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63-3-1

CALORIMETRIC MEASUREMENT
OF FLUORESCENCE YIELD
- USSR -

TRANSLATION NO.

725

FEBRUARY 1963

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

CALORIMETRIC MEASUREMENT OF FLUORESCENCE YIELD

- USSR -

by M. N. Alentsev

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CALORIMETRIC MEASUREMENT OF FLUORESCENCE YIELD

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A method for measuring fluorescence yield of solutions is described, through which the heating resulting from absorbed light in fluorescing and nonfluorescing solutions is compared. Determination of the yield is based on the fact that for the same absorption in a nonfluorescing solution all the absorbed light energy is converted into heat, and the heating of the fluorescing solution takes place by means of that portion of the energy of the absorbed light which is not emitted in the form of fluorescence. Results of fluorescence yield measurements are presented for several solutions of dyes. The precision of measurements for substances with yields close to unity is of the order of 5 percent.

1. Introduction

The photoluminescence yield, that is, the ratio of energy radiated in the form of luminescence, to the absorbed energy of the exciting light [See Note] serves as one of the chief measures quantitatively characterizing the phenomenon. Knowledge of the yield is necessary both in solving several problems of the theory of luminescence as well as in its practical applications.

([NOTE]: Here and in the following treatment the word 'yield' designates the energy yield determined above in distinction to the quantum yield, which signifies the ratio of the number of emitted quanta to the number absorbed.)

However, measurement of the absolute value of the luminescence yield has been made comparatively rarely (1-5). The reason for this consists in the considerable complexity of such measurements. To determine the energy of luminescence, it is necessary to take into account the distribution of the luminescence stream in various directions, the absorption of part of the light in the luminescing body itself, and losses through reflection and refraction. In this connection, as a rule (with the exception of resonance fluorescence of atoms), the spectral composition of the exciting light and the light of luminescence differs, which calls for still another correction for the

selective sensitivity of the radiant energy receiver used. Therefore, one is usually limited to a considerably simpler relative comparison of the relevant measurement of yield with the luminescence yield of one of a few substances for which this quantity has been determined by direct measurement.

In practice, this substance is almost always fluorescein. The luminescence yield of a fluorescein solution in water when excitation using the undecomposed light of an incandescent lamp is used, according to the measurement of Vavilov (3) is taken as equal to 0.80. However, the measurements of Vavilov which were made 25 years ago do not aspire to high precision. The author himself evaluated the possible error as 10-15 percent. The main significance of the results obtained by him for several fluorescing solutions consists in that this was the first time that it was shown that the luminescence yield in condensed systems can reach values close to unity, in spite of the widespread opinion at that time that only a negligible portion of the absorbed exciting light was transformed into the light of luminescence.

The method of Vavilov, which is very simple in concept, consists in a spectral comparison of the brightness of luminescence excited by the undecomposed light of an incandescent lamp, and the brightness of a diffusing white surface illuminated by the same light. However, to obtain a higher precision than that indicated above, it is necessary to introduce many corrections which call for additional measurements.

The calorimetric method described below is sufficiently precise for the determination of the absolute fluorescence yield of dye solutions [See Note], exhibiting high yield, and can be used in establishing standard substances for relative measurements.

([NOTE:]   For measuring the fluorescence yield of fine-crystalline powders, the most reliable method is the sphere method developed by Antonov-Romanovskiy and Epshteyn (5))

2.   General Description of the Method

To determine the photoluminescence yield, the energy of luminescence F and the absorbed energy A of the exciting light are usually measured using one or another method. The yield η is determined as the ratio of these quantities:

$$\eta = F/A \tag{1}$$

However, the yield can be determined by another method. The difference between the absorbed and the emitted energy remains in the luminescing body in the form of thermoenergy and is used in heating the body. If this difference is designated as Q, then F = A - Q, and the yield equals

$$\eta = \frac{A - Q}{A} = 1 - \frac{Q}{A.} \tag{2}$$

In the method described the ratio Q/A is determined in the following manner. The exciting light falls on a layer of fluorescing solution in a flat cuvette. The energy of the light passing through the solution is controlled by a thermopile placed behind the cuvette. The heating of the solution, proportional to the amount of heat Q [See Note] released in it, is measured by using a thermocouple fastened to the cuvette. Then the fluorescing solution is replaced by a solution of nonfluorescing ("Black") dye. The energy of the exciting light absorbed in this solution is transformed entirely into heat. If the concentration of the solution is so selected such that the absorption of the exciting light will be equal in both cases (which is controlled by the thermopile placed behind the cuvette), then the amount of heat released in the solution, measured by the thermocouple fastened to the cuvette, serves as a measure of the energy of the exciting light A that has been absorbed in the fluorescing solution.

([NOTE]: The reading of the galvanometer is recorded with a steady difference in the temperatures of the cuvette and of the surrounding air (thermostat). In this case, the amount of heat released in the cuvette is equal to the amount of heat going from the cuvette to the thermostat. The latter quantity of heat is proportional to the difference in temperatures. Therefore, the reading of the galvanometer measuring this difference in temperatures is proportional to the amount of heat released in the cuvette.)

The quantity Q and A are measured in this case in the same units (thermal). Their ratio is equal to the ratio of the corresponding deflections of the galvanometer which is fixed to the thermocouple. The yield is calculated from formula (2).

The convenience of this method lies in the fact that the difference in the spectral composition of the exciting light and the light of fluorescence is not involved. Unessential also is the distribution of the fluorescence radiation according to direction, provided that all the light energy emerging in the bulk of the solution has left the cuvette.

However, the last condition can be satisfied only for solutions with very low concentration. As a consequence of frequent reflections from the boundaries of the solution and the walls of the cuvette, the path length travelled by the fluorescence light in the solution can prove to be very much larger than the dimensions of the cuvette, which can lead to its noticeable absorption. In this way, part of the original stream of light does not depart from the solution and results in an additional warming of the solution, as a result of which the measured value of the yield proves to be decreased. Lowering the concentration also reduces the absorption of the exciting light, which affects the precision of the measurements.

A rational choice of the cuvette form also can reduce the losses referred to. However, they will always be present to some extent, and their value will remain indeterminate.

To determine the ratio of energy actually leaving the limits of the cuvette to the total original beam of fluorescence emerging in the solution, the following procedure was used. A photometric measurement of the relative distribution of light energy in various directions from the cuvette was made, and by integration the total departing beam expressed by the energy of the light in a direction perpendicular to the plane of the cuvette was determined. On the other hand, the original beam emerging in the solution can be easily calculated from the value of this energy of light (of Section 5). The ratio of the two beams also determines the correction which must be introduced into the value η determined by the above-described method.

3. Apparatus

The diagram of the apparatus used in the calorimetric measurements is presented in Figure 1. The light source 1 is placed in the housing 2. Either a projection lamp of 300 watts or the SVD-Sh 750 superhigh pressure mercury lamp is used as a light source. The light from the source is collected by the condenser 3, which is a round, water-filled flask. In order to filter all the infrared and part of the red light a little copper sulfate is dissolved in the water. In front of the condenser is placed a diaphragm 4, a light filter 5, and a shutter 6. The light collected by the condenser is directed by a mirror 7 to the lens 8, yielding an image of the circular diaphragm 4 on the flat layer of the solution in the cuvette 9. The light passing through a layer of the solution impinges on the thermopile 10, connected to the galvanometer 14. The cuvette is located within a copper container 13 with thick (1 cm) walls. During the period of measurement the box is kept at constant temperature, for which purpose it is surrounded by a heat-insulating covering consisting of two layers of glass wool, between which is placed still another metal wall 11, and this entire arrangement is enclosed in a wooden box 12. With the maintenance of a constant ambient temperature within the limits of $0.1^{\circ}C$, the fluctuations in temperature of the copper case do not exceed $0.05^{\circ}C$.

The details of the construction of the cuvette and copper case are shown in Figure 2. The cuvette consists of three flat quarts round sheets of 1.5 mm thickness. In the center of the sheet is an aperture of 15 mm diameter, which also serves as the recess for the cuvette. The lateral surface is made conical in order to avoid repeated internal reflections of the fluorescence light: light undergoing complete reflection from the flat surfaces of the cuvette impinges on the lateral wall at a small angle and leaves the cuvette.

The cuvette is not cemented, but when filled with liquid is easily held together and due to capillarity is kept sufficiently strong. The cuvette is placed in a copper polished and nickel-plated ring 15 with a conical recess at the same angle as in the cuvette. The ring is secured within the copper case by using a celluloid collar 16. To measure the

difference in temperatures between the ring and the copper case, about the ring in a star-like fashion are placed ten successively joined thermocouples of copper-constantan 17. The thermocouples are joined to the mirror galvanometer (55 ohms, 10^{-8} amp/mm at a meter's distance from the scale). With a temperature difference of $0.1^{o}C$, corresponding to average heating in the measurements described, the reading on the galvanometer scale equalled 120 mm.
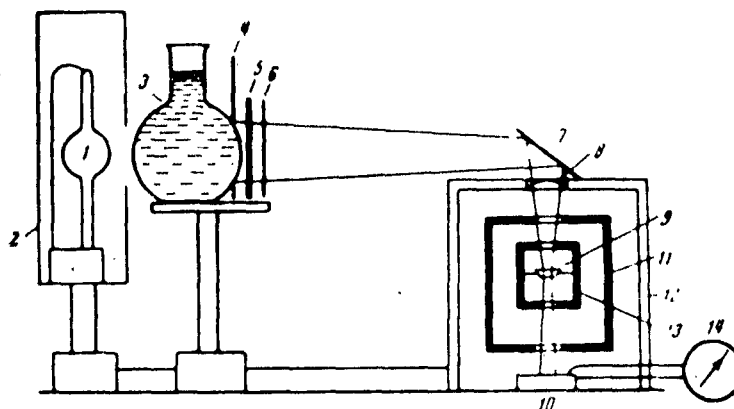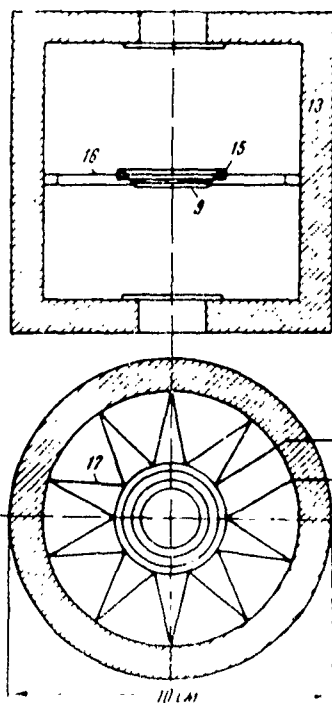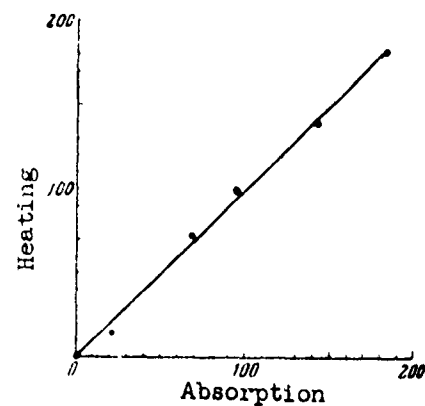

Figure 1.

Figure 2.

Figure 3. Readings of gal-
vanometer deflections
placed along the axes.

- 5 -

The inner walls of the copper case are blackened and covered with carbon black. The entire fluorescence light impinging on the walls is practically completely absorbed [See Note]. Due to the large mass of the copper container its heating is negligible and can be neglected.

([NOTE]. The coefficient of diffuse reflection of a black smoky surface, measured by comparison with the white surface of magnesium oxide, proved to be equal approximately 5 percent. Consequently, 5 percent of the fluorescence light impinging on the walls of the case are scattered. Only a small portion of the light returns to the cuvette, since the solid angle with which the cuvette is accessible from any point of the case walls is small compared to the entire angle 2π, through which the scattered light is sent. With the relative dimensions of our cuvette and case this angle does not exceed 1/10, and therefore from the entire beam of the fluorescence light leaving the cuvette not more than 1 percent can be reversed.)

4. Thermal Measurements

In the illumination of the solution in the apparatus described the temperature of the solution and the cuvette is raised. This temperature rise continues for about half hour, after which a steady difference of temperatures between the cuvette and the copper case (thermostat) is established.

In order to verify whether the deflections of the galvanometer secured to the thermocouples measuring this temperature difference are proportional to the amount of heat evolved in the solution per unit time the following measurements were made. The cuvette was filled with a water solution of a black dye for various concentrations --- from pure water up to a solution resulting in the complete absorption of impinging light. A reading of the galvanometer indication was taken at a steady difference in temperatures for each concentration. The relative amount of light absorbed in each case was determined by using the thermopile. This procedure showed that the readings of the galvanometer secured to the thermocouples is sufficiently precisely proportional to the energy absorbed in the solution through the entire interval of possible absorptions (Figure 3).

These measurements were later used to obtain the value A required to determine the yield according to formula (2). It is obvious that in this case each reading of the galvanometer expresses the amount of heat released in the solution at a certain absorption recorded by the thermopile. Since the solution is nonfluorescing, this amount of heat also determines the value of A. Later, it was sufficient by using an analogous measurement with the fluorescing solution, to take the reading of the steady difference in temperature in cur. of Q and the value of A was determined on the basis of the previous measurements from the thermopile reading.

A small amount of heating of the cuvette preceding even in the absence of dye in solution was subtracted from the corresponding readings in order to specify the heating caused by the absorption of a single dye.

Strictly speaking, the proportionality coefficient between the steady difference in temperatures of the cuvette and the thermostat and the amount of heat released in the solution per unit of time must be somewhat differentiated in the case of the fluorescing and of the dark solution. With the same steady difference in temperatures, the dark solution must lose somewhat more heat through temperature emission according to Kirchhoff's law, since its absorptive ability is greater (the losses through thermoconductivity and convection, of course, are the same for both cases).

In order to verify how much this fact is reflected in the precision of the measurements, a measurement of the drop in cuvette temperature following the end of heating was made. This measurement was taken using several fluorescing and nonfluorescing solutions. The different heat transfer would affect the different rate of temperature drop. All the curves proved to be practically coinciding. This shows that the difference in temperature radiation of the various solutions can be disregarded.

Several difficulties were faced in the selection of the non-fluorescing dye. The solution of this dye cannot have any visible or ultraviolet fluorescence, which is easily noted, nor infrared, which is quite difficult to detect. To select such a dye, the following procedure was used. Several solutions nonfluorescing in the visible region were selected at concentrations adequate for complete absorption of impinging light, and were illuminated in the apparatus described. For several of these solutions, the heating proved to be the highest and within the limits of error of the measurements identical. The presence in such various dyes (India ink, Aquadag [a graphite lubricant], a solution of fluorescein darkened by potassium iodide, and an aniline black dye) of infrared fluorescence with the same yield is completely improbable, and they can be regarded as nonfluorescing. Later, the dye "Anil black FF" was used as the most convenient (not settling out of solution and not changing with time).

5. Determination of the Fluorescence Energy Losses

To carry out the angular measurements of the light strength referred to above, an apparatus was arranged, whose diagram is shown in Figure 4. A cuvette with a ring was placed on an immobile axis 1 around which rotated a lever 2 with a photometer 8 at the end. The angle a which represented the direction of the axis of the photometer from the normal to the cuvette plane were read from the dial 4.

The front lens of the photometer gave such a reduced image of the cuvette and the ring (about 3 mm), that this image fit completely in the pupil of a dark-adapted eye. With this conditions, the brightness

of the front lens observed by the eye was proportional to the strength of the light (and not the brightness) of the source in the given direction.
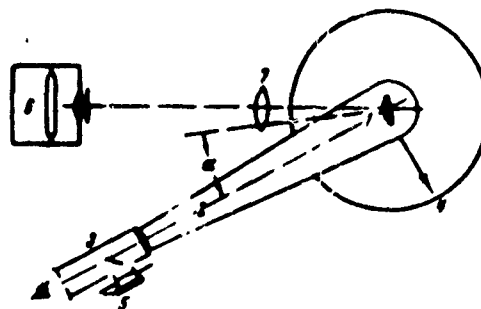

Figure 4.

As a comparison source, a phosphor of constant action, covered by a polaroid film, and placed in a rotating attachment 5 located at the side sleeve, was used. A second fixed polaroid film was placed in the sleeve. The head could be turned and its rotation read off from the table dial secured to the arm. A mirror was placed in the tube of the photometer opposite the sleeve, directing light from the phosphor to the eye of the observer and forming a comparison field. Upon rotating the eyepiece the brightness of the comparison field changed.

Illumination of the solution was accomplished by means of the PRK-4 mercury lamp placed in the case 6, light from the lamp passing through the condenser, the ultraviolet filter, and the lamp 7, giving an image of the circular opening of the condenser on the surface of the solution. In view of the axial symmetry of distribution of the radiation the measurement was adequately taken for a single plane.

The determination of the fluorescence energy losses was done on the basis of the following considerations. If no absorption of the fluorescence light had taken place and the reflection and refraction of the light at the interface with the air were absent, when the entire beam of fluorescence F_0 emerging within the bulk of the solution would be uniformly distributed in all directions, and the strength of the light I in all directions would be the same and would equal

$$I = F_0 / 4\pi.$$ (3)

For the case of a flat layer of the solution not absorbing the light of fluorescence, having an index of refraction n and

with the original beam F_O of the strength of light in a direction perpendicular to the plane of the layer, would be reduced in comparison with I, due to the refraction of light at the flat interface solution-air. The beam passing within the medium with some small solid angle ω , following refraction would travel within a larger angle ω' . From the law of refraction, it follows that the second solid angle will be n^2 times larger than the first. Therefore, the strength of the light I_O along the direction perpendicular to the interface is related to the original strength of light I by the ratio

$$I_0 = I / n^2 \tag{4}$$

In this way, knowing the strength of the I_O, the total original beam of fluorescence F_O can be calculated. From (3) and (4) it follows that

$$F_0 = 4\pi n^2 I_0. \tag{5}$$

The angular distribution of the strength of the light thus obtained from the angular measurements makes possible through graphic integration, a determination of the beam actually emerging from the cuvette:

$$F = 2\pi I_0 \int_0^\pi (I_\alpha / I_0) \sin \alpha \, d\alpha, \tag{6}$$

which is the same as function I_O.

The ratio of the beams F_O and F determines the quantity by which it is necessary to multiply the calorimetrically measured value of the yield in order to obtain the original yield.

In regard to the ratio (5), two more comments must be made. In the first place, the presence of a flat-parallel wall of the cuvette having any index of refraction does not change this ratio, where the index of refraction of the solution remains as the n. This can be easily proved by means of an uncomplicated geometrical calculation.

In the second place, it is easy to show that in the absence of absorption of fluorescence light in the solution, the presence of light reflection from the walls of the cuvette and the solution does not reduce the amount of emerging light. Actually, the light reflected from a single face impinges on another, again is partially reflected, etc. But sooner or later it inevitably leaves the cuvette, since there is no absorption.

If the light in the solution is absorbed, which always takes place to a certain extent, then the expression (5), and consequently, also (6), will be imprecise. The intensity of light I_0 leaving in a perpendicular direction will be less than I/n^2 for the original beam of fluorescence. However, in concentrations reaching 10^{-3} g/cm^3, the layer of a solution of 1.5 mm thickness is colored so faintly that it can be regarded, with sufficient accuracy, that the light of fluorescence passing perpendicularly to the surface of the layer is practically undiminished.

6. Results

All the preliminary experiments on the development of a method were connected with a fluorescein solution in water. The solutions were alkalinized by gradual addition of KOH with the simultaneous control of brightness of the fluorescence until the maximum yield had been reached.

Measurement of the angular distribution of the intensity of light from the cuvette filled with highly diluted (approximately

10^{-3} g/cm^3) solution, in which the absorption of the fluorescence light can be neglected showed that practically speaking, the entire beam emerging in the solution leaves the cuvette. The same measurements, but this time with the cuvette placed in its nickel-plated ring, showed that in spite of the mirror polish of the ring, in this case 11 percent of the original beam is lost. Upon increasing the

concentration up to 10^{-5} g/cm^3, no diminishing in the emergent beam

is noted, for 10^{-4} g/cm^3 0.79 of the original beam emerges, and at

5×10^{-4} the losses equal 52 percent.

The first measurement of the yield was conducted with excitation of fluorescein fluorescence by using the light of a projection lamp without a light filter (except for the solution of copper sulfate). This measurement was undertaken to compare the value of the yield determined by the method described with the value obtained by Vavilov. Two series were run on five individual measurements:

with a concentration of 10^{-4} g/cm^3, and with a concentration of

5×10^{-4} g/cm^3. The results are presented in Table 1.

Table 1

Concentration	5×10^{-4}	10^{-4}
Results of separate measurements	0.85; 0.75; 0.77; 0.80; 0.73	0.87; 0.52; 0.70; 0.79; 0.82
Average of five measurements	0.78	0.74
Mean error	4%	10%

At a concentration of 5×10^{-4}, the mean error does not exceed 5 percent of the measured value. The coincidence, within the limits of possible errors, of the values obtained at the two concentrations shows that the ratio (5) is with sufficient precision suitable even when the loss of the fluorescence light in the solution exceeds half of its total energy.

The resulting values of the yield within the limits of their precision agree with the value of 0.80 that Vavilov obtained. However, for another preparation of fluorescein of unknown origin available to the author, the yield proved to be equal to 0.67, and for uranil -- 0.63. This shows that the yield depends to a considerable extent on the chemical purity of the preparation.

Subsequently, the method described was used for determining the yield of fluorescence of several substances. These measurements were carried out using monochromatic excitation. As a light source the SVD-Sh 250 mercury lamp was used. The Shottovskiy UG-1 filter together with a copper sulfate solution, cutting off the red and infrared regions of the spectra, passed only the line 336 mμ and a small section of the spectra (discontinuous) on the long wavelength side of 366 mμ . The effective wavelength of the entire transmitted section was approximately 372 mμ.

The energy of the exciting light was less than for the film projector lamp illumination. Due to this, the precision of the measurements proved to be somewhat less, and the possible error of the values presented in Table 2 is of the order of 8-10 percent.

Table 2

Substance	Solvent	Energy Yield
Fluorescein	Water	0.63
Rhodamine B-extra	Water	0.43
Anthracene	Benzene	0.28
Acridine sulfate	Ethanol	0.31
3-Aminophthalimide	Ethanol	0.45

The present study was carried out upon the suggestion and under the supervision of Academician S. I. Vavilov.

LITERATURE

1. Paschen, F., Ann. D. Phys, 45, 625 (1914).
2. Wood, R. W., Phil. Mag., 27, 1025 (1914).
3. Vavilov, S. I., Zs. f. Phys., 22, 266 (1924).
4. Shklover, D., A., Zhurnal Tekhnicheskoy Fiziki [Journal of
     Technical Physics], 17 1239 (1947).
5. Antonov-Romanovskiy, V. V., and Epshteyn, M. I., Doklady
     Akademii Nauk SSSR [Reports of the Academy of Sciences USSR],
     64, 483 (1949).

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