Inner-filter effect as a function of the diameter of an exciting beam in fluorescence emitting spectra

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A formula is obtained allowing calculation of the fluorescence intensity in an absorbing medium as a function of the laser beam diameter, the distance between the laser beam center and the cell exit wall, and optical density per unit of the length. The formula was applied to calculations of corrections, which make it possible to take into account the effect of the secondary inner-filter (IFE) in particular experiments. These results are compared with revealing of differences, with the results of IFE calculations performed under the assumption of an infinitely narrow exciting beam, when the IFE can be considered strictly through the use of the Bouguer–Lambert–Beer law. A possibility of determining optical densities in a spectrometer, detecting fluorescence spectra, is demonstrated by the method of variation of the distance between the laser beam and the wall of the working cell.

Introduction

Fluorescent spectroscopy is a sensitive and express method for analysis of small concentrations of substances in the environment. It can be applied in the regimes of single measurements, regular monitoring, sampling, and analysis of samples in stationary or mobile laboratories.[1,2]

The problem of taking into account the inner-filter effect (IFE) arises in determining actual fluorescence spectra in absorbing and, in particular, water media. The effect appears due to passing of exciting and emitted fluorescence light through a non-excited volume of the studied sample (primary and secondary IFE, respectively. In this paper, only the secondary IFE is discussed). Thus, if the optical density of a studied sample differs from zero in the spectral range of the fluorescence emission, then the emitted light is absorbed by the medium, the magnitude of this effect being different for different wavelengths of the fluorescence spectrum. As a result, IFE causes distortion of actual intensities and contours of fluorescence spectra. Therefore, a special attention is paid to this important practical problem in generalized monographs.[3–5]

If the possibility of IFE is ignored, the experimental data, for instance, on the depth dependence of variation of the contour of fluorescence spectrum of water,[6] can be interpreted unambiguously. This is connected with the fact that the "reddening" of the fluorescence spectrum with increasing water depth, when sampling, can be explained by IFE, rather than by natural processes in the photic zone of the sea.[6]

It should be noted that in the presence of a liquid-drop aerosol IFE also takes place in monitoring ground objects from some air carrier. This effect can be considerable due to long optical path of the sensing beam. In this connection, the data obtained by means of the fluorescent lidar monitoring are corrected for influence of the atmospheric absorption spectrum on the amplitude attenuation and the distortion of the spectral composition of the fluorescence signal.[7]

Standard recommendations on exact allowance of IFE for the condensed phase are absent in the literature.[3,5] As a rule, rather rough correction for the IFE at laser excitation is realized through the multiplier 10^{OD} [Refs. 3–5]. This multiplier takes into account the value of optical density of an OD sample at a certain wavelength λ ($OD = \varepsilon_\lambda C a$, where ε_λ is the absorption coefficient at the wavelength λ; C is the concentration of the absorbing centers; a is the distance between the laser beam center and the cell wall). However, this approach is not sufficient for exact allowance for IFE, what is often necessary, for instance, in quantitative determination of quantum yield or first moments of fluorescent spectra. This is connected with the fact that the particular length of the optical path a is difficult to determine exactly in the actual excitation geometry, which, as is known, differs from the form of an infinitely thin beam. This fact is the main reason for giving up the *a priori* use of the multiplier 10^{OD} in the correct allowance for IFE. There is information in the literature on the influence of the exciting beam diameter on the primary IFE magnitude.[8] Data on such influence on the secondary IFE are absent.

In this paper, we determine the coefficient of correction for IFE in fluorescence emission as a function of the diameter of an exciting laser beam and compare the obtained functions with the correction coefficients calculated with the use of the commonly accepted multiplier 10^{OD}.

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Calculation scheme and experiment

The schematic view of the calculation is presented in Fig. 1. A laser beam of the radius R, whose direction is parallel to the entrance slit of the spectrometer, excites the fluorescence in the absorbing medium at the distance a between the laser beam center and the cell wall. The direction of observation of the fluorescence light is parallel to the X axis.

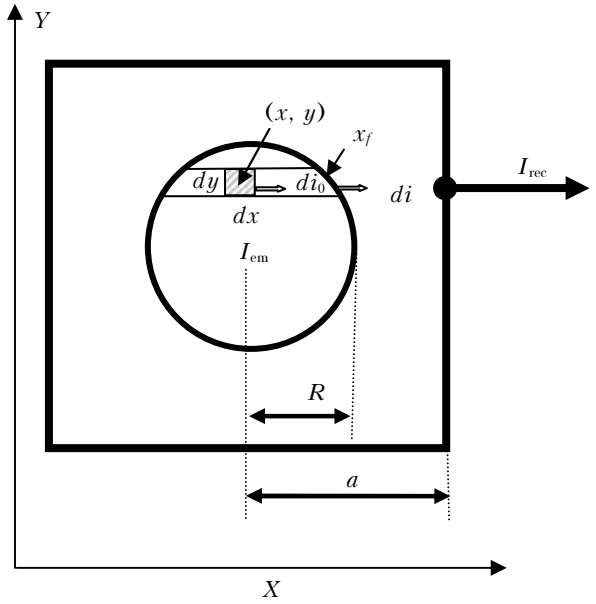

Fig. 1. The scheme of the calculation model: the top view of the working cell.

Inside the light generation zone each differential volume $dV = dxdy$ located at the point (x, y) irradiates light of the intensity $di_0 := \rho I_0 dV$, where ρ is the intensity density of irradiating centers and I_0 is their radiation intensity. Consider a differential layer at the height y from the center of the active zone. According to the Bouguer–Lambert–Beer law (BLB), the light emitted at the point (x, y) is attenuated to $di(x, y) = di_0 \cdot 10^{-\varepsilon C(x_f - x)}$ at the exit from the light spot (when $x = x_f(R, y) = \sqrt{R^2 - y^2}$). All the centers with a given y yield the following total intensity in the point $x_f(R, y)$:

$$i(y) = \int_{-x_f}^{x_f} di(x,y)dx = \frac{(1 - 10^{(-\varepsilon C 2x_f)})\rho I_0}{\varepsilon C \ln 10}. \qquad (1)$$

Further, when passing non-excited medium (passive zone) of length $(a - x_f)$, the light intensity $i(y)$ is additionally attenuated to

$$I(y) = i(y) \cdot 10^{-\varepsilon C(a - x_f)}. \qquad (2)$$

Substituting the values of $i(y)$ from Eq. (1) and $x_f = \sqrt{R^2 - y^2}$ to Eq. (2), we obtain the final expression for the light intensity gone out of the cell at the height y from the laser beam center:

$$I(y) = \frac{\rho I_0 (10^{[-\varepsilon C(a - \sqrt{R^2 - y^2})]} - 10^{[-\varepsilon C(a + \sqrt{R^2 - y^2})]})}{\varepsilon C \ln 10}. \qquad (3)$$

Finally, taking into account all layers dy from $y = -R$ to $y = R$, we find the total intensity of the recorded light I_{rec} at the exit of the cell as a definite integral of the expression (3) with respect to y:

$$I_{rec} = \frac{2\rho I_0}{\varepsilon C \ln 10} \int_0^R (10^{[-\varepsilon C(a - \sqrt{R^2 - y^2})]} - 10^{[-\varepsilon C(a + \sqrt{R^2 - y^2})]}) dy. \qquad (4)$$

From Eq. (4) we can easily obtain the multiplier α, which is the ratio of the intensity of the recorded light I_{rec} to the intensity of the emanating light I_{em}:

$$\alpha = I_{rec}/I_{em} = \frac{2\rho I_0}{\varepsilon C \ln 10} \times$$

$$\times \int_0^R (10^{[-\varepsilon C(a - \sqrt{R^2 - y^2})]} - 10^{[-\varepsilon C(a + \sqrt{R^2 - y^2})]}) dy / (\pi R^2 \rho I_0). \qquad (5)$$

As is seen from Eq. (5), parameters ρ and I_0, which are common for the emanating and recorded light, vanish and α depends only on εC, a, and R. Integrals of Eqs. (4), (5) cannot be found analytically and should be calculated numerically.

The above-mentioned model contains three explicit assumptions: 1) distribution of intensity density in the lateral section of a laser beam is uniform; 2) falling of "slant" beams to the spectrometer is ignored; 3) reradiation of fluorescence light caused by its reabsorption is not taken into account. The above reradiation can be ignored in the case of small quantum fluorescence yield or low absorption,[9] that often occurs in case of natural samples. Just this class of samples is under study in monitoring of natural objects (water solutions of humic acids, organic substances, chlorophyll, plankton, etc). The quantum fluorescence yield for such samples is less than 1% [Ref. 10]. Experimental feasibility of the first and second assumptions is considered below.

The spectra were obtained at the N_2 laser strob-fluorimeter designed on the base of the DFS-12 spectrometer (λ_{exc} = 337.1 nm).[11] A beam diameter of the LGI-21 laser was 0.3 cm. The intensity distribution in the laser beam section was uniform due to laser resonator adjustment, setting the laser beam diaphragm, and was observed visually. Thus, the feasibility of the first assumption in the IFE calculation is reachable.

The exciting beam direction is parallel to the line, conventionally passing through the spectrometer's entrance slit center. The laser beam center coincides with the center of a working cell of

1×1 cm section (Fig. 1). An advantage of the proposed geometrical scheme (the exciting beam is parallel to the entrance slit of the spectrometer, "vertical excitation") is the fact that the position of the maximally illuminated volume after adjusting, "seen" by the spectrometer, remains the same with respect to the slit when changing the optical density.[3] In this connection, it is not necessary to change the position of the cell when varying OD, as it was done in experiments on observation of fluorescence in absorbing media for the geometry "with the right (left) angle."[12]

The cell has been filled with the solution so that the meniscus was absent. A sample of a water solution of humic acid (HA) was used as a fluorophore. The solution was prepared by the procedure described earlier in Ref. 13. The solution concentration was 165 mg/liter. In the experiments on studying the dependence of fluorescence intensity on the distance between the laser beam's center and the cell front wall the distance was controlled with a micrometer. Calculations were performed by a program written in the Maple package.

Calculational and experimental results. Discussion

Calculations

Figure 2 presents the calculated ratios of the intensity I_{rec} of the light, which has left the cell, to the intensity I_{em} of the emitted light as functions of optical density for different R/a. It is seen that R/a tends to $10^{-\varepsilon C a}$ with a decrease of this quantity (see curves from top to bottom), and deviates from it more and more at $R \to a$ (increase of the laser beam diameter or decrease of the distance from the cell exit wall). The larger is the absorption coefficient εC, the more pronounced are deviations.

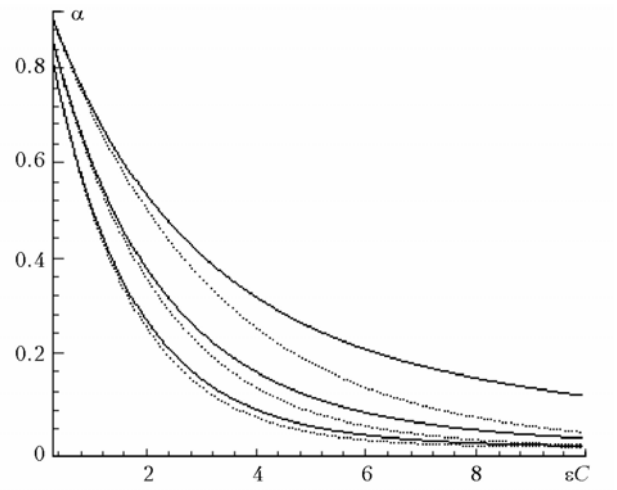

Fig. 2. The value α as a function of the absorption coefficient εC calculated by the formula (5) (solid lines) and by the Bouguer–Lambert–Beer law, $\alpha = 10^{-\varepsilon C a}$ (dashed lines). $R = 0.15$ cm, the curves (from bottom to top) correspond to $a = R$; $1.5R$; $2R$.

Experiment

The contribution of slant beams into the total fluorescence intensity can be minimized by diaphragming the condensing lens diameter, because the contribution depends on the ratio of the condensing lens diameter D to the distance between its position and the illuminated volume L. The independence of the contour shape of the condensing lens diameter serves a criterion for the absence of contribution from the slant beams. Experiments on diaphragming the condensing lens diameter within 0.5–3.5 cm, accurate to ±3%, revealed no changes in spectral fluorescence parameters of HA solutions. This means that the contribution of slant beams to the general observed intensity of fluorescence in specific geometrical conditions is small even for a non-diaphragmed lens, i.e., the second assumption on applicability of the used model for IFE calculation is also feasible experimentally. Note that another way to eliminate the falling of slant beams into the spectrometer is forming a parallel beam falling onto the entrance slit with the help of a collimator.

Figure 3 presents fluorescence spectra of the HA water solution with allowance for the IFE by the formula (5) (Fig. 3, *1*) and with allowance for the IFE by the BLB law (Fig 3, *2*). (The absorption spectrum of the HA molecule is a function, whose values monotonically decrease with the increase of the wavelength. For this sample, the value of optical density OD for $a = 0.5$ cm varies from 3 to 0.12 in the spectral range 360–630 nm). As is seen from the comparison of the presented fluorescence spectra, exact allowance for the correction for IFE leads to a pronounced decrease of the fluorescence spectrum intensity relative to that calculated by the BLB law. This is explained by the fact that the distance to the active zone center in the presence of absorption is not a but $a_{eff} = a - \gamma$ ($\gamma > 0$), i.e., the fluorescent volume is at the lesser distance for the observer, and the correction, which takes into account the IFE, must be smaller than the correction calculated by the BLB law.

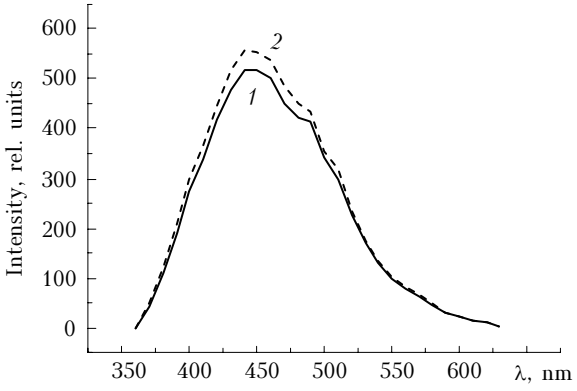

Fig. 3. Fluorescence spectra of the water solution of humic acid extracted from black soil, with allowance for IFE: the spectrum with a correction by the formula (5) (*1*); the spectrum with a correction by the Bouguer–Lambert–Beer law (*2*).

Thus, allowance for the IFE with the help of the BLB law leads to overestimation of the integral intensity (6.7%) for a given sample under conditions of the experiment. Neglect of the correction for IFE correspondingly leads to incorrect values for other parameters of the fluorescence spectrum as well. In particular, for the studied HA sample, the difference in the values of the first moment (center of gravity) of the experimentally measured fluorescence spectrum and the fluorescence spectrum with allowance for the correction for IFE by the formula (5) is 7 nm.

As follows from the results of the performed calculations, the values of εC can be determined by the fluorescence method. This problem can arise for verification (refinement) of data obtained spectrophotometrically or for estimation of εC in the absence of the spectrophotometer. The ratio β of recorded fluorescence intensities at excitation by laser light at the distances $a_i = R + \Delta_i$ and $a_j = R + \Delta_j$, respectively appears to be independent of R. This value well agrees (to an accuracy of $\sim 10^{-9}$) with the value β calculated by the ratio

$$\beta = I(a_j)/I(a_i) = 10^{-\varepsilon C\,(\Delta j - \Delta i)}, \qquad (6)$$

i.e., the inner filter effects have no influence on β. (It should be noted that the expression (6) can be obtained analytically under a hypothetical assumption that the section of an exciting beam is square).

From Eq. (6) it is easily to obtain

$$\log\beta = \log((I(a_j)/I(a_i)) = -\varepsilon C \Delta a. \qquad (7)$$

Then, measuring experimentally $\log\beta$ as a function of $\Delta a = \Delta j - \Delta i$, we obtain εC values from the slope of this function. If necessary, knowing the value of C, one can determine the absorption coefficients ε.

Applicability of the formula (7) for obtaining the information on absorptions from experimental data on fluorescence was verified by a sample of humic acid. We studied $\log\beta$ as a function of Δa for the fluorescence wavelengths 440 and 490 nm. The obtained values of εC were (0.084 ± 0.002) and (0.06 ± 0.002) cm^{-1} for 440 and 490 nm, respectively. These values almost coincide with the data obtained by a Hewlett Packard spectrophotometer: (0.082 ± 0.001) and $(0.056 \pm 0.001)\,\mathrm{cm}^{-1}$. The closeness of εC values, which were obtained by different methods, confirms the applicability of the fluorescent method in estimating $\varepsilon C(\varepsilon)$ values.

Conclusion

To take into account IFE in fluorescence emission, we have obtained a formula, which permits one to calculate the fluorescence intensity in an absorbing medium as a function of the laser beam diameter, distance from the laser beam center to the cell exit wall, and the optical density per unit of length. Using the formula, we have calculated corrections, which make it possible to take into account the inner filter effect on the emission of fluorescence in specific experiments. The results have been compared with calculations obtained under the assumption of an infinitely narrow exciting beam, when IFE can by taken into account rigorously by the Bouguer–Lambert–Beer law. Comparison of the obtained data demonstrates that the exciting beam size can introduce noticeable distortions into the observed fluorescence contour under conditions of high optical density of the sample.

The calculated corrections for IFE were obtained under "vertical excitation" with the use of a laser. A sufficiently simple calculation of corrections seems to be possible only at this way of excitation, because the calculation is a considerably more complicated physical-mathematical problem at the standard lamp way of excitation and observation ("right angle") due to the presence of the caustic (which, as a rule, is very perceptible even in modern spectrometers, such as LS-50, LS-55, or elder ones MPF-31, MPF-2A). However, if the lamp light is collimated into a maximally parallel light beam and, for the fluorescence excitation, directed in parallel to the entrance slit of the spectrometer, the application of the proposed formula is quite reasonable.

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