

DETERMINATION OF THE DEGREE OF POLARIZATION OF FLUORESCENCE WITH A POLARIZATION FILTER EFFICIENT OVER A WIDE WAVELENGTH RANGE

by

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E. D. Cehelnik et al. published a paper in 1975, where the sources of error involved in the measurement of fluorescence and in the determination of the degree of polarization are dealt with in detail. Considering the fact that the procedure described cannot be applied unless comparatively expensive polarizing prisms are used and almost all of the instruments available through trade channels are equipped with polarization filters, the modification of the correction procedure has seemed advisable to take into account the extinction ratio of polarization filters, which is generally far from negligible. We have succeeded in giving the correction formula for the degree of polarization as well as for the emission anisotropy; these formulae express parameters definable with precision, such as the ratio of polarization of the exciting light, the extinction ratio of the polaroid and the sensitivity to polarization of the detector.

Introduction

E. D. CEHELNIK *et al.* [1] dealt in a paper published in 1975 in detail with the sources of error involved in fluorescence measurements and found that the measured intensities of fluorescence are considerably affected by the emission anisotropy of the material under investigation, the direction of detection, the degree of polarization of the exciting light, as well as the dependence on polarization of the detection system. Also, they supplied the correction formulae in terms of which the degree of polarization of the sample can be calculated by measuring the proper intensity and with full knowledge of the polarization properties of the exciting and detection systems.

Considering the fact the procedure described cannot be applied over a wide wavelength range unless some comparatively expensive polarizing prisms are used; and as almost all of the instruments available through trade channels are equipped with filters, it seems advisable to modify the correction procedure described in [1], in order to take into account the extinction ratio of the polarization filters, being far from neglectable in most cases.

Theory

The notations of a part of the quantities involved in dealing with the subject are shown in Fig. 1. The exciting light falling to sample M and passing through monochromator Mo and polarization filter P_1 is not completely polarized; for this reason, it seems expedient to split this beam into two incoherent plane-polarized components with horizontal and vertical electric vectors and with photon flux densities $S^{V'}$ and $S^{H'}$. The beam emitted from the sample for detection has also to be split into components with intensities I_V and I_H , these components, having passed through polarization filter P_2 , are denoted by $I_{V''}$ and $I_{H''}$ in a vertical polaroid position, by $I_{V'}$ and $I_{H'}$ in a horizontal position, and the signals detected with D are denoted by R_V and $R_{H'}$, respectively.

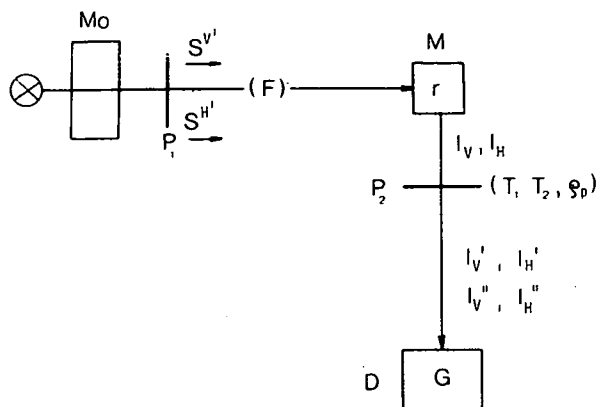


Fig. 1. Principal diagram

o experimental arrangement with the required notations

In addition to these notations, the following quantities and functions are used.
a) Let F be the polarization ratio of the exciting light falling to the sample:

$$F = \frac{S^{V'}}{S^{H'}} \quad (1)$$

b) To characterize the polarization filter; the extinction ratio

$$e = \frac{T_2}{T_1} \quad (2)$$

is given, as a rule, where T_1 and T_2 denote the principal transmittance to the plane-polarized-light with electric vectors parallel as well as perpendicular to the direction of polarization.

c) The emitted beam can be well characterized by emission anisotropy r , closely related to the structure of the molecule as defined by A. JABLONSKY [2], and by

the average emission effect cross section τ . In terms of Eqs. (16 (a—d)) of [1]:

$$I_V = \frac{1}{3} k S^{V'} \tau (1 + 2r) + \frac{1}{3} k S^{H'} \tau (1 - r), \quad (3)$$

and

$$I_H = \frac{1}{3} k \tau (1 - r) (S^{V'} + S^{H'}),$$

where k is a constant.

d) The sensitivity to polarization G of the detection system is

$$G = \frac{T_V}{T_H}, \quad (4)$$

where the sensitivity of the detector to vertically and horizontally polarized light is denoted by T_V and T_H , respectively.

When measuring the degree of polarization, the direction of polarization of the polarizer P_1 is vertical, let R_V and R_H be the recorded signals for the horizontal and vertical positions of polarizer P_2 , which can be expressed in terms of the quantities introduced as follows:

$$R_V = T_V I_V + T_H I_H = T_V T_1 I_V + T_H T_2 I_H, \quad (5)$$

$$R_H = T_V I_V + T_H I_H = T_V T_2 I_V + T_H T_1 I_H. \quad (6)$$

In terms of Eqs. (3), (5), (6)

$$r = \frac{(G\varrho + 1)x - (G + \varrho)}{G \frac{2F-1}{F+1} - \varrho - x \left(\frac{2F-1}{F+1} G\varrho - 1 \right)} \quad (7)$$

each quantity, to be found on the right of the above form obtained for emission anisotropy, can be measured: where $x = \frac{R_V}{R_H}$ is the quotient of the signals recorded. As the well-known connection

$$p = \frac{3r}{2+r} \quad (8)$$

between the degree of polarization and emission anisotropy is a function of excitation by vertical polarization and of a viewing angle perpendicular to the direction of the exciting beam, the degree of polarization can also be stressed by the above quantities as follows:

$$p = \frac{(\varrho G + 1)x - (\varrho + G)}{(1 - \varrho G \Phi)x + (G \Phi - \varrho)}, \quad (9)$$

where

$$3\Phi = 2 \frac{2F-1}{F+1} - 1. \quad (10)$$

As an accurate evaluation can be given for ϱ , G , Φ , through successive measurements for calibration, the degree of polarization can be calculated quite easily by making use of the measurement for x .

Experimental procedures

The possibilities of determining ρ , G , and Φ are treated below.

A) The extinction ratio ρ proper to the polaroid can be given by using one of the methods described in handbook [3]. The measurement can be carried out more easily if we have got a second polarizer; for this purpose, good quality prism polarizer may be used. If we have a prism polarizer like this at our disposal, functions T_1 and T_2 can be given for the polarization filter, with the aid of plane-polarized light emitted by the prism and with an equipment for measuring transmittance. The functions ρ , T_1 , T_2 of the filters employed by us are shown in Fig. 2.

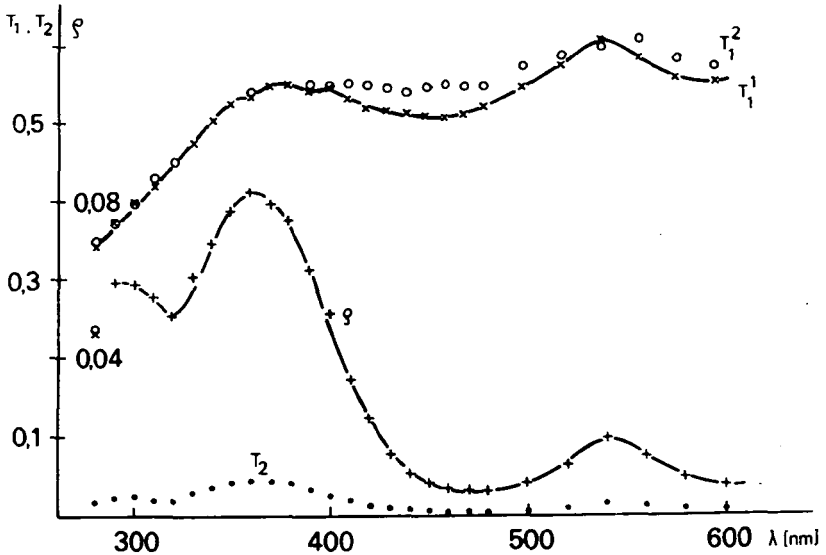


Fig. 2. Characteristic functions of the polaroids of Perkin-Elmer MPF 44 A

B) The sensitivity of detector G_2 to polarization is used to characterize the detector; this sensitivity, in terms of Eq. (4) is the quotient of the sensitivity of the detector to vertically and horizontally polarized light. Fig. 3 illustrates the principal arrangement of the measurement made to determine G with the aid of a polarization filter.

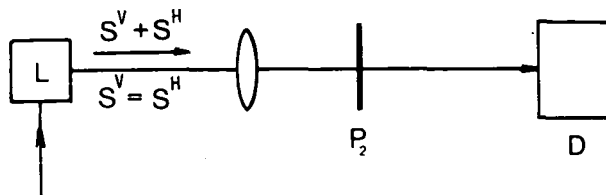


Fig. 3. Principal diagram of the experimental arrangement used for determining the sensitivity to polarization of the detector

Depolarized light was focused on the polarization filter. This light can be produced by using a fluorescent material, polarized but to a low degree, such as a solution of quinine sulfate, excited by light with a horizontal electric vector. The signal recorded in the vertical position of polaroid P_2 is denoted by R_V , that in the horizontal position by R_H , and their quotient by ω . In this instance

$$R_V = T_V T_1 S^V + T_H T_2 S^H, \quad (11)$$

$$R_H = T_H T_1 S^H + T_V T_2 S^V.$$

Owing to excitation by unpolarized light $S^V = S^H$, for this reason

$$\omega = \frac{G + \varrho}{1 + G\varrho}, \quad \text{as well as} \quad G = \frac{\omega - \varrho}{1 - \omega\varrho} \quad (12)$$

expressions are derived; starting from these, G can be calculated from the readings of ω and ϱ .

C) Quantity is interpreted in terms of Eq. (10), this provides information about the polarization of the exciting light falling to the sample by expressing F . F the ratio of polarization of the light falling to the sample, *i.e.*

$$F = \frac{S^{V'}}{S^{H'}} = \frac{T_1 S^V}{T_2 S^H} = \frac{1}{\varrho} \frac{S^V}{S^H} = \frac{f}{\varrho}, \quad (13)$$

where $f = \frac{S^V}{S^H}$ is the ratio of polarization of the light emerging from the monochromator. The experimental arrangement shown in Fig. 4 have been used to express F ,

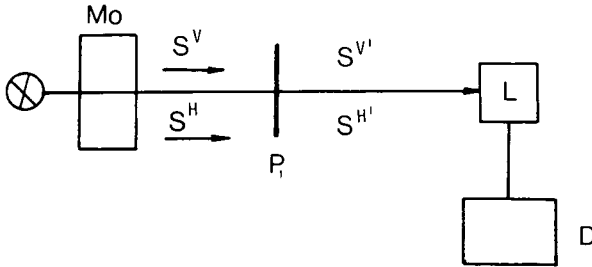


Fig. 4. Principal diagram of the experimental arrangement used for determining the ratio of polarization of exciting light emerging from monochromator Mo

where L is an unpolarized luminescent solution emitting fluorescence, which forms with the detector a detection system insensitive to polarization. Denoting the detector by R^V in the vertical position of polaroid P_1 , and by R^H in the horizontal position, form

$$\frac{R^V}{R^H} = \frac{k'(S^{V'} + S^{H'})}{k'(S^{V''} + S^{H''})} = \frac{T_1 S^V + T_2 S^H}{T_2 S^V + T_1 S^H} \quad (14)$$

is found to express the quotient of the two signs, this leads to

$$f = \frac{1 - \frac{R^V}{R^H}}{\frac{R^V}{R^H} - 1}, \quad (15)$$

where ϱ and $\frac{R^V}{R^H}$ are readings of measurement. Functions Φ and G , with respect to the equipment used by us, are shown in Fig. 5.

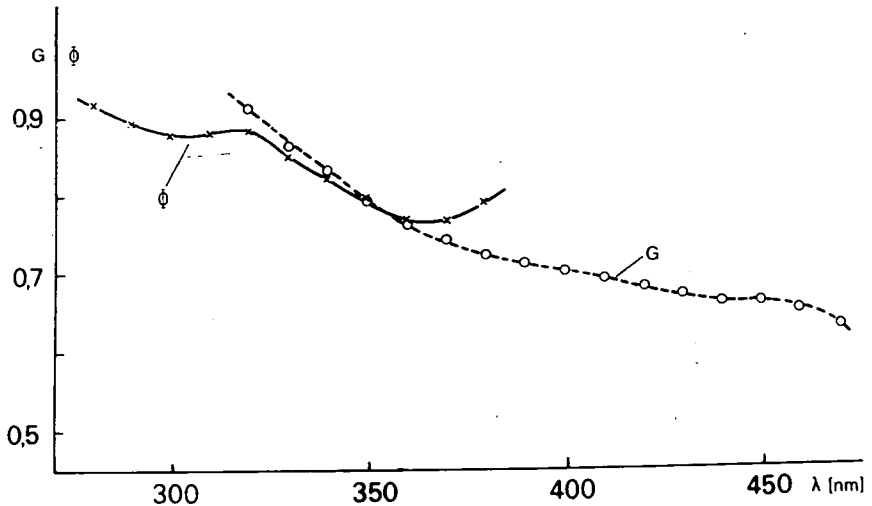


Fig. 5. Functions Φ and G characteristic of the Perkin-Elmer MPF 44 A equipment

Results

The importance of the correction procedure described above will be illustrated by particular instances. The measurements were made by a Perkin-Elmer 44 A spectrometer, and the readings for a bichromophoric dye PT-CH₂-DMP, paraphenylene-bis (5-phenyl-4-methyl)-4'-terphenyl(-2-oxazole) are listed in Table I. At

Table I

C (M)	Solvent	λ_{ex} (nm)	Φ	p_m	p	error (%)
$5 \cdot 10^{-6}$	60% glycerol 40% methanol	290	0.891	0.309	0.341	10
$1 \cdot 10^{-6}$	dioxane	280	0.918	-0.017	-0.026	38
		330	0.846	0.104	0.107	3

wavelength $\lambda_m=425$ nm, where the observations were carried out, $q=0.0173$ and $G=0.681$. The readings tabulated imply that the error in p_m calculated without correction (taking into account the sensitivity to polarization of the detector) is a function of the degree of polarization as well as of exciting wavelength λ_{ex} . The absolute value of the corrected polarization degree p tends to be higher than p_m .

The significance of the above correction procedure is also shown by the reading obtained by determining the fundamental polarization value of *p*-phenylene-bis-(5-phenyl-4-methyl-2-oxazole), (dimethyl-POPOP). The fundamental polarization value given in terms of Perrin and Levshin's equation has been found to be $P_0=0.47$, derived from corrected values, while p_m readings, interpreted as above, do not allow for this quantity more than 0.34. The linear functions we are supposed to derive from Perrin and Levshin's equation are shown in Fig. 6.

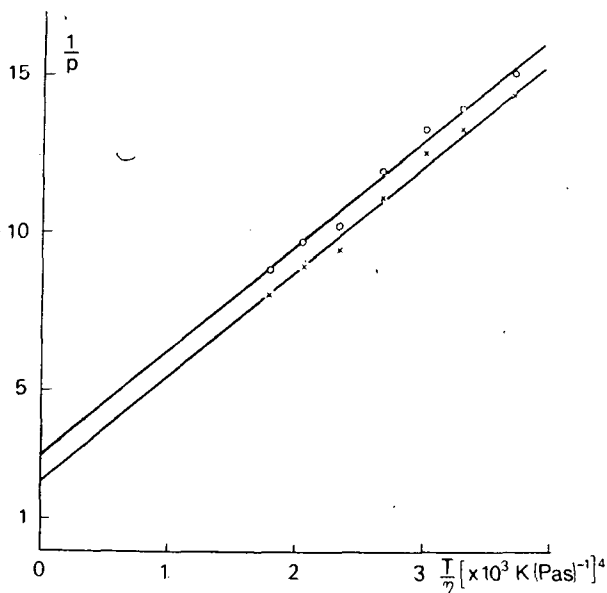


Fig. 6. Functions for determining the fundamental polarization value. The values derived from the corrected degrees of polarization are denoted by x , those derived from p_m are denoted by o , the solid lines are connected to the readings by the least squares method

To conclude, we should like to observe that, for measuring the degree of polarization with polarization filters efficient over a wide wavelength range, we gave the correction formula — for emission anisotropy and for the degree of polarization; these formulae take into account the parameters which can be recorded with precision by the above-described experimental procedure.

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ОПРЕДЕЛЕНИЕ СТЕПЕНИ ПОЛЯРИЗАЦИИ С ПОМОЩЬЮ
ПОЛЯРИЗАЦИОННОГО ФИЛЬТРА ШИРОКОГО ДИАПАЗОНА

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Цехелник и его сотрудники в 1975 г. сообщили о возможных ошибках в измерении степени поляризации. Исходя из того, что описанный способ используется только с помощью дорогостоящих поляризационных призм, а коммерческие приборы снабжены поляризационными фильтрами, явилось целесообразным введение коррекционного метода, содержащего в себе непренебрегаемую долю отношения экстинкции. Нам удалось построить коррекционную формулу для степени поляризации и анизотропии излучения, в которых отношение поляризации определяется точно, а отношение экстинкции и поляризационная чувствительность детектора выступают как параметры.