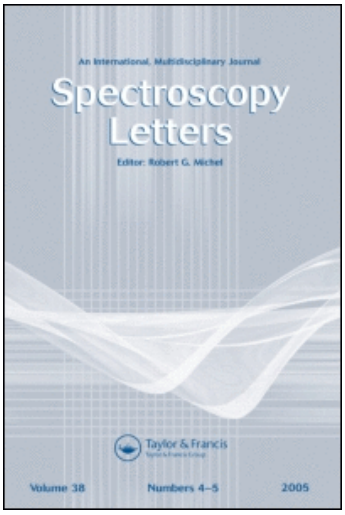

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M. A. Rebolledo[a]; J. C. Abad[a]
[a] Departamento de Física Fundamental. Facultad de Ciencias, Universidad de Santander, España

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DETERMINATION OF LENGTHS AND DIAMETERS OF ROD-LIKE MACROMOLECULES BY INTENSITY FLUCTUATION SPECTROSCOPY.

M.A. Rebolledo and J.C. Abad

Departamento de Física Fundamental. Facultad de Ciencias.Universidad de Santander.España.

This work presents a numerical study on the possibility of identifying the length and the diameter of rod-like macromolecules in diluted solution,when the laser light scattered by the solution is analyzed by means of intensity fluctuation spectroscopy (IFS).This study is made by computer-simulation of the second order normalyzed factorial moment ,$n^{(2)}(T)$, of the laser light scattered by the macromolecules.The results are presented and discussed.

The spectroscopic information in IFS is usually obtained from the second order temporal coherence degree $g^{(2)}(\tau)$. For the case of rod-like macromolecules [1] it is accomplished

$$g^{(2)}(\tau)=1+C\left|(B_0/B)\exp(-D_T K^2\tau)+(B_1/B)\exp\left[-(D_T K^2+6D_R)\tau\right]\right|^2 \qquad (1)$$

where C is a spatial coherence factor, K the modulus of the scattering vector (that depends on the scattering angle θ), D_T the translational diffusion coefficient, D_R the rotational diffusion coefficient and B_0 , B_1 , B the well-known coefficients of Pecora [2] given by the following equations

$$B_0=\left[(2/KL)\int_0^{KL/2}(\sin x/x)dx\right]^2 \qquad (2)$$

$$B_1 = 5\left\{(1/KL)\left[-3j_1(KL/2) + \int_0^{KL/2} (\sin x/x)dx\right]\right\}^2 \qquad (3)$$

$$B = (2/KL)\int_0^{KL} (\sin x/x)dx - \left[(\sin KL/2)/(KL/2)\right]^2 \qquad (4)$$

L being the length of the rod and j_1 the Bessel spherical function of first order. The information can also be obtained from the second order normalyzed factorial moment, $n^{(2)}(T)$, of the counting distribution, which is related immediately to $g^{(2)}(\tau)$ through [3]

$$\partial^2[T^2 n^{(2)}(T)]/\partial T^2 = 2g^{(2)}(T) \qquad (5)$$

where T is the counting time.

Since the objective of this work is the measurement of the length L and the diameter D of rods, it is necessary to know how D_T and D_R depend on L and D. In spite of the fact that no exact solution is known for this problem, several approximate models have been developed, like the equivalent ellipsoid model [4], models based on the internal rod structure [5,6,7], and empirical models like that of Broersma [8,9]. It seems to us that this last model is the most suitable of them and is the one we have utilized. According to it

$$D_T = [K_B T(2\sigma - \gamma_T)]/(6\pi\eta L) \qquad D_R = [3K_B T(\sigma - \gamma_R)]/(\pi\eta L^3) \qquad (6)$$

where

$$\sigma = \ln(2L/D)$$
$$\gamma_T = 1.65 - 4\left[(1/\sigma) - 0.43\right]^2 - 8\left[(1/\sigma) - 0.30\right]^2 \quad , \text{ when } \sigma > 2 \qquad (7)$$
$$\gamma_R = 1.57 - 7\left[(1/\sigma) - 0.28\right]^2$$

K_B being the constant of Boltzmann, T the temperature in Kelvin degrees and η the solvent viscosity.

Tobacco mosaic virus TMV (L=2980 Å , D=180 Å) is the rod-like macromolecule to which the most works on this area have been devoted. Cummins et al. [10] performed experimental measurements on TMV, obtaining for D_T and D_R values that approximate to those predicted by the theoretical models, but

which did not agree with them as much as it could be expected. Maeda and Saito [11] suggested that this disagreement could be due to a phenomenon of anisotropy in the movement of the particles. But the measurements carried out by Schaefer et al. [12] showed that this phenomenon was not enough to explain the discrepancy. On the other hand the results for D_R obtained from the analysis of the depolarized component of the scattered light [13, 14, 15] showed the same divergence between experiment and theory. Though this divergence could be attributed to deficiencies in the theoretical models, it seems more reasonable to attribute it to the characteristic peculiarities of TMV [4] (internal cylindrical hole, not homogeneous distribution of its mass, etc). We think that this last hypothesis is confirmed by Newman et al. [16] results. These authors found a good agreement between the experimental and the tehoretical results obtained from Broersma's model for the fd-DNA cylindrical virus (L=9150 Å , D=85 Å). This is why we consider that Broersma's model gives an adequate description of the diffusion coefficients D_T , D_R of a rod-like particle and it is the model we have utilized in this work.

Equations (2), (3), (4), (6) and (7) substituted into eqs. (1) and (5) provide us with expressions of $g^{(2)}(\tau)$ and $n^{(2)}(T)$ as functions of L and D. In this article we just try to study the possibility of determining L and D from one of these expressions in an experiment of IFS. We have utilized $n^{(2)}(T)$ and have made a computer-simulation of the values of $n^{(2)}(T)$ that would be obtained in an experiment by means of a fast simulation method developed by us [17]. These simulated values include the fluctuations due to the detection noise.

We have selected several cases for different values of L, D and of the scattering angle θ. Ten series of values of $n^{(2)}(T)$ have been simulated for each case, each one of them in five different values of the counting time. The simulation of a value of $n^{(2)}(T)$ is carried out starting from its theoretical value, calculated from L , D , θ and eqs. (1) to (7). Afterwards the ten series of values of $n^{(2)}(T)$ corresponding to each case are fitted to the theoretical expression by a least squares procedure. A value of L and another of D are obtained from the fitting of each series. With the values of L and D given by the ten series we have calculated $\langle L \rangle$, $\langle D \rangle$, $\sigma(L)/\langle L \rangle$

and $\sigma(D)/\langle D\rangle$,being these two last parameters the errors with which L and D would be determined from a real experiment.All the simulations have been performed for 10^6 samples in each value of the counting time,for 10 photons per coherence time and for the data that correspond to a solution temperature of 25°C.Owing to the complexity of the expressions to be fitted it has been necessary to spend a long calculation time for each fitting,what has restricted us to the study of a small number of cases.

Table I shows the results obtained for rods of fixed external dimensions,equal to those of TMV,at different scattering angles θ.Table II shows the results obtained for a fixed scattering angle and different rod dimensions.Results in table III have been obtained for a constant value of KL.The parameter R that appears in tables II and III indicates the relation L/D of the rod dimensions.It can be seen in table I that the values obtained for $\langle L\rangle$ and $\langle D\rangle$ agree satisfactorily with the expected values,within the limit of errors.We do not consider necessary to include $\langle L\rangle$ and $\langle D\rangle$ in tables II and III since we have always observed the same agreement as in table I.

The study of the three tables leads to the following conclusions:

It is possible to determine the length and diameter of a rod-like macromolecule,with errors even inferior to 1% from 10^6 samples,when we measure at the adequate scattering angle (if the approximate dimensions of the rod are unknown we can get the ideal angle by trial and error).This is an

Table I.Results obtained from the fitting of 10 simulated series for a rod-like macromolecule with TMV dimensions.

		L=2980 Å	D=180 Å		
θ	KL	$\langle L\rangle$(Å)	$\langle D\rangle$(Å)	$\sigma(L)/\langle L\rangle$	$\sigma(D)/\langle D\rangle$
170°	7.86	2986.1	180.5	0.82 %	0.47 %
135°	7.29	2999.6	180.2	2.04 %	0.66 %
100°	6.04	2916.4	185.3	13.8 %	10.4 %
65°	4.24	2825.3	211.4	32.9 %	36.5 %
30°	2.04	2865.7	223.7	42.3 %	54.4 %

Table II. Results for $\theta=135^\circ$. The asterisk (*) indicates that these data are the same of the preceding table and that have been reproduced here again in order to make easier the comparison.

$\theta=135^\circ$	R= 8.28	R=16.56	R=33.11
L=2980 Å KL=7.29	D=360 Å	D=180 Å	D= 90 Å
$\sigma(L)/\langle L\rangle$	2.01 %	2.04 % *	2.10 %
$\sigma(D)/\langle D\rangle$	0.74 %	0.66 % *	0.72 %
L=1490 Å KL=3.64	D=180 Å	D= 90 Å	D= 45 Å
$\sigma(L)/\langle L\rangle$	26.2 %	25.9 %	19.4 %
$\sigma(D)/\langle D\rangle$	18.4 %	27.9 %	32.7 %

Table III. Results for KL=3.64. The asterisk (*) means the same as in table II.

KL=3.64	R= 8.28	R=16.56	R=33.11
L=3725 Å $\theta=43^\circ 23'$		D=225 Å	
$\sigma(L)/\langle L\rangle$		26.6 %	
$\sigma(D)/\langle D\rangle$		29.2 %	
L=2980 Å $\theta=55^\circ 1'$	D=360 Å	D=180 Å	D= 90 Å
$\sigma(L)/\langle L\rangle$	26.3 %	26.9 %	19.3 %
$\sigma(D)/\langle D\rangle$	18.5 %	30.0 %	31.9 %
L=2235 Å $\theta=76^\circ 2'$		D=135 Å	
$\sigma(L)/\langle L\rangle$		26.1 %	
$\sigma(D)/\langle D\rangle$		28.7 %	
L=1490 Å $\theta=135^\circ$	D=180 Å	D= 90 Å	D= 45 Å
$\sigma(L)/\langle L\rangle$	26.2 % *	25.9 % *	19.4 %*
$\sigma(D)/\langle D\rangle$	18.5 % *	27.9 % *	32.7 %*

interesting conclusion since diameters as small as 180 Å and 90 Å can be measured with a standard of length of λ=6328 Å.

For a fixed kind of rods the errors decrease when the scattering angle θ increases.

For different kinds of rods the errors depend on KL and on the relation R=L/D exclusively,and not on the absolute dimensions of the rod.

For a fixed number of samples there exists a minimum value of L under which the errors are too big.For instance,the error obtained for KL=3.64, L=3725 Å and D=225 Å is the same as for KL=3.64, L=1490 Å and D=90 Å (table III),but whereas the error can be considerably reduced by increasing θ in the first case,it can hardly be reduced in the second case since θ already takes a high value of 135°.

Finally,for a certain value of L,the error in D seems to have a tendency,not completely proved,to increase when D decreases.

REFERENCES

1. H.Z. Cummins,"Photon correlation and light beating spectroscopy", Ed. H.Z. Cummins and E.R. Pike,Plenum Press,London (1974).
2. R. Pecora, J. Chem. Phys. 48,4126 (1968).
3. B. Saleh, "Photoelectron statistics",Springer-Verlag,Berlin (1978).
4. C. Tanford,"Physical chemistry of macromolecules",John Wiley & Sons, New York (1961).
5. D.P. Filson and V.A. Bloomfield, Biochem 6,1650 (1967).
6. V. Bloomfield,W.O. Dalton and K.E. Van Holde, Biopolymers 5,135 (1967).
7. V. Bloomfield,K.E. Van Holde and W.O. Dalton, Biopolymers 5,149 (1967).
8. S. Broersma, J. Chem. Phys. 32,1626 (1960).
9. S. Broersma, J. Chem. Phys. 32,1632 (1960).
10. H.Z. Cummins,F.D. Carlson,T.J. Herbert and G. Woods, Biophys. J. 9,518(1969).
11. H. Maeda and N. Saito, J. Phys. Soc. Japan 27,984 (1969).
12. D.W. Schaefer,G.B. Benedek,P. Schofield and E. Bradford, J. Chem. Phys. 55,3884 (1971).
13. A. Wada,N. Suda,T. Tsuda and K. Soda, J. Chem. Phys. 50,31 (1969).
14. J.M. Schurr and K.S. Schmitz, Biopolymers, 12,1021 (1973).
15. T.A. King,A. Knox and J.D.G. McAdam,Biopolymers 12,1917 (1973).
16. J. Newman,H.L. Swuinney and L.A. Day, J. Mol. Biol. 116,593 (1977).
17. J.C. Abad and M.A. Rebolledo, Spectrosc. Lett. vol. 14,nº 2 (1981).

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