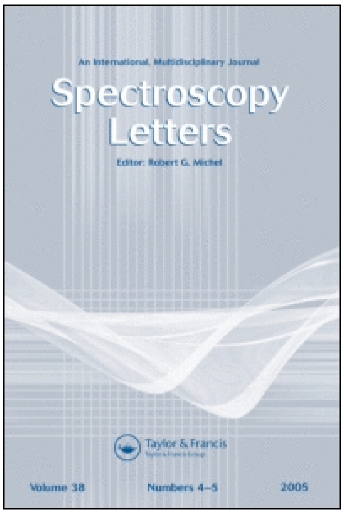

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Determination of Fluorescence Quantum Yields Using a Spontaneous Raman Scattering Line of the Solvent as Internal Standard

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DETERMINATION OF FLUORESCENCE QUANTUM YIELDS USING A SPONTANEOUS RAMAN SCATTERING LINE OF THE SOLVENT AS INTERNAL STANDARD

Key words: Fluorescence quantum yield; quantum yield measurement; spontaneous Raman scattering; internal standardization

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ABSTRACT

A method for determination of molecular quantum yields of fluorescent compounds in solution by internal calibration with the signal of spontaneous Raman scattering of the solvent is proposed. An experimental verification of the method is carried out for substances with known quantum yields and satisfactory agreement between experimental results and literature values is obtained.

INTRODUCTION

The problem of acurate and convenient measurement of fluorescence quantum yields has been discussed on many occasions in the scientific literature. Its importance is determined by the wide theoretical and practical interest in finding the values of fluorescence quantum yields of different substances.

The existing modern methods for measurement of fluorescence quantum yields have been reviewed by several authors[1 - 4]. In their majority they concern measurement of quantum yields in single-component solutions, rather than in solid materials.

Nowadays relative methods of quantum yield measurements of substances in solution prevail over absolute ones. Using them the yield of the unknown is compared with the quantum yield of a standard material. There are a number of difficulties, however, which must be overcome in order to obtain the molecular fluorescence quantum yield (the quantum yield of a fluorescent molecule in a very dilute solution - $C < 10^{-6}$ M [4]). They include corrections for concentration quenching, for self-absorption and secondary fluorescence - in optically dense measurements, together with refractive index corrections, if the standard and the unknown are compared in different solvents[4]. Strict preservation of experimental geometry and high optical identity between the cuvettes for the standard and the unknown are essential even when optically dilute solutions are used.

The aim of this letter is to report a new method for determination of fluorescence quantum yields[5,6], which is free of the inconveniencies mentioned above. It is based on the use of the spontaneous Raman scattering signal of the solvent as an internal standard. An experimental verification is carried out and the accuracy of the method is discussed.

THEORETICAL CONSIDERATIONS

Application of lasers as excitation sources in luminescence analysis has led to a considerable increase in sensitivity of detection of trace fluorescent substances in solution. Refering to quantum yield measurements this means a complete elimination of concentration effects as concentrations of $\sim 10^{-10}$ M are readily detectable. The influence of experimental geometry on the accuracy of the analysis, however, still presents an obstacle for the true registration of emission spectra of the fluorescent standard and that of the substance with unknown quantum yield.

A solution of this problem has been proposed[7,8] for the case of quantitative analysis of fluorescent admixtures in water. It consists in calibration of the laser fluorometer by the spontaneous Raman scattering signal of the solvent (water). The following formula has been derived for optically thin solutions ($\sigma_a n l \ll 1$, σ_a absorption cross section (cm^2), n admixture concentration (cm^{-3}), l optical path of the laser beam in the solution)[9]:

$$n = \frac{4\pi n_s \sigma_{rs}}{\sigma_a q} \Phi_0 \qquad (1)$$

where n_s (cm^{-3}) is the concentration of the solvent (water), σ_{rs} ($cm^2 sr^{-1}$) total differential Raman scattering cross section of a suitable line of the solvent, q fluorescence quantum yield. $\phi_o = N^o_{fl} / N_{rs}$ is the ratio of the number of detected fluorescent photons of the admixture to the number of detected Raman photons of the solvent.

Equation (1) can be used for determination of q if a solution with known concentration of fluorescent molecules is prepared:

$$q = \frac{n_s}{n} \frac{4\pi \sigma_{rs}}{\sigma_a} \phi_o \tag{2}$$

In this way by simultaneous registration of the Raman line of the solvent and the fluorescence band of the solution ϕ_o can be found and q can be calculated from (2) if all other constants are known.

Even when fluorescence is excited by low power pulsed lasers, fluorescence saturation effects may introduce a nonlinear dependence of the number of emitted fluorescent photons on the photon flux density of the exciting laser radiation[10]. Fluorescence nonlinearity is expressed by the fluorescence saturation factor Γ : $\Gamma = N^o_{fl} / N_{fl}$, where N_{fl} is the number of detected fluorescent photons, N^o_{fl} is the number of fluorescent photons which would have been detected at the same intensity of excitation, but in the abscence of saturation. In order to determine ϕ_o in such situations we have to determine Γ first as $\Gamma = \phi_o / \phi$, where $\phi = N_{fl} / N_{rs}$. When Γ linearly depends on the photon flux density F

of the exciting laser radiation ($\Gamma = 1 + \mu F$), φ_o can be found by the method of internal standard too[11]:

$$\varphi_o = \frac{\varphi'(1 - N''_{rs}/N'_{rs})}{\varphi'/\varphi'' - N''_{rs}/N'_{rs}} \qquad (3)$$

where φ' , N'_{rs} and φ'' , N''_{rs} correspond to excitation at F' and F'' , respectively.

EXPERIMENTAL

Fluorescence and Raman spectra of the following solutions have been investigated: rhodamine 6G (R6G) in water, fluorescein in 0,1 N NaOH, 4-methylumbelliferone (4-MU) in 0,01 M NaOH and quinine sulfate in 1 N H_2SO_4. Rhodamine 6G and 4-methylumbelliferone were laser grade purity, produced by Eastman Kodak Co., fluorescein and quinine sulfate were analytical grade purity.

The experimental setup used in the registration of spectra of R6G solution was the same as that shown in reference 11. Excitation of the solution was accomplished by the second harmonic of an YAG:Nd laser (λ = 532 nm, τ_p = 12 ns, P = 1 MW) and the spectra were detected by the help of an optical multichannel analyser OMA-I (EG&G Princeton Applied Research). An example of the spectra obtained in conditions of fluorescence saturation is shown in Fig.1,a .

All necessary corrections of the spectra and calculations of the areas under the contours of the two bands were accomplished by the help of a microcomputer TRS-80 (Radio Shack). For this purpose data about the

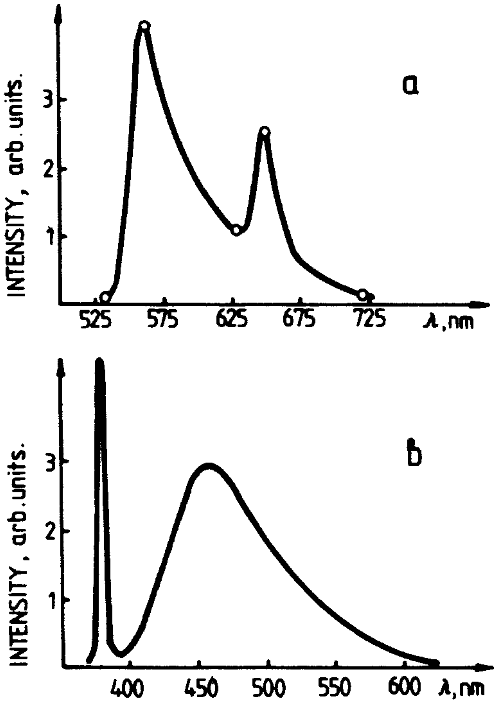

Fig.1. Fluorescence and Raman scattering spectra of:
a) rhodamine 6G in water, excitation at 532 nm ;
b) quinine sulfate in 1 N H_2SO_4, excitation at 337 nm.

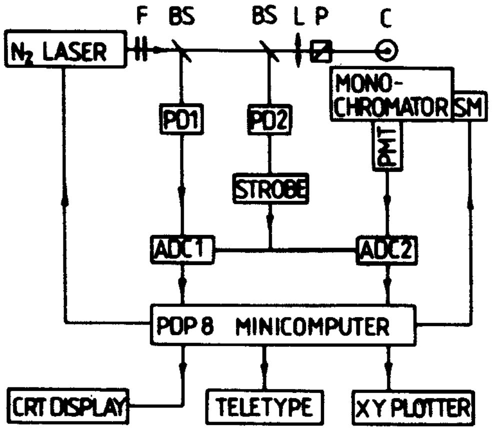

Fig.2. Scheme of the automated laser fluorometer. BS - beam splitter, L - lens, P - polarizer, C - cuvette, SM - stepping motor.

intensities in five characteristic points of the spectrum (Fig.1,a) were introduced in its memory. This information was read from the digital display of the contents of OMA-I memory. A specially developed programm allowed us to do the following: (a) correction of the spectra for the spectral transmittance of the receiving system; (b) calculation of peak intensities of fluorescence and Raman bands, taking into account baseline corrections; (c) calculation of N_{fl} and N_{rs} by the help of effective halfwidths of the corresponding bands; (d) calculation of saturation factors, ϕ_o and determination of fluorescence quantum yield by formula (2).

Spectra of fluorescein, 4-methylumbelliferone and quinine sulfate solutions were registrated by the help of a fully automated laser fluorometer developed in our laboratory - Fig.2 . The excitation source is a low pressure TE nitrogen laser (λ = 337 nm, τ_p = 6 ns, P = 300 kW). The scanning monochromator is a single grating one, of the Cherny-Turner type, model MDR-2. A minicomputer PDP-8 controlls the operation of the system and is used for processing of the spectra. There are two registration channels. In the signal channel the spectral information detected by a PMT, type FEU-51 is transformed in digital form by ADC2 and is stored in the computer's memory. The reference channel consists of a photodiode PD1 and an analogue-to-digital convertor ADC1. It serves to monitor the output power of the laser. A strobe generator, initialized by the laser pulse through a photodiode PD2, "opens" ADC1 and ADC2 for a period of 1 μs. In this way high signal-to-noise ratios are achieved.

Before each run the operator selects by means of the teletype the number of repetitive scans, the step-size of scanning, the total number of points in the spectrum, etc. All necessary corrections and processing of the spectra, as well as their visualization or plotting are accomplished by the help of a specialized programm.

The spectra of the three water solutions were obtained on our automated fluorometer in the case of weak excitation, when formula (2) can be directly applied. This was achieved by introducing neutral density filters F in the path of the laser beam. An example of the spectra is shown in Fig.1,b for quinine sulfate in 1 N H_2SO_4.

RESULTS AND DISCUSSION

The results of our investigation are summarized in Table 1. The values of the total differential Raman scattering cross section of the 3440 cm^{-1} line of water, used in calculations of q are: $\sigma_{rs}(337\ nm) = 4,5.10^{-29}$ $cm^2 sr^{-1}$ and $\sigma_{rs}(532\ nm) = 0,53.10^{-29} cm^2 sr^{-1}$. They have been calculated on the base of the value of $\sigma_{rs}(488\ nm)$, reported in reference 12. Absorption cross sections of the investigated compounds were measured on a spectrophotometer Specord UV-VIS (Zeiss - Jena).

Comparison between the values of q obtained by the method of internal calibration with the Raman line of water and those cited by other researchers shows good agreement. The total relative error of the method proposed

TABLE 1

Name of compound	Solvent	Concentration	q	q ref.
R6G	water	$1,0.10^{-10}M$	0,86	0,82[13]
4-MU	0,01 M NaOH	$1,6.10^{-8}M$	0,62	0,64[14]
Fluorescein	0,1 N NaOH	$1,2.10^{-9}M$	0,91	0,88[15] 0,90[2]
Quinine sulfate	1 N H_2SO_4	$2,5.10^{-8}M$	0,51	0,54[16] 0,55[17]

here can be estimated to about ± 10 % . This is not so bad remembering that the accuracy in determination of fluorescence quantum yields by other methods is seldom better than ± 5%[2] . The error in our method may be attributed mainly to the uncertainty in measurement of Raman scattering cross section values, which is ± 8% in the case of the 3440 cm^{-1} line of water[12]. This error can explain the differences between the values of q obtained by us and those cited from the literature.

The method for determination of fluorescence quantum yield discussed here has several advantages over conventional methods. As the standard and the substance with unknown quantum yield are in one and the same cuvette there is no need to controll experimental geometry or to make refraction index corrections. The main advantage of the method, however, is the complete elimination of the influence of concentration effects since spectra are registrated at extremely low concentrations of fluorescent molecules (10^{-8} - 10^{-10} M).

Fluorescence quantum yields can be measured using internal calibration by suitable Raman lines of other solvents, whose Raman scattering cross sections have been determined with good precizion - benzene[18], cyclohexane[19] etc. It is preferable to employ tunable lasers for excitation of the solutions as in this case the line of Raman scattering of the solvent can be shifted in relation to fluorescence band and thus optimal conditions of excitation and measurement of ϕ_o can be reached.

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