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ALGORITHMS FOR MULTIPARAMETER RECONSTRUCTION OF SPECTRAL AND LUMINESCENCE CHARACTERISTICS BY THE FLUORESCENCE SATURATION METHOD

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Considered is the possibility of using fluorescence saturation curves to find the following molecular parameters: (i) the quantum yield of triplet states, (ii) the lifetime of the first excited singlet state S_1, and (iii) the absorption cross section for a transition from the ground state to S_1 at the wavelength of excitation. Algorithms for a simultaneous determination of two and three parameters have been proposed. Requirements to the accuracy of saturation curve measurements were found by mathematical modeling. The results of an experimental check-up of the proposed solution algorithm for a three-parameter problem are presented for the case of eosine aqueous solution.

INTRODUCTION

In a number of studies devoted to the determination of the spectroscopic parameters of molecules by the method of nonlinear laser fluorometry, a three-level energy diagram was shown to be rather accurate for description of fluorescence phenomena. This enables one to solve inverse problems, i.e., to determine the spectroscopic parameters of molecules from measurements of fluorescence saturation curves.

It was shown in [1] that in the three-level energy diagram, the fluorescence response of the medium depends on three parameters: η_T, α, and p, where η_T is the quantum yield into a triplet state; α is the ratio of the excited singlet state lifetime to the exciting laser pulse duration: $\alpha = t_3 t_p^{-1}$; and $p = t_p \sigma_{13} F$, where σ_{13} is the absorption cross section at the exciting radiation wavelength, F is the effective photon flux density of the exciting laser pulse. Adequacy of this model was proved in [1] by experimental data on fluorescence saturation of organic dyes upon pulsed photoexcitation. According to the number of parameters to be determined simultaneously we distinguish single-, two-, and three-parameter problems. Some approaches to the solution of single-parameter problems were described previously in [2]. This work is devoted to the solution of two- and three-parameter problems.

SOLUTION ALGORITHMS FOR TWO-PARAMETER PROBLEMS

Within the framework of the three-level model, three types of two-parameter problems may be formulated:

(i) Simultaneous search for the parameters α and p when the parameter η_T is given: the so-called two-parameter α, p-problem.

(ii) Simultaneous search for the parameters η_T and p when the parameter α is given: the so-called two-parameter η_T, p-problem.

(iii) Simultaneous search for the parameters η_T and α when the parameter p is known: the so-called two-parameter η_T, α-problem.

To obtain optimized solution algorithms for inverse problems, some specific features of the procedure of fluorescence saturation spectroscopic measurement should be taken into account. One of the features is that measurements taken to build the fluorescence saturation curve are relative since calibration of the equipment for absolute measurements of fluxes of incident radiation and fluorescence emission is a complicated experimental job per se. Therefore, the number of fluorescence photons N_{fl} is determined not in absolute but in relative units, i.e., with an accuracy up to some factor constant for each point of the saturation curve. This factor remains constant in the case of a linear response of the photodetector in the range of fluorescence intensities measured. In what follows we shall assume this response to be linear.

To exclude the unknown factor for experimentally measured fluorescence intensities N_{fl}, we shall use normalized values, i. e., instead of $N_{\text{fl}\,i}$ $(i = 1, 2, 3, \ldots, n)$, we introduce the quantities

$$W_i = \frac{N_{\text{fl}\,i+1}}{N_{\text{fl}\,1}}, \qquad i = 1, 2, \ldots, n-1. \tag{1}$$

Here and henceforth we arrange $N_{\text{fl}\,i}$ in order of increasing value.

To solve the inverse problem, the intensity of incident radiation has to be known for each point of the saturation curve. In our experiments, we used the so-called method of internal reference [3] when the signal of Raman scattering by a solvent, e. g., by water, is used to obtain information about the intensity of incident radiation. An advantage of the internal reference method is that the Raman scattering signal is proportional to the incident radiation intensity in the wide range of radiation intensities, and that the Raman scattering line can be measured simultaneously with the fluorescence spectrum of the compound under study, which reduces possible measurement errors. Just as measurements of the number of fluorescence photons, N_{fl}, measurements of the number of Raman scattering photons, N_{Rs}, are also relative. Similarly, we introduce normalized radiation intensity values for N_{Rs}:

$$k_i = \frac{N_{Rs\,i}}{N_{Rs\,n}}, \qquad i = 1, 2, \ldots, n. \tag{2}$$

Here $N_{Rs\,i}$ are also arranged in increasing order, and $N_{Rs\,n}$ is the maximum value among the set of $N_{Rs\,i}$ values.

The parameter p, which is determined in solving the inverse problem is proportional to the intensity of incident radiation. For a given saturation curve, we assume the parameter p to be equal to that for the nth point, i. e., this value is maximum for a given saturation curve. For the ith point, the parameter p is given by

$$p_i = k_i p, \qquad (k_n = 1). \tag{3}$$

It should be noted that N_{fl} and N_{Rs} denote the overall (integrated over time) quantities, the time of integration being much greater than the laser pulse duration but much smaller than the phosphorescence time, i. e., the time of the $T_1 \to S_0$ transition. The latter restriction follows from the fact that, within the three-level model under consideration [1], the contribution of phosphorescence photons to the measured signal is not taken into account. The fulfillment of this condition in our experiments is achieved by photodetector gating. The gating time is chosen to be about 10^{-6} s, which is much greater than the laser pulse duration (about 10^{-8} s) and the lifetime of the S_1 level, but much smaller than the time of the $T_1 \to S_0$ relaxation, which exceeds 10^{-4}–10^{-3} s.

The solution of two-parameter problems is described by a system of two equations. Since we deal with normalized values, three points on the saturation curve are needed to write down this system:

$$\begin{cases} \dfrac{I(\eta_T,\,\alpha,\,k_2 p)}{I(\eta_T,\,\alpha,\,k_1 p)} = \dfrac{N_{\text{fl}\,2}}{N_{\text{fl}\,1}} = W_1, \\[2ex] \dfrac{I(\eta_T,\,\alpha,\,k_3 p)}{I(\eta_T,\,\alpha,\,k_1 p)} = \dfrac{N_{\text{fl}\,3}}{N_{\text{fl}\,1}} = W_2, \end{cases} \tag{4}$$

where k_1, k_2, and k_3 are the fractions of the radiation intensity; $N_{\text{fl}\,1}$, $N_{\text{fl}\,2}$, and $N_{\text{fl}\,3}$ are the fluorescence responses of the medium to photoexcitation with intensities $k_1 p$, $k_2 p$, and $k_3 p$, respectively.

In the case of a rectangular distribution (over time and space) of photons in the incident beam, the response function I has an analytical expression (see [1]). In the general case, I is obtained after a numerical integration of the system of equations that describes the three-level model [1].

When two parameters, e. g., η_T and α, are known, each of equations (4) is an equation with one variable with respect to p; hence, in the space $(\eta_T,\,\alpha,\,p)$ each of equations (4) defines a surface. The line of intersection of these surfaces, γ, represents all possible sets of $(\eta_T,\,\alpha,\,p)$ values that are solutions to (4). For instance, if the quantum yield into the triplet state $\eta_{T\,0}$ is known (a two-parameter α, p-problem), the intersection of the line γ with the plane $\eta_T = \eta_{T\,0}$ will give a solution to the α, p-problem.

If system (4) is considered as a two-parameter η_T, p-problem, i.e., the value of the parameter α is known, then to find a solution to (4) it is necessary to find the intersection of the line γ with the plane $\alpha = \alpha_0$, where α_0 is known. Similarly, for the η_T, α-problem, it will be the intersection with the p plane.

We cannot give here a detailed analysis of solution uniqueness for two-parameter problems for reasons of space so we shall only present the results. It was found that for the two-parameter α, p-problem system (4) has no more than two solutions. In the other two cases (η_T, p- and η_T, α-problems) the solution, if there is one, is unique.

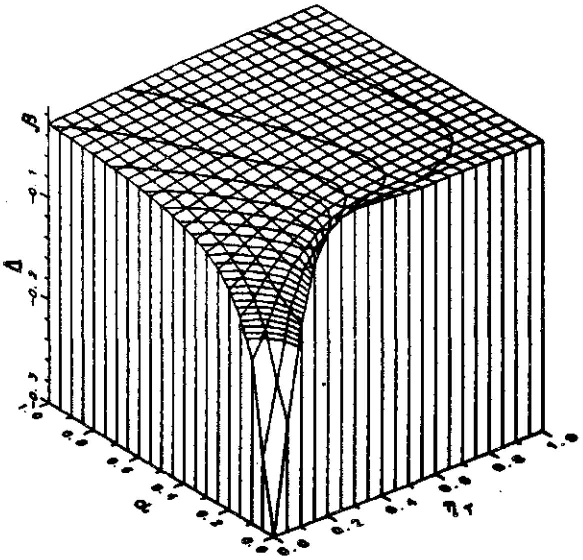

Fig. 1

The distance $\Delta = p_1(\eta_T, \alpha) - p_2(\eta_T, \alpha)$ between the characteristic surfaces p_1 and p_2 as a function of the triplet quantum yield η_T and the nonstationarity parameter α.

This is illustrated by Fig. 1. Here for different pairs of the η_T, and α parameters, the distance is shown between the surfaces $p_1(\eta_T, \alpha)$ and $p_2(\eta_T, \alpha)$ which are formed by equations (4) for a certain saturation curve. The line of zeroth difference β along which p_1 and p_2 coincide is indicated in the figure. One has to find the point of intersection of the line β and the straight line $\eta_T = \eta_{T\,0}$ to solve the α, p-problem and the point of intersection of β and the straight line $\alpha = \alpha_0$ to solve the η_T, p-problem. To find a solution to the η_T, α-problem one has, moving along the β line, to determine the point for which $p = p_0$; the coordinates η_T, α of this point will give a solution to the η_T, α-problem.

One can see that at certain values of the quantum yield into the triplet state $\eta_{T\,0}$, the $\eta_T = \eta_{T\,0}$ plane intersects the β line twice, while the $\alpha = \alpha_0$ plane intersects it only once, which is in line with the concept of solution uniqueness for two-parameter α, p- and η_T, p-problems.

One can see that solution of the α, p-problem requires a proper choice of root. This can be achieved by examining how a saturation curve calculated by three points would fit a fourth point. The root that gives the best fitting to the fourth point is chosen.

Therefore, the minimum number of points needed for determining parameters is four for the α, p-problem and three for other two-parameter problems.

In practice, more points are measured (8 in our experiments). This may by used to improve the accuracy of solving inverse problems. Having performed a numerical analysis, we have found that the best results are given by the following way of utilizing excess information.

When the number of points, n, of the saturation curve is greater than the minimum required number m, one can solve C_n^m problems. All the solutions obtained (not all of these problems may have solutions) are averaged, and the mean value is taken as a result for a given saturation curve.

RESULTS OF TWO-PARAMETER MODELING

To elucidate the potentialities of the method, we carried out mathematical modeling over a wide range of parameter variation.

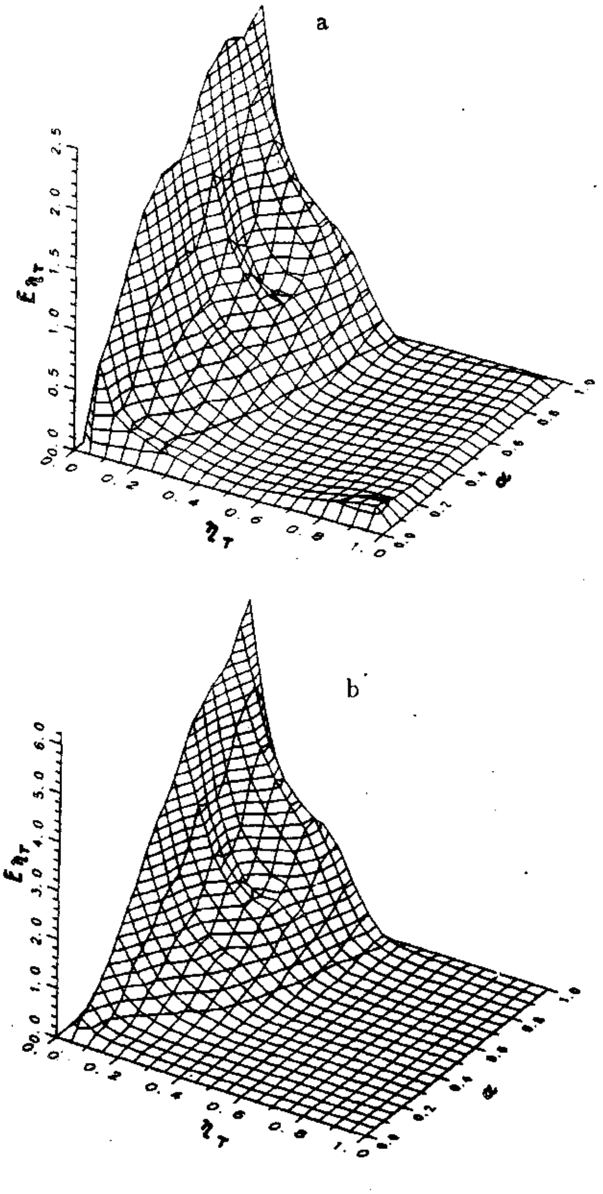

Fig. 2

Relative error of the triplet quantum yield determination, E_{η_T}, as a function of real values of the nonstationarity parameter α and the triplet quantum yield η_T in solving the two-parameter η_T, p-problem (a) and the η_T, α-problem (b).

Figure 2 a shows the relative error of η_T determination in the η_T, p-problem when the relative error of saturation curve measurements is 10^{-3}. One can see that when the quantum yield into the triplet state $\eta_T > 0.5$, the error of η_T measurements does not exceed 5% in a wide range of nonstationarity parameter α. But as η_T decreases, the error of the reconstructed η_T grows sharply and reaches 240%.

Figure 2 b shows the relative error of η_T determination in the η_T, α-problem for the relative error of saturation curve measurements of 10^{-3}. As in the preceding case, there is a wide region of good η_T reconstruction. For example, with a nonstationarity factor $\alpha \approx 1$, the quantum yields into the triplet state $\eta_T > 60\%$ are reconstructed with an accuracy better than 10%. Upon excitation by long pulses (i. e., at $\alpha < 0.1$), the region of η_T reconstruction with an accuracy better than 10% markedly broadens and falls within the interval of $\eta_T > 15\%$. An unfavorable situation for solving the η_T, α-problem is that of small η_T and large α.

SOLUTION ALGORITHMS FOR THREE-PARAMETER PROBLEMS

Within the framework of the three-level model adopted, the three-parameter η_T, α, p-problem provides the greatest amount of information about the spectroscopic parameters of the fluorescing substance. In the three-parameter problem, unknown are all the three parameters and they are determined simultaneously by solving the following system of nonlinear equations:

$$\begin{cases} \dfrac{I(\eta_T,\, \alpha,\, k_2 p)}{I(\eta_T,\, \alpha,\, k_1 p)} = \dfrac{N_{\mathrm{fl}\,2}}{N_{\mathrm{fl}\,1}} = W_1, \\ \dfrac{I(\eta_T,\, \alpha,\, k_3 p)}{I(\eta_T,\, \alpha,\, k_1 p)} = \dfrac{N_{\mathrm{fl}\,3}}{N_{\mathrm{fl}\,1}} = W_2, \\ \dfrac{I(\eta_T,\, \alpha,\, k_4 p)}{I(\eta_T,\, \alpha,\, k_1 p)} = \dfrac{N_{\mathrm{fl}\,4}}{N_{\mathrm{fl}\,1}} = W_3, \end{cases} \tag{5}$$

where the same notation is used as in (4).

To construct system (5), we now need four points of the fluorescence saturation curve. System (5) forms three characteristic surfaces in the η_T, α, p space. The points belonging to all the three surfaces represent a solution to (5).

Analysis shows that if there is a solution to (5) in the range of possible values of the parameters $0 < \eta_T < 1$, $\alpha > 0$, $p > 0$, this solution will be unique.

RESULTS OF THREE-PARAMETER MODELING

The relative error of the parameter α determination in solving the three-parameter η_T, α, p-problem is shown in Fig. 3. It has been found that to obtain results comparable in accuracy with those of the two-parameter problem, the accuracy of measurements should be improved by an order of magnitude. For this reason, the calculations were performed for a relative error of 10^{-4}.

Figure 3 shows that in the range of $\alpha = 0.3$–0.7 and any quantum yield into the triplet state, the parameter α is reconstructed with an accuracy better than 30%.

Figure 4 shows the results of η_T reconstruction for the same problem. Here a situation seems to be much better. At $\alpha \approx 10^{-1}$, the quantum yield into the triplet state is reconstructed with an accuracy better than 20% over the entire range of its values. For $\alpha \approx 1$, this accuracy is observed for quantum yields into the triplet state greater than 50%.

For brevity's sake, we omit the results of p determinations for the case of the three-parameter problem. It should be noted only that of the three parameters the accuracy of p determination is the best. For instance, at $\alpha > 0.3$, p is determined with an accuracy better than 30% at any value of the quantum yield into the triplet state.

Therefore, in the three-parameter case, for $\eta_T \approx 0.5$ and $\alpha \approx 0.5$, the error of determination of any of the three parameters does not exceed 15% provided the fluorescence saturation curve is measured with an accuracy of about 10^{-4}.

DISCUSSION

At present, errors of measuring the saturation curve constitute several percent [1]. Theoretical analysis has shown that such an accuracy is insufficient to solve two-parameter problems for arbitrarily chosen substances by the proposed method. However, the experience shows [4] that, with optimal values of the

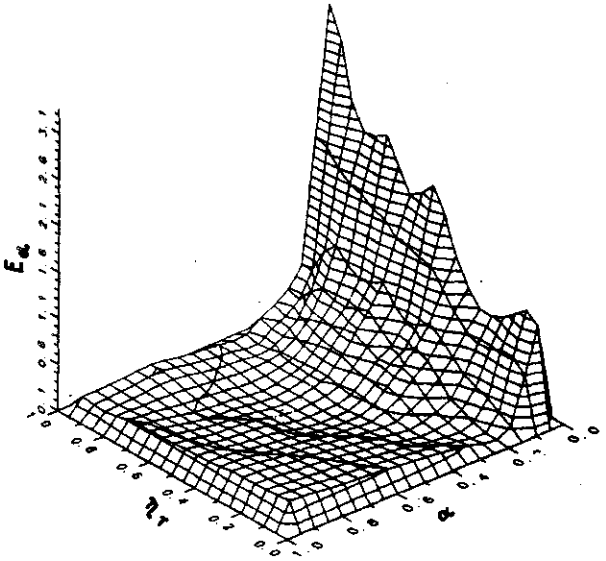

Fig. 3

Relative error of the nonstationarity parameter determinations, E_α, as a function of real values of the nonstationarity parameter α and the triplet quantum yield η_T in solving the three-parameter η_T, α, p-problem.

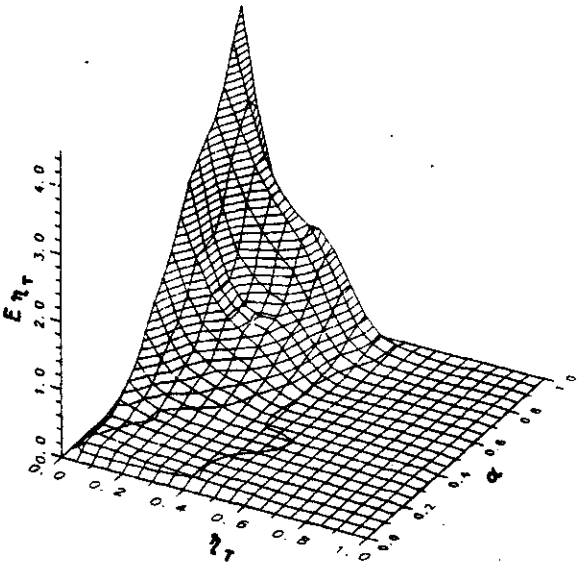

Fig. 4

Relative error of the triplet quantum yield determinations, E_{η_T}, as a function of real values of the nonstationarity parameter α and the triplet quantum yield η_T in solving the three-parameter η_T, α, p-problem.

parameters, two- and even three-parameter problems can be adequately solved based on experimental data available.

Upon treatment of the fluorescence saturation curve for eosine solutions in water plotted by using 16 experimentally obtained points, the parameters η_T, α, and p shown in Table 1 were obtained by solving the

Table 1

Comparison of Spectral and Luminescence Parameters
for Aqueous Solutions of Eosine
Found in the Literature and Reconstructed
by the Three-Parameter Algorithm

Parameters	η_T	α	p	t_3, ns	σ_{13}, cm^2
Calculation (published data)	0.68	0.13	1.9	1.4	1.1×10^{-16}
Reconstruction	0.20	0.23	2.6	2.5	1.5×10^{-16}

three-parametric problem. Comparison with the values of the same parameters calculated using independent measurements [5] of η_T, t_3, and σ_{13} shows that there is a satisfactory reconstruction of the parameters α and p. The slightly greater error in reconstructing the quantum yield into the triplet state, η_T, is in line with the results of mathematical modeling given above.

To solve two and three-parameter problems in the wide range of parameter values, the accuracy of measurements must be improved to tenth and hundredth fractions of a percent, respectively. Now the work is in progress aimed at improvement of experimental facilities and processing procedure for fluorescence saturation curves in order to raise the quality of input data for the algorithm of parameters reconstruction.

One of possible approaches to the problem seems to be using two lasers with greatly different pulse durations. The use of two pulses will not restrict the versatility of the method, but will provide additional information needed to improve the procedure for calculation of spectroscopic parameters.

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