99974
	3.0	12.0	3.00009	11.9996
	3.0	30.0	2.99999	29.9998
	3.0	60.0	3.00011	59.9987
Oblate	11.11	10.0	11.1064	9.99860
			14.1828*	0.708762*
	14.8	10.0	14.8269	9.99694
			17.0887*	3.18927*
	10.0	2.45	9.99897	2.45295*
			9.03218	5.36248
	5.145	1.0	5.14442*	1.00088
			4.48876*	2.97451*
	10.0	0.0	9.99995	0.00000

* Truncated at 25 iterations.

more precise estimates for the dimensions of oblate ellipsoids could be obtained. However, all estimates are within the range of precision of experimentally determined rotational diffusion coefficients.

APPLICATION

The dielectric dispersion data presented by Oncley (9) are readily applicable to the estimation procedure developed above. Those proteins which required two relaxation times to fit the dispersion curves were interpreted by Oncley to be ellipsoids of revolution. The relaxation times reported by Oncley were used to calculate the reduced rotary diffusion coefficients for ellipsoids of revolution given by Eq. 1. The estimates for the semimajor and minor axes obtained by the above procedure are shown in Table IV.

By comparison of the observed dispersion curves with theoretical dispersion curves, which are defined in terms of the parameters $\rho = a/b$, the axial ratio, and τ_0, the relaxation time for the sphere of equal volume, Oncley obtained values of these parameters for each protein studied. The semiaxial lengths can be obtained from ρ and τ_0 according to Eqs. 6.

$$\begin{aligned} a_1 &= (kT\tau_0/4\pi\eta\rho)^{1/3}, \\ a_3 &= (KT\tau_0\rho^2/4\pi\eta)^{1/3}. \end{aligned} \tag{6}$$

Values of a_1 and a_3 calculated by Eqs. 6 are also given in Table IV. The estimation procedure based on the observed relaxation times yields values of the semiaxial lengths which are in agreement with those based on the parameters ρ and τ_0. The concept of the sphere of equal volume is not necessary to the estimation procedure in terms of the observed relaxation times.

TABLE IV

COMPARISON OF SEMIAXIAL LENGTHS IN NANOMETERS OBTAINED BY DIFFERENT ESTIMATION PROCEDURES FOR DATA REPORTED BY ONCLEY

	$a_1(\tau_1\ \tau_2)$	$a_3(\tau_1, \tau_2)$	$a_1(\tau_0, \rho)$	$a_3(\tau_0, \rho)$
β-Lactoglobulin (4, 25)	1.54	6.32	1.56	6.23
β-Lactoglobulin (2, 25)	1.60	6.33	1.60	6.39
β-Lactoglobulin (2, 0)	1.22	4.94	1.22	4.88
Egg albumin	1.41	7.09	1.39	6.97
Horse serum albumin	1.55	9.21	1.54	9.25
Horse serum pseudoglobulin-γ (25)	2.06	18.7	2.08	18.7
Edestin	1.85	16.8	1.88	16.7
Gliadin	0.81	6.37	0.82	6.55
Secalin	0.66	6.77	0.67	6.67
Zein (25)	0.93	6.29	0.92	6.44
Zein (0.8)	0.65	4.36	0.64	4.49

* Numbers in parentheses characterize the different protein solutions as presented by Oncley.

TABLE V

COMPARISON OF SEMIAXIAL LENGTHS IN NANOMETERS OBTAINED BY DIFFERENT ESTIMATION PROCEDURES FOR DATA OF ROSSENEU-MOTREFF ET AL.

	$a_1(\tau_1, \tau_2)$	$a_3(\tau_1, \tau_2)$	$a_1(\delta, V_e)$	$a_3(\delta, V_e)$
Apotransferrin	2.72	6.20	2.46	6.20
Transferrin	2.85	5.77	2.76	5.52

A second application is based on the interpretation of the data reported by Rosseneu-Motreff et al. (10) for dielectric dispersion of apotransferrin and transferrin monomers. For each protein, they reported two relaxation times which were extrapolated to infinite dilution. At infinite dilution the viscosity of the solvent may be taken to be that of water at the temperature of the solution, which was 25°C. The fast and slow relaxation times, in microseconds, were 0.135 and 0.215 for apotransferrin, and 0.135 and 0.195 for transferrin, respectively. These values were used to calculate the reduced rotary diffusion coefficients, as above, which lead to estimates of the semiaxial lengths shown in Table V.

Rosseneu-Motreff et al. reported values of the intrinsic viscosity for these proteins, and, based on the theory of Scheraga and Mandelkern, calculated the dimensions of the ellipsoid of revolution. These dimensions were obtained from the δ function of Scheraga and Mandelkern, and the equivalent hydrodynamic volume V_e and are also shown in Table V. It is noted that the dielectric dispersion data is sufficient for the determination of the semiaxial lengths and therefore precludes the necessity of determining the intrinsic viscosity.

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1. SADRON, C. 1953. *In* Progress in Biophysics and Biophysical Chemistry. J. A. V. Butler and J. T. Randall, editors. Pergamon Press, New York. 3:237.
2. SCHERAGA, H. A., and L. MANDELKERN. 1953. *J. Am. Chem. Soc.* **75**:197.
3. BRADBURY, J. H. 1970. *In* Physical Principles and Techniques of Protein Chemistry. S. J. Leach, editor. Academic Press, Inc., New York. Pt. B, Chapter 11.
4. FERRY, J. D., and J. L. ONCLEY. 1938. *J. Am. Chem. Soc.* **60**:1123.
5. RIDGEWAY, D. 1966. *J. Amer. Chem. Soc.* **88**:1104.
6. TAO, T. 1969. *Biopolymers.* **8**:609.
7. PERRIN, R. 1934. *J. Phys. Radium.* **5**:497.
8. EDSALL, J. T. 1965. *In* Proteins, Amino Acids, and Peptides. E. J. Cohn and J. T. Edsall, editors. Hafner Publishing Co., Inc., New York. Chapter 21.
9. ONCLEY, J. L. 1965. *In* Proteins, Amino Acids, and Peptides. E. J. Cohn and J. T. Edsall, editors. Hafner Publishing Co., Inc., New York. Chapter 22.
10. ROSSENEU-MOTREFF, M. Y., F. SOETEWEY, R. LAMOTE, and H. PEETERS. 1971. *Biopolymers.* **10**:1039.