A NUMERICAL INVERSION OF THE PERRIN EQUATIONS FOR ROTATIONAL AND TRANSLATIONAL DIFFUSION CONSTANTS BY ITERATIVE TECHNIQUES

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ABSTRACT An iterative numerical technique is presented which allows the semiaxes for prolate and oblate ellipsoids to be determined from the Perrin equations for rotational and translational diffusion constants. The use of this inversion technique is illustrated by application to the proteins: lysozyme, bovine serum albumin, human transferrin, and bovine rhodopsin solubilized in digitonin.

INTRODUCTION

The relatively new technique of laser beat frequency spectroscopy has greatly facilitated experimental determination of the translational diffusion coefficient, D, for macromolecules in solution (Dubin et al. [1], and Cummins et al. [2]). Additionally, the spectrum of the depolarized scattered light has been shown to contain information on the rotational diffusion coefficient, R_1, for macromolecules (3–5). R_1 denotes the rotational diffusion coefficient for an ellipsoid of revolution rotating about an axis normal to its symmetry axis.

These hydrodynamic parameters, D and R_1, contain sufficient information to allow determination of the dimensions of the hydrodynamic equivalent ellipsoid presented in the theory of Scheraga and Mandelkern (6). Dubin et al. (7) determined these parameters for lysozyme and calculated the dimensions of the prolate and oblate equivalent ellipsoids consistent with Perrin's equations (8). However, their method of inverting the Perrin equations was not discussed.

Benoit et al. (9) have presented a graphical procedure for the inversion of the Perrin equations for D and R_1. However, such procedures are not easily converted for use with small digital computers. For this reason, we present in this paper a numerical procedure for the inversion of the Perrin equations for D and R_1, and demonstrate the accuracy of the method. Also, literature data are used to illustrate the application of this method. This procedure is similar to that previously published for inversion of the Perrin equations for R_1 and R_3, where R_3 denotes the rotational diffusion constant for rotation about the symmetry axis of the ellipsoid of revolution (10).

THEORY

Since the Perrin equations for D and R_1 are functions of the solution conditions η and T, it is convenient to remove these conditions by defining reduced diffusion coefficients,

$$D' = (6\pi\eta/kT)D, \text{ and } R_1' = (16\pi\eta/3kT)R_1. \tag{1}$$

Case I: Prolate Ellipsoid, $a_1 < a_3$

The Perrin equations for the reduced rotational diffusion constants are

$$D' = d(\epsilon)/a_3$$
$$R_1' = f(\epsilon)/a_3^3, \tag{2}$$

where[1]

$$d(\epsilon) = ln[(1 + \epsilon)/(1 - \epsilon^2)^{1/2}]/\epsilon$$
$$f(\epsilon) = 2/(2 - \epsilon^2)(1 - \epsilon^2) - [(1 + \epsilon^2)/(2 - \epsilon^2)]g(\epsilon)$$
$$g(\epsilon) = (1/2\epsilon^3)[2\epsilon/(1 - \epsilon^2) - ln(1 + \epsilon)/(1 - \epsilon))] \tag{3}$$

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. Although the interpolation procedures for the table are similar to those presented in the earlier paper (10), we present them here for completeness: (*a*) Determine the bounds of the ratio, D'^3/R_1' from Table I and the corresponding bounds of the eccentricity. (*b*) Determine $f(\epsilon)$ for the two values of ϵ and calculate a_3 utilizing Eqs. 2 which is consistent with the reduced rotational diffusion coefficient R_1'. Using this a_3, determine 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 $d(\epsilon)$ as above, computing values of a_3 and a_1 for the bounds consistent with the reduced translational diffusion coefficient D'. 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 chords AB and UV of the contours $R_1'(a_1, a_3)$ = constant and $D'(a_1, a_3)$ = constant shown in Fig. 1. Straight lines through the origin correspond to constant eccentricities. Therefore, intersections of lines corresponding to the bounding eccentricities with the curves R_1' and D' determine the points A, B and U, V, respectively. The intersection of the chords AB and UV determine a new eccentricity ϵ' which approximates the true value determined by the intersection of the curves R_1' and D'. ϵ' intersects these curves at

[1] The equations for f and g were presented earlier (10); however, the equation for f contained a typographical error which is corrected here.

TABLE I

TRANSLATIONAL AND ROTATIONAL DIFFUSION COEFFICIENTS RATIO FUNCTIONS FOR PROLATE ELLIPSOIDS

ε	$f(\varepsilon)$	$d(\varepsilon)$	D'^3/R_1'
0.1	0.6727	1.0034	1.5015
0.2	0.6914	1.0137	1.5063
0.3	0.7247	1.0317	1.5153
0.4	0.7764	1.0591	1.5301
0.5	0.8532	1.0986	1.5541
0.6	0.9674	1.1552	1.5938
0.7	1.1435	1.2390	1.6633
0.8	1.4386	1.3733	1.8002
0.9	2.0342	1.6358	2.1517
0.91	2.1316	1.6786	2.2188
0.92	2.2420	1.7272	2.2982
0.93	2.3688	1.7832	2.3938
0.94	2.5169	1.8490	2.5116
0.95	2.6940	1.9282	2.6611
0.96	2.9130	2.0270	2.8590
0.97	3.1977	2.1570	3.1385
0.98	3.6017	2.3444	3.5778
0.99	4.2953	2.6734	4.4483
0.991	4.4008	2.7241	4.5934
0.992	4.5188	2.7810	4.7596
0.993	4.6525	2.8457	4.9529
0.994	4.8069	2.9206	5.1826
0.995	4.9895	3.0095	5.4631
0.996	5.2130	3.1188	5.8192
0.997	5.5010	3.2602	6.2991
0.998	5.9069	3.4603	7.0143
0.999	6.6004	3.8040	8.3397
0.9991	6.7058	3.8564	8.5523
0.9992	6.8237	3.9150	8.7935
0.9993	6.9572	3.9814	9.0713
0.9994	7.1114	4.0581	9.3978
0.9995	7.2938	4.1490	9.7919
0.9996	7.5170	4.2602	10.2860
0.9997	7.8047	4.4037	10.9418
0.9998	8.2102	4.6060	11.9022

points B' and V', respectively, as shown in Fig. 2. Pairs of coordinates a_3, a_1 are calculated for the two points B' and V'. Choosing a_3 from the point B' and a_1 from the point V' determines a new estimate of the true semiaxes. This procedure may be repeated until some fixed convergence criterion is satisfied.

Case II: Oblate Ellipsoid, $a_1 > a_3$

The method of analysis for the oblate ellipsoid is similar to that for the prolate ellipsoid. The reduced rotational and translational diffusion constants are (8)

$$D' = t(\epsilon)/a_1$$

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

where

$$t(\epsilon) = \tan^{-1}[\epsilon^2/(1-\epsilon^2)]^{1/2}/\epsilon$$

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

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

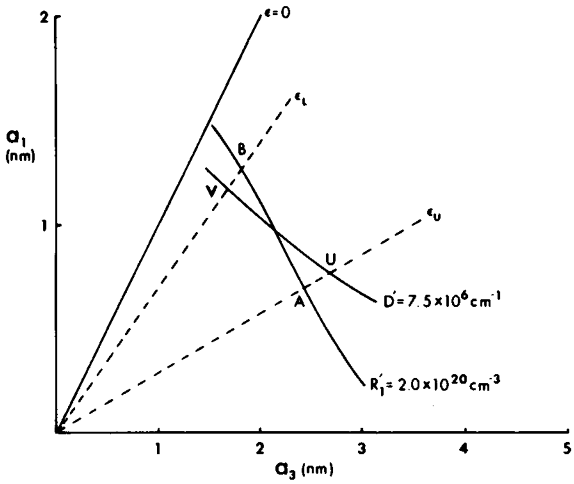

FIGURE 1

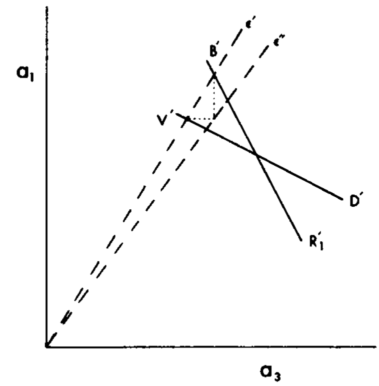

FIGURE 2

FIGURE 1 Contours of reduced rotational and translational diffusion constants and lines of constant eccentricity for prolate ellipsoids.
FIGURE 2 Diagram of an intermediate step in the iterative procedure for the prolate case.

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 interpolations procedures for Table II are similar to those for Table I.

Figure 3 shows the contours for several values of Perrin's reduced equations for translational and rotational diffusion. Interesting differences between cases I and II are made apparent by this figure. The lower octant corresponds to case I and the upper octant to case II. For case I, only a single solution exists for a given pair of values D' and R_1'. For case II, two, one, or no solutions exist for a given pair of values D' and R_1', depending on the value of the ratio D'^3/R_1'. This is more clearly described in terms of this ratio considered as a function of eccentricity. For case I:

$$\lim_{\epsilon \to 0} D'^3/R_1' = 1.5$$
$$\lim_{\epsilon \to 1} D'^3/R_1' = 51$$

and for case II:

$$\lim_{\epsilon \to 0} D'^3/R_1' = 1.5$$
$$\lim_{\epsilon \to 1} D'^3/R_1' = 2.467.$$

Examination of Table II shows that the ratio passes through a minimum value as the eccentricity varies from 0 to 1. Therefore, for ratios greater than 1.5 and less than 2.466, only one solution exists. For ratios greater than 1.4532 and less than 1.5, two solutions exist. Case II is therefore ruled invalid for ratios less than 1.4532 and greater than 2.467.

TABLE II

TRANSLATIONAL AND ROTATIONAL DIFFUSION COEFFICIENTS RATIO FUNCTIONS FOR OBLATE ELLIPSOIDS

ε	$k(\varepsilon)$	$t(\varepsilon)$	D'^3/R_1'
0.1	0.6707	1.0017	1.4985
0.2	0.6830	1.0068	1.4941
0.3	0.7046	1.0156	1.4870
0.4	0.7369	1.0288	1.4777
0.5	0.7827	1.0472	1.4672
0.6	0.8464	1.0725	1.4576
0.7	0.9352	1.1077	1.4533
0.8	1.0622	1.1591	1.4661
0.9	1.2525	1.2442	1.5377
0.91	1.2768	1.2564	1.5532
0.92	1.3023	1.2697	1.5717
0.93	1.3291	1.2843	1.5939
0.94	1.3574	1.3007	1.6210
0.95	1.3874	1.3192	1.6547
0.96	1.4191	1.3406	1.6978
0.97	1.4529	1.3662	1.7552
0.98	1.4890	1.3984	1.8366
0.99	1.5279	1.4437	1.9694
0.991	1.5320	1.4496	1.9882
0.992	1.5361	1.4559	2.0089
0.993	1.5402	1.4626	2.0315
0.994	1.5444	1.4700	2.0568
0.995	1.5487	1.4781	2.0854
0.996	1.5530	1.4873	2.1184
0.997	1.5573	1.4978	2.1577
0.998	1.5617	1.5106	2.2070
0.999	1.5662	1.5276	2.2760
0.9991	1.5667	1.5297	2.2850
0.9992	1.5671	1.5320	2.2945
0.9993	1.5676	1.5345	2.3048
0.9994	1.5680	1.5371	2.3160
0.9995	1.5685	1.5399	2.3283
0.9996	1.5689	1.5431	2.3421
0.9997	1.5694	1.5468	2.3580
0.9998	1.5699	1.5511	2.3772
0.9999	1.5703	1.5568	2.4028

In order to demonstrate the accuracy of the numerical procedure presented in this paper, translational and rotational diffusion coefficients were calculated for several values of typical semiaxial lengths. The diffusion coefficients were then used as data for the estimation procedure. All computations were carried out on a DEC PDP/11 computer. The chosen convergence criterion was $|D^3/R_1 - \hat{D}^3/\hat{R}_1| \leq 10^{-4}D^3/R_1$ or 50 iterations, whichever came first. $\hat{D}$ and $\hat{R}_1$ are the calculated diffusion constants corresponding to the last estimates calculated for a_1 and a_3. Table III shows the comparisons of the exact dimensions with the first order estimates and limiting estimates obtained in the above manner. From the table it appears that the first order estimates are within 0.5% of the exact dimensions for case I. Therefore, for practical purposes, first order estimates obtained according to this procedure are well within the accuracy obtainable by present experimental methods of determining diffusion coefficients.

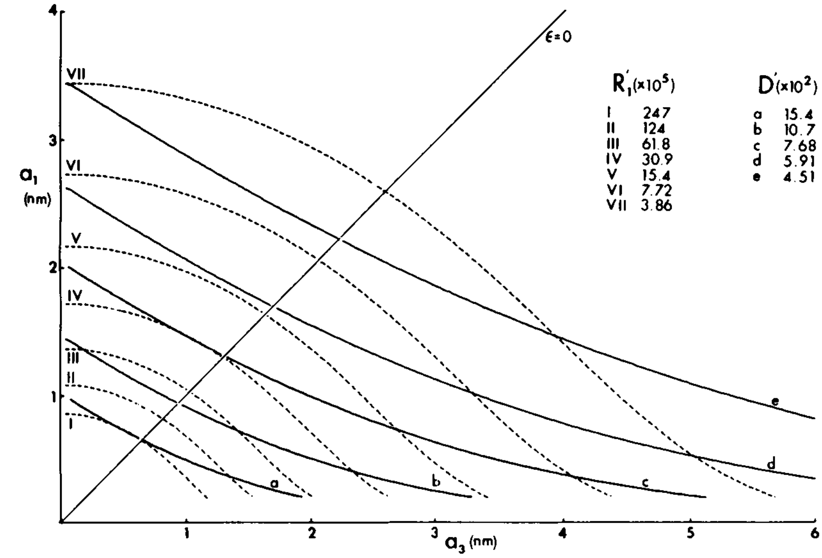

FIGURE 3 Parametric family of curves for reduced rotational and translational diffusion constants for prolate and oblate ellipsoids.

TABLE III
COMPARISON OF EXACT DIMENSIONS WITH ESTIMATES OBTAINED BY INVERSION PROCEDURES

	Exact dimensions		First-order dimensions		Estimated dimensions	
	a_1	a_3	a_1	a_3	a_1	a_3
	nm		*nm*		*nm*	
Prolate	1.5	2.0	1.5014	1.9975	1.5001	1.9998
	1.5	2.5	1.5001	2.4999	1.5000	2.4999
	1.5	3.0	1.5076	2.9884	1.5001	2.9998
	1.5	6.0	1.5007	5.9996	1.5001	5.9998
	1.5	15.0	1.5000	15.0001	1.5000	15.0000
	1.5	30.0	1.5085	30.0071	1.5002	29.9994
	3.0	3.8	3.0007	3.7985	3.0002	3.7995
	3.0	5.0	3.0002	4.9997	3.0001	4.9997
	3.0	6.0	3.0151	5.9767	3.0002	5.9996
	3.0	12.0	3.0014	11.9993	3.0003	11.9996
	3.0	30.0	3.0000	30.0001	3.0000	30.0000
	3.0	60.0	3.0170	60.0142	3.0004	59.9989
	100.0	200.0	100.5034	199.2246	100.0075	199.9883
Oblate	11.11	10.0	11.1158	9.9854	11.1126	9.9953
			12.5490	7.2816	12.5976	7.1928*
	14.8	10.0	14.3979	10.7586	14.3494	10.8512*
			14.6678	10.2489	14.7207	10.1540*
	10.0	2.45	9.9996	2.4505	9.9999	2.4505
	5.145	1.0	5.1530	0.9442	5.1452	0.9988*

*Truncated 50 iterations.

APPLICATION

The values for the translational and rotational diffusion constants determined by Dubin et al. (7), using techniques of quasielastic light scattering, for lysozyme are $D_{20,w} = (10.6 \pm 0.1) \times 10^{-7}\,\text{cm}^2/\text{s}$ and $R_{1_{20,w}} = (16.7 \pm 0.8) \times 10^6/\text{s}$. Using these data they calculated, according to their inversion procedure, the following ellipsoids of revolution:

$$\text{prolate:}\quad 2a_3 = (55 \pm 1)\,\text{Å},\qquad 2a_1 = (33 \pm 1)\,\text{Å},$$

$$\text{oblate:}\quad 2a_3 = (12.5 \pm 0.3)\,\text{Å},\qquad 2a_1 = (55 \pm 1)\,\text{Å}.$$

Substitution of these diffusion constants into the iteration procedure presented here yields the following ellipsoids of revolution:

$$\text{prolate:}\quad 2a_3 = (55 \pm 1)\,\text{Å},\qquad 2a_1 = 34.1\,\text{Å},$$

$$\text{oblate:}\quad 2a_3 = 13.6\,\text{Å},\qquad 2a_1 = 55.1\,\text{Å}.$$

The differences in the two sets of estimates reflect the different inversion procedures and, based on the inherent accuracy of the inversion procedure presented here (see Table III), show the advantage of using this procedure.

Additional examples of the results of the inversion procedure presented here are shown for bovine serum albumin, human transferrin, and bovine rhodopsin solubilized in digitonin.

The rotational diffusion constant for BSA determined by transient birefringence is $R_{1_{20,w}} = 1.93 \times 10^6/\text{s}$ (11). The translational diffusion constant has been determined to be $D_{20,w} = 6.15 \times 10^{-7}\,\text{cm}^2/\text{s}$ (12, 13). These values yield the following dimensions for a prolate ellipsoid: $2a_3 = 144.5$ Å and $2a_1 = 38.1$ Å. These values are in excellent agreement with those reported earlier (11, 14, 15, 16).

The rotational diffusion constant for human transferrin determined by transient birefringence is $R_{1_{20,w}} = 2.80 \times 10^6/\text{s}$ (17). Roberts et al. (18) reported the translational diffusion constant to be $D_{20,w} = 5.85 \times 10^{-7}\,\text{cm}^2/\text{s}$. According to these data the dimensions of the prolate ellipsoid are $2a_3 = 97.76$ Å and $2a_1 = 61.2$ Å. These values are in good agreement with those reported by Rosseneu-Motreff et al. (19) and by Wright (17).

The rotational diffusion constant for bovine rhodopsin solubilized in digitonin determined by transient birefringence is $R_{1_{25,\eta}} = 1.16 \times 10^6/\text{s}$ (20), where the solvent viscosity was determined to be 0.8782 cP. Hubbard (21) has reported the translational diffusion constant $D_{20,w}$ for rhodopsin solubilized in digitonin to lie within the range 3.5 to $4.9 \times 10^{-7}\,\text{cm}^2/\text{s}$. Choosing the upper value and adjusting to the solvent conditions 25° C, 0.8782 cP results in the following dimensions for the prolate ellipsoid; $2a_3 = 194.4$ Å and $2a_1 = 34.9$ Å. These values are in good agreement with those calculated by Wright (22) using data reported by Strackee (23), and by Wright (2) based on transient birefringence studies. These latter dimensions are $2a_3 = 200$ Å and $2a_1 = 29.8$ Å.

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REFERENCES

1. DUBIN, S. B., J. H. LUNACEK, and G. B. BENEDEK. 1967. Observation of the spectrum of light scattered by solutions of biological macromolecules. *Proc. Natl. Sci. U.S.* **57**:1164.
2. CUMMINS, H. Z., F. D. CARLSON, T. J. HERBERT, and C. WOODS. 1969. Translational and rotational diffusion constants of tobacco mosaic virus from Rayleigh linewidths. *Biophys. J.* **9**:518.
3. WADA, A., N. SUDA, T. TSUDA, and K. SODA. 1969. Rotatory-diffusion broadening of Rayleigh lines scattered from optically anisotropic macromolecules in solution. *J. Chem. Phys.* **50**:31.
4. PECORA, R. 1964. Doppler shifts in light scattering from pure liquids and polymer solutions. *J. Chem. Phys.* **40**:1604.
5. CAROLI, C., and O. PARODI. 1969. Frequency spectrum of the depolarized light scattered by a rigid molecule in solution. *J. Phys.* **B2**:1229.
6. SCHERAGA, H. A., and L. MANDEKERN. 1953. Consideration of the hydrodynamic properties of proteins. *J. Am. Chem. Soc.* **75**:179.
7. DUBIN, S. B., N. A. CLARK, and G. B. BENEDEK. 1971. Measurement of the rotational diffusion coefficient of lysozyme by depolarized light scattering: configuration of lysozyme in solution. *J. Chem. Phys.* **54**:5158.
8. PERRIN, F. 1934. Mouvement Brownien d'un ellipsoide (I). Dispersion dielectrique pour des molecules ellipsoidales. *J. Phys. Radium.* **5**:497.
9. BENOIT, H., L. FREUND, and G. SPACH. 1967. Dilute solutions of polypeptides: light scattering and hydrodynamics. *In* Poly-α-Amino Acids. G. D. Fasman, editor. Arnold, London. 105.
10. WRIGHT, A. K., R. C. DUNCAN, and K. A. BEEKMAN. 1973. A numerical inversion of the Perrin equations for rotational diffusion constants for ellipsoids of revolution by iterative techniques. *Biophys. J.* **13**:795.
11. WRIGHT, A. K., and M. R. THOMPSON. 1975. Hydrodynamic structure of bovine serum albumin determined by transient electric birefringence. *Biophys. J.* **15**:137.
12. CREETH, J. M. 1952. The use of the Gouy diffusiometer with dilute protein solutions. An assessment of the accuracy of the method. *Biochem. J.* **51**:10.
13. RAJ, T., and W. H. FLYGARE. 1974. Diffusion studies of bovine serum albumin by quasielastic light scattering. *Biochemistry.* **13**:3336.
14. MOSER, P., P. G. SQUIRE, and C. T. O'KONSKI. 1966. Electric polarization in proteins—dielectric dispersion and Kerr effect studies of isoionic bovine serum albumin. *J. Phys. Chem.* **70**:744.
15. SQUIRE, P. G., P. MOSER, and C. T. O'KONSKI. 1968. The hydrodynamic properties of bovine serum albumin monomer and dimer. *Biochemistry.* **7**:4261.
16. ROSSENEU-MOTREFF, M. Y., F. SOETEWEY, R. LAMOTE, and H. PEETERS. 1973. Dielectric study of the urea denaturation of delipidated and relipidated bovine serum albumin. *Biopolymers.* **13**:1259.
17. WRIGHT, A. K. 1976. Shape studies on macromolecules in aqueous and detergent solvents by transient electric birefringence. *J. Colloid Interface Sci.* **55**:109.
18. ROBERTS, R. C., D. G. MAKEY, and U. S. SEAL. 1966. Human transferrin: molecular weight and sedimentation properties. *J. Biol. Chem.* **241**:4907.
19. ROSSENEU-MOTREFF, M. Y., F. SOETEWEY, R. LAMOTE, and H. PEETERS. 1971. Size and shape determination of apotransferrin and transferrin monomers. *Biopolymers.* **10**:1039.
20. WRIGHT, A. K. 1976. A study of rhodopsin-detergent micelles by transient electric birefringence. *Biophys. Chem.* **4**:199.
21. HUBBARD, R. 1953. The molecular weight of rhodopsin and the nature of the rhodopsin-digitonin complex. *J. Gen. Physiol.* **37**:381.
22. WRIGHT, A. K. 1974. Ellipsoid models for rotational diffusion of rhodopsin in a digitonin micelle and in the visual receptor membrane. *Biophys. J.* **14**:243.
23. STRACKEE, L. 1971. Rotational diffusion of rhodopsin-digitonin micelles studies by transient photodichroism. *Biophys. J.* **11**:728.