A POLARIZATION SPECTROFLUORIMETER

By L. VIZE

Institute of Experimental Physics, Attila József University, Szeged

With the apparatus described, polarization spectra, polarization of emission, and diagrams of polarization can be measured. The apparatus, containing an electro-magnes wich allows to adjust the twist of the polarizator with great accuracy, is suitable to measure signals of 10^{-15}. A with a signal to noise ratio = 100 in the wavelength interval from 360 nm to 800 nm. The apparatus can also be used or measuring excitation and emission spectra.

Introduction

Examination of the polarization of luminescence, together with other luminescence-characteristics, can give valuable informations concerning the structure of molecules [1], migration of energy [2] and interactions between the molecule capable of luminescence and the solvent [3]. It seemed therefore justified to build an apparatus, wich can be considered as more accurate than those constructed earlier [4—9], using some recent results of experimental technics.

1. *The principle of measurements* is based on the following definition given e.g. in [10]. Let us consider a part of the solution to be studied, contained in a volume element ΔV of parallelepiped form in a system of co-ordinates $OXYZ$, so that O be comprised in ΔV, the edges of ΔV being parallel to the corresponding axes of the system. Let the solution in ΔV be irradiated with monochromatic, linearly polarized light, the electric vector of which is parallel to the X-axis. Let us consider a narrow beam of fluorescence-light of wavelength λ', emitted parallel with the Z axis and passing through a polarizator, the intensities of the beams with electric vectors parallel to the X and Y axis being denoted by I_1 and I_2 respectively. The degree of polarization $p(\lambda, \lambda')$ expressed in terms of I_1 and I_2 is then given by the following formula:

$$p = \frac{I_1 - I_2}{I_1 + I_2}. \tag{1}$$

It would however be inexact to make measurements on a quantity of solution of such a little volume, because the conditions in the volume-element ΔV would be altered among others by surface effects. In order to obtain the true degree of polarization given by Eq. (1) from the measured degree of polarization p' determined in the same way from the intensities I_1 and I_2 measured on macroscopic quantitites of solution (p' being therefore not generally to be considered as true degree of polarization), the measurements are to be corrected according to [10]. One of the

conditions necessary for this correction is to comply with the conditions of excitation and observation described in [10] during the measurement of p'. Therefore we took care that in our instrument the sample could be excited on a surface of 0.01 cm^2, and the luminescence beam emitted under a beam angle of 0.05 rad could be observed on a surface of about 0.80 cm^2.

2. *Optical arrangement*. The plane of reference of the apparatus serving to measure successively the intensities I_1 and I_2 is the plane determined by a_1 and a_2 the axis of the cone of exciting light and that of observed radiation respectively (see Fig. 1). In Fig. 1 L denotes a high-pressure xenon arc. lamp (type Osram XBO 450); L' a high pressure mercury lamp (type Osram HBO 500), or a halogen (iodine) lamp of 150 W; M_1 and M_2 are monochromators (type Zeiss SPM-2) L_1, L_2, L_3 and L_4 fused silica lenses for projection; C_1 and C_2 cells containing CuSO$_4$ solution for absorbing heat radiation; F_1 is a Schott-filter absorbing most part of the exciting light, F_2 an interference filter transmitting the band selected from the spectrum of L'; S_1 and S_2 metal mirrors; Em is an electro-magnet serving to adjust the polaroid filter P_1 around axis a_1 with high accuracy; H_1 and H_2 are handles to turn the sample-holder and the polaroid filter P_2 in the desired position; CH is the sample-holder; V a dc amplifier (Clamann—Grahnert, type MV-4); DV a digital voltmeter (EMG 1361) which can be coupled with a printing recorder; Th denotes an ultra-thermostat; V_1 the vacuum pump; D_1, D_2 and D_3 are diaphragms; Ph a photo-multiplier (EMI 9558 a). Concerning the part of Fig. 1 enclosed by a broken line see part 4.

In *adjusting the apparatus*, the first step was to determine the plane (a_1, a_2). This was performed with a cathetometer for observing the light beam from a pinhole source of light in the sample holder passing through diaphragms of small aperture placed in the channels of excitation and observation. The electric vector **E** of the exciting light was adjusted in position I, perpendicular to the plane (a_1, a_2), with a Sénarmont-prism. The electric vector **E** can be brought from position I into the plane (a_1, a_2) (position II) with the electro-magnet Em, this method being more convenient and giving better reproducibility. In order to secure practical immobility of the projection on the cell of the exit slit of the monochromator M_1 when turning the polarizator P_1, this latter must be plane-parallel within some minutes and perpendicular to axis a_1. After projecting the light of the halogen lamp P_2 with the aid of a metal mirror placed in the position of the xenon lamp L, the polaroid P_2 was turned around a_2, until the instrument coupled with the photo-multiplier Ph indicated a minimal value. This operation could be performed with the aid of the range-switch of the dc amplifier with a mean relative error of about 0.1%.

In the *course of measurements* the vector **E** of exciting light was brought first into position I perpendicular to plane (a_1, a_2), then into position II parallel to it with the aid of the electro-magnet Em, and the intensities $I'_\parallel$ and $I'_\perp$ of the luminescence light corresponding to positions *I* and *II* of **E**, passing through the fixed polaroid P_2 in the direction of a_2, were measured. The light from monochromator M_1 being partially polarized and its degree of polarization depending on the wavelength λ, the intensities I_1 and I_{11} passing P_1 in position *I* and *II* are generally different. To take into account this fact in measuring the degree of polarization, the quotient $c(\lambda) = I_{11}(\lambda)/I_1(\lambda)$ is to be measured. To this end a metal mirror was put in the place of the cell and parchment paper before the diaphragm D_1; P_2 thus obtained always

Fig. 1

natural light. The measured degree of polarization p' (which is not generally to be considered identical with p defined in Eq. (1)) can be calculated from the intensities $I'_{\parallel}$ and $I'_{\perp}$ measured directly (provided that the intensity of dispersed light can be neglected against I' and $I'_{\perp}$) with the following formula:

$$p'(\lambda, \lambda') = \frac{I'_{\parallel} c(\lambda) - I'_{\perp}}{I'_{\parallel} c(\lambda) + I'_{\perp}}. \qquad (2)$$

If the intensities $I_{\parallel d}$ and $I_{\perp d}$ of the dispersed light cannot be neglected in comparison with $I'_{\parallel}$ and $I'_{\perp}$, the solution is to be replaced by a solution of chinese ink which has the same absorption at wavelength λ; then p' can be calculated from the measured intensities $I_{\parallel d}$ and $I_{\perp d}$, with the following formula (the intensities of dispersed light including the dark current of the multiplier):

$$p'(\lambda, \lambda') = \frac{(I'_{\parallel} - I_{\parallel d}) c(\lambda) - (I'_{\perp} - I_{\perp d})}{(I'_{\parallel} - I_{\parallel d}) c(\lambda) + (I'_{\perp} - I_{\perp d})}. \qquad (3)$$

We had also to check the linearity of the dc amplifier, the error of which was found to be less than 0.5%. For this measurement the digital voltmeter was used.

3. The degree of polarization depends, besides the wavelengths λ and λ' of exciting and observed light, also on the *temperature of the sample*. The sampleholder *CH*, together with the thermostat *Th* and the vacuum-pump V_1, an electric heater in the sample-holder and a thermocouple measuring the temperature serve to ensure constancy of the temperature desired. The rod *b* of the revolving cell-holder *a* made of metal (see Fig. 2), capable of receiving 6 cells simultaneously and mounted on a plastic plate, was i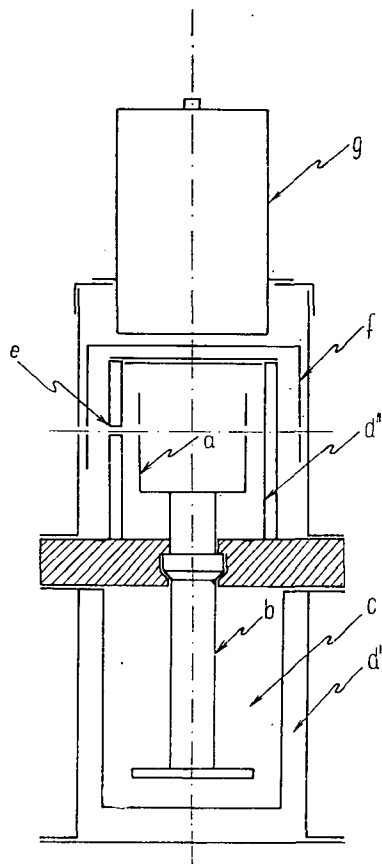mmersed in liquid air (or water) in the container *c* (or flowing through it). The spaces *d'* and *d"* could be evacuated. The exciting light passing through the double window *e* made of glass (or of fused silica) fell on the solutionr and the luminescence light through the same window on the polaroid P_2. The cylinde- *f* of the easily demountable mechanical phosphoroscope was rotated by the electro- motor *g*.

The electromagnet *Em* serves to adjust the position of the polaroid P_1 fitted with high accuracy in a mild-iron holder. In order to obtain the angles 0°, 90°, 180° and 270°, and for measurements according to [4], also 45°, 135°, 225° and 315°, between E and the vector E' of the observed light, E has to be adjusted in the angles 0°; 45°30'; 90°; 134°30'; 180°; 224°30'; 270°; 314°30' with respect to position I, as can be shown by trigonometrical calculation. (These angles are determined by

Fig. 2

the condition that for measurements according to [4], the components in the directions I and II of E, which encloses the angle α with position I, viewed from the direction of the axis a_2 enclosing an angle of $10°$ with axis a_1, have to be equal.)

4. *The sensitivity of the apparatus* is determined by the sensitivity of the photomultiplier (EMI 9558 a, which is 200 μA/lumen for the photocatode) and that of the Clamann—Grahnert dc amplifier (type MV 4, with an input resistivity of 17 MΩ and bandwidth of about 0.5 Hz) which is 10^{-12} A. According to our measurements the fluctuation of the dark current amounts to about 10^{-12} A. The quantum yield of the photocathode of the multiplier is about 0.1 for blue and green light, the gain can be estimated to 10^5 with the anode voltage 1200 volts employed, thus our observing system indicates quantum current densities of 10^3 photon/cm² sec. For reliable measurements a signal to noise ratio of ~ 100 is necessary, *i.e.* luminescence light fluxes down to 10^5 photon/cm² sec can be measured.

When measuring solutions of high concentration, highly quenched solutions or solutions of low-yield by themselves, the desired signal to noise ratio from the monochromator M_1 cannot be secured. In these measurements the light of source L' is to be projected on the sample along a shorter path, through an interference filter, with the aid of mirror S_1 (see the part enclosed by a broken line in Fig. 1). The beam angle of the exciting light cone is then 1 rad.

Accuracy of the apparatus. Using an iodine lamp, which practically shows no fluctuations, as exciting light source, the relative error of the measured degree of polarization *vs.* degree of polarization is shown by curve A of Fig. 3; according to this the relative error is 3% for 10% degree of polarization and 30% for 1% degree of polarization. If xenon-arc or mercury lamps must be used for excitation, the accuracy of the apparatus will be impaired, the fluctuation of the light flux from the lamps amounting to 1—1.5%, (see curve B in Fig. 3). In this case the random error due to the fluctuation of the light flux can be diminished by increasing the time constant of the observing system to 3 sec by a condensator shunted with the working resistance of the multiplier, then compensating $I'_\perp$ with a simple compensator of great resistance and measuring $I'_\parallel - I'_\perp$ directly in position I of the polaroid P_1, after increasing the amplification in a small but known degree. Curve C in Fig. 3 shows that the error of measurement can be considerably reduced in this way. As an example the very low ($\sim 0.6\%$) degree of polarization of an aqueous fluorescein solution of 10^{-4} mole/litre concentration has been measured with and without compensator, five times in each position of P_1 and for all six positions of the cell holder, *i.e.* 120m easurements were made with and without compensator, respectively. Our results are shown in Fig. 4, where the ordinate y gives the number of the results in the corresponding interval divided by the total number of measurements.
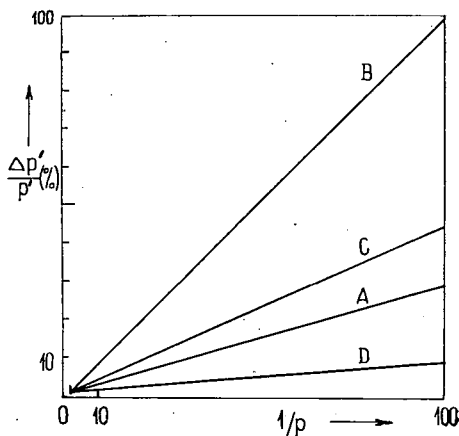

Fig. 3

Whereas the half-width of this function measured with compensator is only about 0.4% (in degrees of polarization), it is 1.2% without compensator.

The accuracy of measurements can be also defined as the error $\Delta \bar{p}'$ in determining the mean value $\bar{p}'$ of a sequence of measurements concerning the quantity studied, divided by $\bar{p}'$. According to our measurements, this relative error amounts to $\sim 0.1\%$, and is due partly to the imperfect linearity of the observing system,
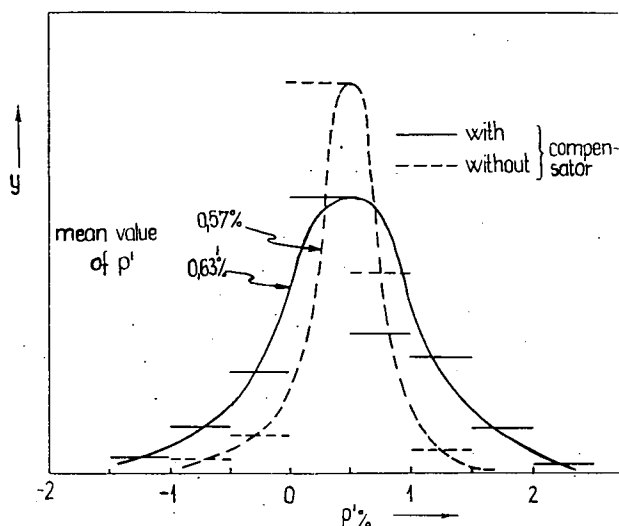

Fig. 4

partly to imperfections in regulation of the polaroids. The accuracy of our apparatus in this sense is shown by curve D of Fig. 3. In determining the accuracy of a measuring device according to this definition, two conditions have to be fulfilled: 1. The speed of measurements has to be high enough, and 2. the distribution has to correspond to a distribution function, characteristic for the apparatus, with adequate accuracy. With our apparatus these requirements are fulfilled, as the results of measurements show Gaussian distribution with an error of less than 10%.

The speed of measurements is about 1 result per second. The measurement of a polarization spectrum consisting of 20 points takes about 30 minutes.

Changes of more than 0.2 sec duration can be well studied with an oscilloscope of adequately low frequency (*e.g.* EMG type 1546) coupled with the amplifier.

Among the corrections to be made on the measured degree of polarization p', the correction for secondary luminescence has already been mentioned. If the exciting and observed light beam is not sufficiently parallel and monochromatic, corrections for parallelism and band width are to be made.

The field of application of the apparatus includes measurements of polarization spectra, polarization of emission, excitation and emission spectra as well as diagrams of polarization in the wavelength interval from 360 to 800 nm in a temperature

range which can be changed from about the temperature of liquid air to that of boiling water, as well as investigations on changes taking place in more than 0.2 sec in fluorescence and phosphorescence phenomena alike.

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ПРИБОР ДЛЯ ИЗМЕРЕНИЯ СТЕПЕНИ ПОЛЯРИЗАЦИИ ЛЮМИНЕСЦЕНЦИИ

Л. Визе

На описанном приборе можно измерять поляризационный спектр, спектр поляризации; получить диаграммы поляризации. Прибор, в котором с помощью электромагнита с большой точностью можно поворачивать поляризатор, пригоден для измерения сигнала 10^{-15} А, при соотношении сигнал/шум-100, в области от 360 до 800 нм. длины волны. На нем можно снимать спектр люминесценции и спектр возбуждения.