ABSORPTION AND FLUORESCENCE SPECTRA AND YIELD OF HIGHLY DILUTED SOLUTIONS

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With a new method, based on the use of an integrating spherical cuvette, absorption and fluorescence spectra as well as yield functions of different, extremally diluted solutions have been investigated. The hypothesis used in developing the method was found to be justified by the experimental results which show, among others, that the yield function breaks down in the anti-stokes region even in case of solutions of 10^{-8} mole/l concentration.

§ 1. In investigating fluorescence of solutions, it soon became clear that the experiments should be extended to solutions of much lower concentration than 10^{-3}—10^{-4} mole/l. Namely, spectrophotometrical results [1] showed that in solutions of several dyestuffs considerable dimerization can occur, which could strongly disturb the determination of the spectra of the monomer. Furthermore, the anti-stokes drop in yield has been ascribed by some authors [2] to the circumstance that in the spectral region of the fluorescence band the inactive absorption due to the presence of dimers cannot be neglected even at concentrations lower than 10^{-4} mole/l. Therefore, it proved necessary to elaborate an experimental method which would allow to determine the fluorescence characteristics of fluorescent solutions of 10^{-7}—10^{-9} mole/l concentrations with acceptable accuracy.

This experimental method has been described in detail in [3]; here we wish only to recall the most important features of the method. The solution is contained in a sphere of glass or quartz so as to fill it completely. On the exterior reflecting coating of the sphere there are apertures to permit entrance and exit of the light. In measuring absorption, the spherical cuvette is filled first with the solvent, then with the solution, and the ratio of the intensities of entering and issuing light is determined in both cases; the absorption coefficient can be obtained from these data in a simple way. The intensity and wavelength of the exciting light entering the sphere is constant in measurements of emission spectra, the spectral distribution of the fluorescence light issuing through the exit aperture is determined with the usual spectrophotometrical method with a monochromator and a photomultiplier. To take into account the effects of reabsorption, the correction described in [3] is used. When measuring the yield function, the wavelength of the exciting light is varied and a part of the issuing fluorescence light, spectrally distant from the exciting light, is observed. In our method the effects of secondary fluorescence are neglected but, as it is easy

to see, this neglection cannot cause noteworth errors in the determination of the emission spectra and the yield function.

In our experimental investigations to be dealt with here, prism and grating Zeiss monochromators SPM 2 were used as spectral filters for the entering and issuing light; Osram xenon and mercury lamps XBO 500 and HBO 500 served as exciting light sources and photomultipliers 1P 28 and EMI 9558 A as photodetectors. The spectra of solutions of high concentration used for comparison were measured with a spectrophotometer Optica (Milano) type CF 4. The temperature was $25 \pm 2°$ C during all measurements.

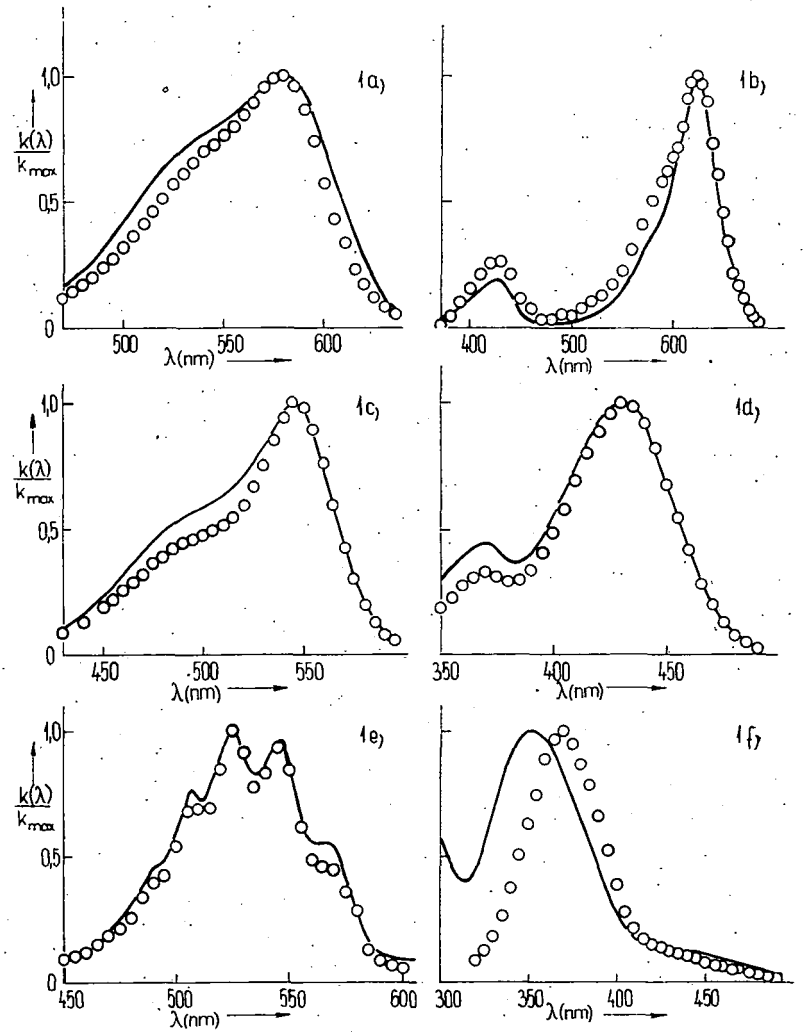

Fig. 1

§ 2. The data of the integrating cuvettes used were the following. G1: Jena molybdenum uviol glass, coated with magnesium oxide, radius $r=3{,}90$ cm; G2: pyrex glass, silver coating, $r=4{,}34$ cm; G3: Jena molybdenum glass, $r=3{,}55$ cm; G4: pyrex glass, silver coating, $r=2{,}48$ cm; G5: pyrex glass, silver coating, $r=1{,}50$ cm; G6: quartz glass, magnesium oxide coating, $r=4{,}89$ cm; G7: pyrex glass, barium sulphate coating, $r=4{,}34$ cm. Finally, a cylindrical integrating cuvette G8 of $V=80$ cm^3 volume, made of pyrex glass and provided with silver coating was also used.

The factor A in the evaluating formula, used for the determination of the absorption coefficient as described in (3) has been chosen for the different cuvettes so that its value when measuring the absorption of an aqueous solution of cobalt ammonium sulphate of 1.10^{-3} and 5.10^{-4} mole/l concentration, containing 5% sulphuric acid, should correspond to the literature data for the molar decadic extinction coefficient of the solution. Because of the wavelength dependence of the reflection coefficient of the coating and of the transmission of the wall, A resulted of course more or less dependent from the wavelength.

§ 3. Some results of our absorption measurements performed with the integrating cuvettes G 1 to G 8 are presented in Figs. 1 to 4. In Figs. 1*a)* to 1*f)* absorption spectra of different non-fluorescent solutions can be seen; the spectra of solutions of higher concentration, measured with the usual methods, are plotted with solid lines, the spectra measured with the integrating cuvettes are designed with little circles. The data pertaining to the figures are given in Table I. It resulted from our measurements that in most cases, the absorption spectra of non-fluorescent materials were left practically unchanged by higher dilutions (e.g. this was the case with solutions of brilliant green, crystal violet, and fuchsin). In some cases, however, the

Table I

Integrating cuvette: G1

N°	Fluorescent compound and concentration (in mole/liter)	Solvent
1a	Crystal violet $1\cdot10^{-4}$; $5\cdot10^{-7}$	H_2O
1b	Brilliant green $1\cdot10^{-4}$; $1\cdot10^{-7}$	H_2O
1c	Fuchsin $1\cdot10^{-4}$; $1\cdot10^{-7}$	H_2O
1d	Auramine $1\cdot10^{-4}$; $2\cdot10^{-7}$	H_2O
1e	Potassium permanganate $1\cdot10^{-3}$; $4\cdot10^{-6}$	H_2O
1f	Potassium bichromate $1\cdot10^{-3}$; $1\cdot10^{-6}$	H_2O

Table II

Integrating cuvette: G1

N°	Fluorescent compound and concentration (in mole/liter)	Solvent
2a	Fluorescein $1\cdot10^{-4}$; $5\cdot10^{-8}$	H_2O $1\cdot10^{-2}$ mole/l 1 NaOH
2b	Rhodamine B $1\cdot10^{-4}$; $5\cdot10^{-8}$	H_2O 6% CH_3COOH
2c	Trypaflavine $5\cdot10^{-5}$: $1\cdot10^{-7}$	H_2O 6% CH_3COOH
2d	Rhodamine 6G $1\cdot10^{-5}$; $5\cdot10^{-8}$	H_2O
2e	Chlorophyll A $1\cdot10^{-4}$; $1\cdot10^{-7}$	CH_3OH
2f	Eosin $1\cdot10^{-5}$; $5\cdot10^{-8}$	H_2O $1\cdot10^{-2}$ mole/l 1 NaOH

spectra show changes not interpreted hitherto (see the spectra of potassium bichromate and potassium permanganate) which may be due to absorption of the solute on the wall of the cuvette.

Absorption spectra of fluorescent solutions are shown in Figs. 2*a)* to 2*f)*. The corresponding data are contained in Table II. In measuring these spectra, a

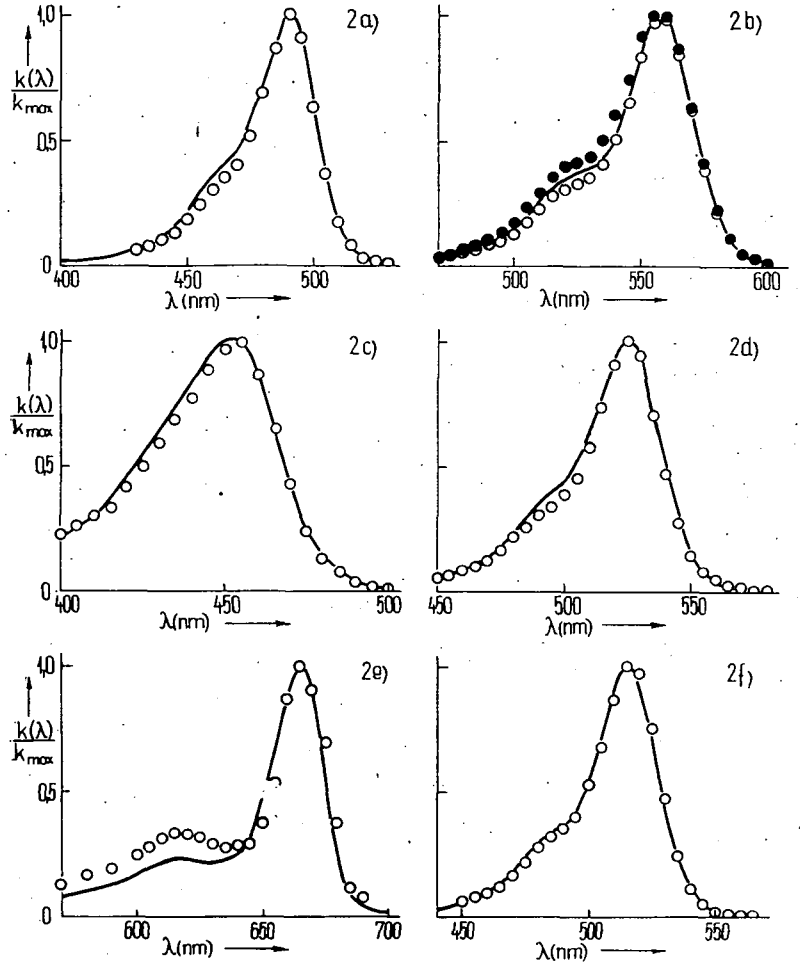

Fig. 2

second monochromator was inserted in the light path in front of the photomultiplier in order to minimize the disturbing effects of fluorescence light. In our figures showing the absorption spectra of fluorescent solutions the results obtained with the integrating spheres are denoted by little circles again, the absorption spectra for higher concentrations are plotted with solid lines in all figures. In order to illustrate the distrubing effects of fluorescence, in Fig. 2*b)* also a spectrum obtained

without inserting a second monochromator in front of the photomultiplier is shown, denoted by filled circles.

It can be seen from the Figs. *2a)* to *2f)* that the shape of the absorption spectra resulting from averaging several measurements on solutions of low concentration is practically almost identical with that obtained for high concentrations. In case of high dilution only a slight decrease in the half bandwidth of the absorption band can be observed in these figures. It should be mentioned that the absorption spectrum of rhodamine B — not shown here — did not change beyond the limits of error in dilutions of $1 \cdot 10^{-4}$ to $2{,}5 \cdot 10^{-8}$ mole/l.

To illustrate the reproductibility of absorption measurements with integrating cuvettes, the results of measurements obtained with different cuvettes are shown in Fig. 3. The absorption spectrum measured with the usual method for the solution of $1 \cdot 10^{-4}$ mole/l concentration is plotted with a solid line, the mean values of the of absorption coefficients obtained with different cuvettes for solutions of $1 \cdot 10^{-7}$ and $5 \cdot 10^{-8}$ mole/l concentration are marked with different signs.

§ 4. For measuring fluorescence spectra of highly diluted solutions, the integrating cuvettes were used as described in (3). The data pertaining to the different spectra (Fig. 4) are to be found in Table III. It can be seen from the experimental results that in solutions of some materials, e.g. of fluorescein and trypaflavine, high dilution does not cause remarkable changes in the emission spectra, whereas in other cases, e.g. in solutions of rhodamine B, dilution results in a marked shift of the emission band towards shorter wavelengths. Finally, there are some materials

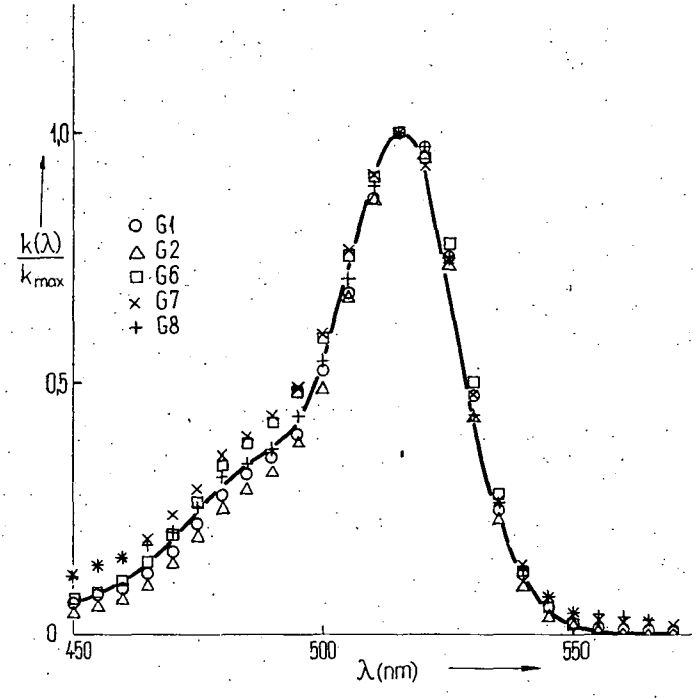

Fig. 3

in the solution of which dilution produces a marked distortion of the emission spectrum. So in methylalcoholic solution of chlorophyll A, it can be well observed that the dilution from 10^{-5} to 10^{-7} mole/l concentration causes the weak band in the long wavelength region of the emission spectrum to vanish almost totally.

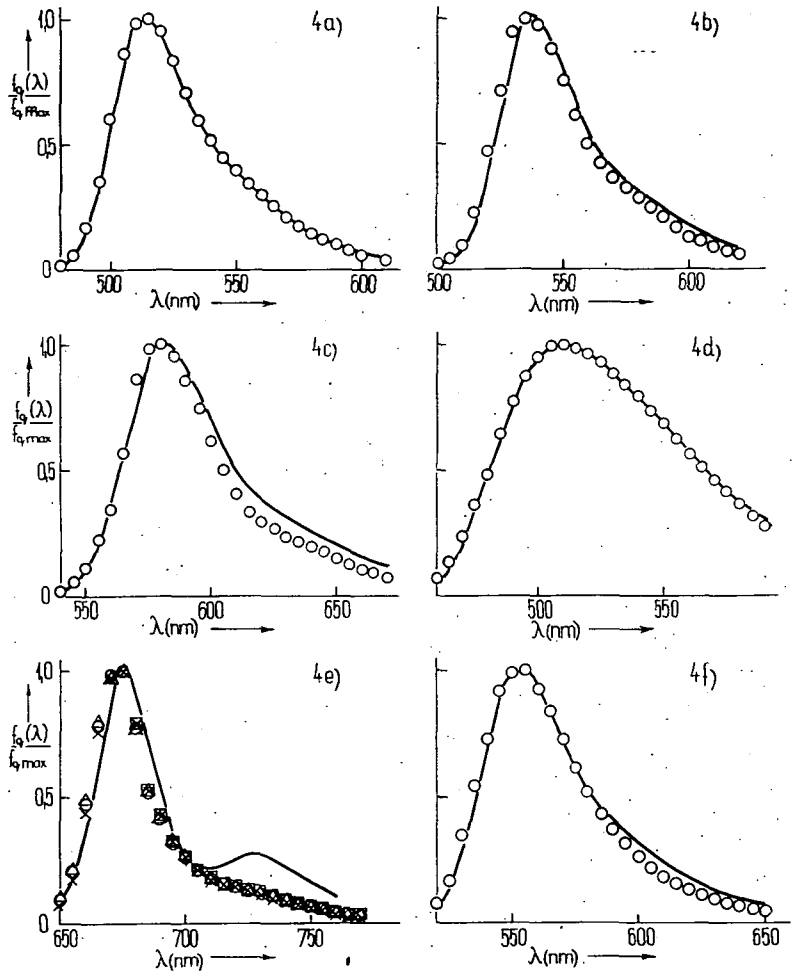

Fig. 4

(In order to clear up the errors due to possible impurities, the emission spectra of the diluted solutions have been measured with four different exciting wavelengths listed in Table III. Changes in the exciting light, however, left the shape of the spectrum practically unchanged.)

A relatively good accordance between emission spectra of one of the used media, namely an aqueous eosin solution of 10^{-7} mole/l concentration, obtained with different integrating cuvettes can be seen from Fig. 5.

Table III

Integrating cuvette: G1

N°	Fluorescent compound and concentration (in mole/liter)	Solvent	Exciting wave-lengths (nm)
4a	Fluorescein $1 \cdot 10^{-4}$; $5 \cdot 10^{-8}$	H_2O $1 \cdot 10^{-2}$ mole/l NaOH	436
4b	Eosin $5 \cdot 10^{-5}$; $5 \cdot 10^{-8}$	H_2O $1 \cdot 10^{-2}$ mole/l NaOH	480
4c	Rhodamine B $1 \cdot 10^{-4}$; $5 \cdot 10^{-8}$	H_2O 6% CH_3COOH	510
4d	Trypaflavine $5 \cdot 10^{-5}$; $1 \cdot 10^{-7}$	H_2O 6% CH_3COOH	436
4e	Chlorophyll A $1 \cdot 10^{-4}$; $1 \cdot 10^{-7}$	CH_3OH	415, 435, 620, 650
4f	Rhodamine 6G $1 \cdot 10^{-5}$; $5 \cdot 10^{-8}$	H_2O	480

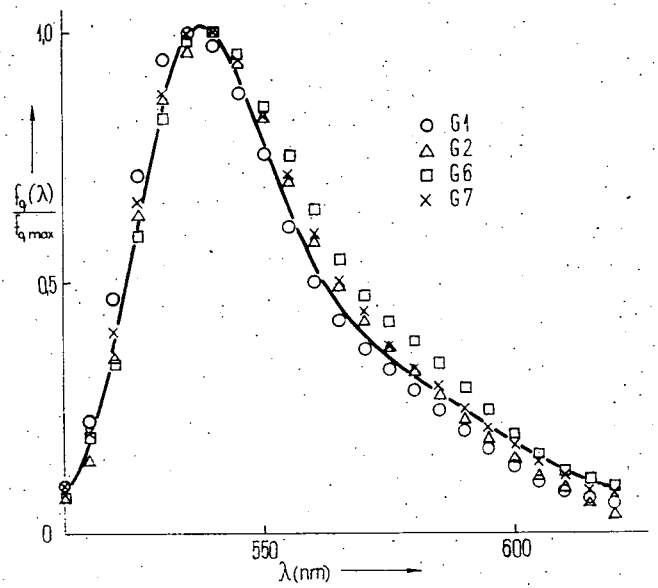

Fig. 5

Table IV

Integrating cuvette: G1

N°	Fluorescent compound and concentration (in mole/liter)	Solvent
6a	Rhodamine 6G $1\cdot10^{-5}$; $5\cdot10^{-8}$	H_2O
6b	Rhodamine B $1\cdot10^{-4}$; $2{,}5\cdot10^{-8}$	H_2O 6% CH_3COOH

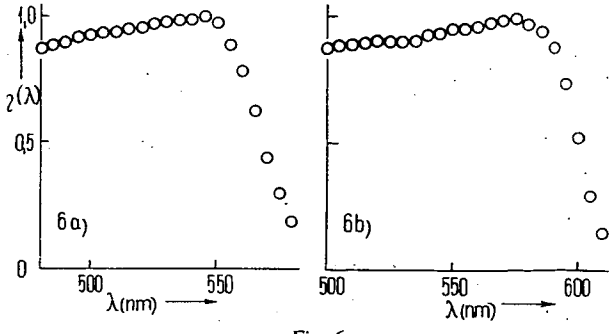

Fig. 6

§ 5. Our results concerning the dependence of the yield on the wavelength of exciting light are shown in Fig. 6. The yield function $\eta(\lambda)$ has been determined on the base of Eq. (10) in (3). The data pertaining to the figures are given in Table IV. The yield functions of highly diluted solutions show a marked drop in the antistokes region.

It appears from the results described above that the absorption and the emission spectra of extremely diluted solutions of dyestuffs generally present only slight deviations from those of solutions of high concentration, and the yield functions show the antistokes drop also in highly diluted solutions. The integrating sphere itself proved to be suitable for practical application.

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О СПЕКТРАХ ПОГЛОЩЕНИЯ И ФЛУОРЕСЦЕНЦИИ И О ВЫХОДЕ ПРИ ОЧЕНЬ МАЛЕНЬКОЙ КОНЦЕНТРАЦИИ РАСТВОРОВ

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С помощью нового метода, основанного на интегрирующую сферу исследовалось спектр поглощения и флуоресценции и функция выхода при чрезвычайно маленькой концентрации растворов. Экспериментальные результаты непосредственного подтверждают гипотезы, предложенные при основании метода, и в том числе показывают, что выход флуоресценции падает в антистоксовой области при концентрации растворов 10^{-8} моль/л.