A POLARIZATION SPECTROFLUORIMETER WITH ON-LINE DATA PROCESSING*

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Semi-automatic set up adaptable for series measurements is described for determination of polarization of fluorescence emission with high precision. Measurements of fluorescence intensities are carried out in a spectrofluorimeter equipped with polarizers. The control of the measurements and on-line data processing is made by a minicomputer WANG-600-14.

Examination of fluorescence polarization is a useful tool for studying the nature of electric transitions associated with absorption and emission of fluorescing molecules [1]. Fluorescence polarization technique is often used for the investigation of intermolecular energy transfer [2], molecular motions in solutions, microviscosity of solvents [3], and for a number of biological problems [4].

The degree of fluorescence polarization p is defined by the following relationship:

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}, \tag{1}$$

where $I_{\parallel}$ is the fluorescence intensity polarized in the same plane as the exciting beam, and $I_{\perp}$ is the fluorescence intensity polarized at right angels thereto. Although several methods were elaborated for determination of p [5—11], computer data handling offers new possibilities for fast and very precise determination of luminescence polarization. A usual method is to measure the fluorescence intensities $I_{\parallel}$ and $I_{\perp}$ either subsequently or simultaneously [6, 8, 10, 12]. By this simple method, however, due to instability in the intensity of exciting light and fluctuations in photomultiplier dark current confident results can only be obtained by statistical data evaluation from a great number of repetitions of the measurement. Especially at low fluorescence intensities this makes the measurements rather tedious.

The aim of the present work was to construct an equipment which connects the simplicity of the direct method with the controlling and on-line data processing facilities of computers.

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Fig. 1 shows the block diagram of the instrument. Exciting light is provided by an 500 W Xe arc lamp L. The polarized monochromatic light incident on the sample, located in a termostated cuvette C, is obtained by a Hilger double grating monochromator M (Typ. D331), and by polarizer P_1, mounted on a hand rotating disc. Before collection at the photomultiplier PM (EMI 9558 QB), the fluorescence light passes through a filter F, to block the scattered light, and polarizer P_2, with

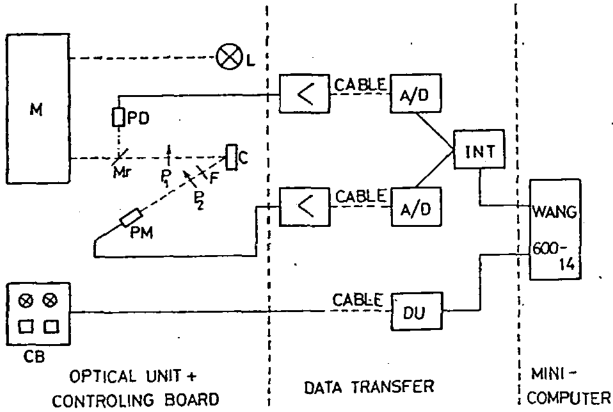

Fig. 1. Block diagram of the equipment

fixed position. Arrows on Fig. 1 indicate the electric vectors of the polarizers P_1 and P_2, respectively. For measuring the intensity of the exciting light a mirror Mr, and a photodiode PD is used. After suitable amplification the signals of photomultiplier and photodiode are transferred separatly to two analog-to-digital converters A/D, which are synchronized to each other with a maximal sampling rate of $50\ s^{-1}$.

The digital data proportional to the exciting and the fluorescent light enter simultaneously the minicomputer WANG—600—14, across a digital microinterface INT.

The semi-automatic measurement is processed by a laboratory-built unit DU, constructed from digital integrated circuits. This unit makes possible to start the computer program at the optical unit — located in about 50 m distance from the minicomputer — and gives information about the run of the program by a controlling board CB.

During the measurement the electric vector of the exciting light passing through the polarizer P_1 was brought first into parallel, then into perpendicular position with respect to the electric vector of fluorescence light passing through the fixed polarizer P_2. The partially polarized light from monochromator M, that also depends on the wavelength λ, has generally different intensity in the two positions of P_1. Taking into consideration this fact in determining p, the quotient $c(\lambda)=I_{\perp,e}(\lambda)/I_{\parallel,e}(\lambda)$ has to be measured. To carry out this, a scrambler was put into the sample holder and in this way P_2 obtained always depolarized light.

Taking into account the dark current of the photomultiplier I_d, the following formula was used for the calculation of p

$$p = \frac{(I_{\parallel}-I_d)c(\lambda)-(I_{\perp}-I_d)}{(I_{\parallel}-I_d)c(\lambda)+(I_{\perp}-I_d)}. \tag{2}$$

The flow diagram of the measurement is shown in Fig. 2. The random errors of this method originate mainly from two sources. The first is the fluctuation of the dark current and the electric noise of the whole equipment. The distribution of a great number of intensities was found to be Gaussian-type, so the relative error of the mean value is $\Delta I_{rel}=\sigma_n/\bar{I}\sqrt{n}$, where n is the number of the measurements, σ_n is the

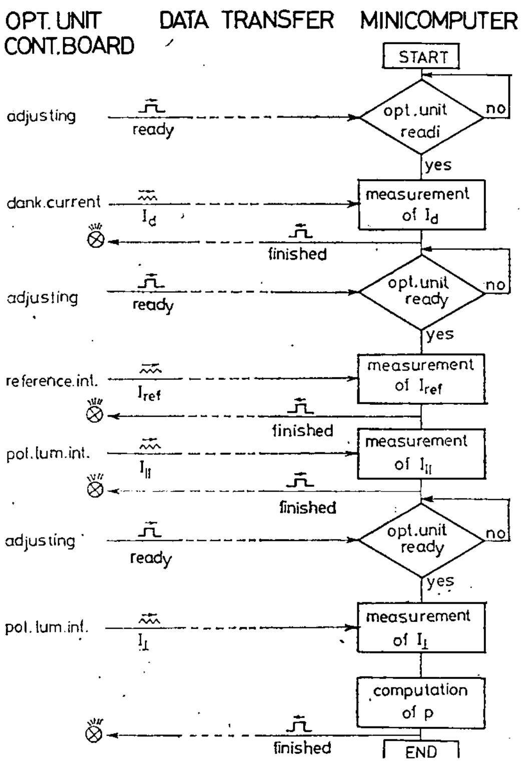

Fig. 2. Flow diagram of the measurement

standard deviation and $\bar{I}$ is the mean value. To reduce the error due to the fluctuation of the dark current, the computer program continously makes the sampling and compute ΔI_{rel}. When ΔI_{rel} is less than a given error limit, δ, the sampling stops. The great advantage of this sampling method is that the sampling number is not fixed, but is always optimal for the given error limit δ. The other source of the errors is the instability of the exciting lamp. To avoid this difficulty, before the measurement of $I_{\parallel}$ and $I_{\perp}$, we determine the intensity of the exciting light and this value is considered as reference I_{ref}, in the following. In determination of $I_\parallel$ and $I_\perp$ the sampling procedure is similar to that of the dark current. Except that in this case, together with the intensity of the polarized fluorescence, the intensity of the exciting light I_g is measured, too. If $|I_g - I_{ref}| < \varepsilon$, where ε is programmed error limit, the measured $I_\parallel$ or $I_\perp$ value is accepted, if $|I_g - I_{ref}| > \varepsilon$ the measurement is repeated. The sampling continues until $\sigma_n / \bar{I} \sqrt{n} < \delta$.

Because of the continuous computations during the measurement the maximal sampling rate was reduced from $50\,s^{-1}$ to $12.5\,s^{-1}$. Fig 3 shows the absolute and relative error of p calculated from ten test measurements at $\delta = \varepsilon = 1\%$. It can be seen that the absolute error Δp is nearly constant independently on the value of p, and so the relative error $\Delta p/p$ is steeply rising at low values of p. With appropriate choice of the error limits δ and ε, the desired absolute or relative error for a certain p can be obtained even at low fluorescence intensities. Setting low error levels the time necessary for the measurement considerably increases. For the degree of polarization of $10^{-4}\,M$ Na-fluorescein solution we obtain $p = 0.0016 \pm 0.0012$, which is similar to earlier results [8].

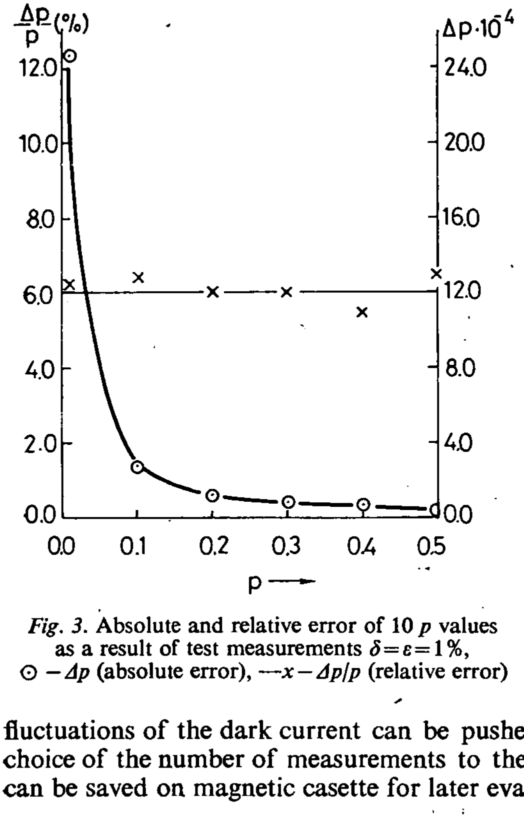

Fig. 3. Absolute and relative error of 10 p values as a result of test measurements $\delta = \varepsilon = 1\%$, ⊙ $-\Delta p$ (absolute error), —x—$\Delta p/p$ (relative error)

In conclusion, we may say that our set up connecting the direct determination of p with an up-to-date data processing method makes possible the fast determination of fluorescence polarization with high precision. The errors due to the instability of the exciting light and fluctuations of the dark current can be pushed under a given level by the optimal choice of the number of measurements to these error limits. The p values obtained can be saved on magnetic casette for later evaluation.

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ПОЛЯРИЗАЦИОННЫЙ СПЕКТРОФЛУОРИМЕТР С НЕПОСРЕДСТВЕННОЙ ОБРАБОТКОЙ ДАННЫХ

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Описан полуавтоматический аппарат применимый к серийным измеремиям поляризации флуоресценции с большой точностю. Проведены измерения интенсивности флуоресценции в спектрофлуориметре с поляризаторами. Контроль измерения и непосредственную обработку данных проводили на настольном вычислителе типа WANG—600—14.