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Numerical Simulation of Chemical Equilibria and Photostationary States Using Spectrophotometric and Spectrofluorometric Data

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Abstract—A mathematical model describing series of experimental absorption and fluorescence spectra of multicomponent systems via equilibrium and photostationary concentrations of components of the systems was constructed. With photostationary concentrations, it is possible to take into account changes in the fluorescence spectra resulting from photochemical reactions. Ther unknown equilibrium and kinetic parameters of the model and also the spectra of pure components are found from experimental data by the nonlinear least-squares method. A software for the model was developed, and its applicability to the determination of the composition of multicomponent systems, resolution of the spectra of pure components, and determination of equilibrium and kinetic constants was demonstrated.

The development of new methods and software for analytical treatment of multicomponent systems using spectral data attracts growing researchers' attention [1–6]. A major advantage of such methods, allowing calculation of the composition of multicomponent systems and spectral characteristics of their components, is that they do not require separation and isolation of individual components, which may be very difficult or even impossible for intermediate or unstable compounds.

However, it should be noted that the available programs have certain limitations determined by the levels of both mathematical models and specific implementations. In particular, the majority of computer methods for resolving the spectra do not distinguish qualitatively the electronic absorption and fluorescence spectra. The intensities of both spectra are considered to be proportional to the concentrations of dissolved substances in the ground electronic states. Such a limited approach results in the inability of these, sometimes rather useful, methods to resolve the fluorescence spectra in the case of adiabatic photochemical reactions. This can be seen from the consideration of even the simplest classic systems such as, e.g., β-naphthol–β-naphtholate anion. In this system, in photodissociation of β-naphthol, the anion in the ground electronic state is virtually fully absent [4]. Moreover, modern programs should involve developed means of the data imaging and the possibility of integration with other popular programs of treating numerical and graphic information [1].

The aim of this work was to construct a mathematical model that would allow determination of the compositions of complex equilibrium and photostationary systems using spectrophotometric and spectrofruorometric data, evaluation of the equilibrium and kinetic constants, assessment of their reliability, and also resolution of the spectra to obtain the spectral characteristics of separate components of the systems. Along with the theoretical construction of the mathematical model, we developed the corresponding software and showed its applicability to examining real systems.

Every physicochemical model should relate properties of separate chemical components, their amount, and also certain physicochemical parameters to a directly observed response function [7]. In particular, for an equilibrium system of absorbing components, we should relate the molar extinction coefficients of separate components, their concentrations, and thermodynamic equilibrium constants to the optical density, which will be the response function. The problem of determining the parameters of the model by the response function is the inverse problem of mathematical physics. The methods of solving inverse problems can be subdivided into three classes: graphical and approximate analytical methods; exact analytical methods; statistical methods (likelihood maximum, entropy maximum, least-squares, regularization, etc.).

Numerous methods from the first two classes, as applied to the treatment of spectrophotometric data, have been described in [8]. At the same time, the methods of the last class are the most universal among the methods of solving the inverse problems. Given a computer, the labor expenses of a researcher for solving an inverse problem are equal to the expenses for a simple reformulation of this problem.

When searching for parameters of a mathematical model describing a series of absorption spectra, we will start from the quest for approaching in some sense a calculated matrix of optical densities **D** depending on the model parameters to an experimental matrix $\mathbf{D}^{\mathbf{e}}$ of dimensionality $l \times n$. This matrix is obtained by quantization of a series of n continuous spectra by l wavelengths. According to the least-squares method, the parameters that minimize functional (1) will be the most probable.

$$\sum_{i=1}^{l}\sum_{j=1}^{n} \mathrm{w}_{ij}(D_{ij} - D_{ij}^{\mathrm{e}})^2 \longrightarrow \min. \tag{1}$$

Here w_{ij} is the statistical weight of the experimental D_{ij}^{e} point, and D_{ij} is the calculated optical density of the jth spectrum at the ith wavelength. The simplest function that qualitatively reflects the dependence of the relative error of a spectrophotometric analysis is the dependence of the type $10^{\mathrm{D}}/D$ [8]. On this basis, we can take weights (25)w_{ij}) equal to the square of transmission [relationship (2)].

$$w_{ij} = 10^{-2D_{ij}}. \tag{2}$$

Similar weights taking into account also the instrumental function of the spectrophotometer were successfully used in [3]. Nevertheless, in the calculation practice the measurements are most often believed to be of equal accuracy [9]; hence, we will assume w_{ij} = 1. In particular, unit weights are commonly used when simulating an experimental three-dimensional matrix of fluorescence excitation and emission by a trilinear form [4].

Following the Bouguer–Lambert–Beer law of light absorption and also the additivity principle, we can write [8] expression (3).

$$\mathbf{D} = \varepsilon \times \mathbf{c} \times d. \tag{3}$$

In this expression, ε is the matrix of the molar extinction coefficients of the absorbing components of the dimensionality $l \times m$ (m is the number of absorbing components); **c**, matrix of concentrations of absorbing components of a series of solutions differing in the extent of chemical transformations (of dimensionality $m \times n$); and d, optical path length (a scalar quantity, if this length does not change from one experiment to another).

For the convenience of practical use and also for scaling of the extinction coefficients, which can differ by several orders of magnitude, we can rewrite expression (3) as (4).

$$\mathbf{D} = \mathbf{f} \times \mathbf{a} \times \mathbf{c}. \tag{4}$$

Here **f** is the matrix of the shapes of the spectra of pure components of dimensionality $l \times m$ (in arbitrary units linearly related to the molar extinction coefficient, e.g., in the optical density units), and **a**, the diagonal matrix of the spectrum amplitudes (i.e., of normalizing factors to the molar extinction coefficients if d is 1 cm) of dimensionality $m \times m$.

The number of light-absorbing components can be predetermined by finding the rank of $\mathbf{D}^{\mathbf{e}}$. Under the conditions of a real experiment, when calculating the rank of matrix, it is also necessary to take into account the errors in measuring spectra (for example, by the Wallace–Katz method [10]). This information can be useful when subsequently constructing a scheme of the occurring processes.

To find the matrix **c** of concentrations, we must prearrange the scheme of the occurring processes as the equations of chemical reactions and also the $\mathbf{c_0}$ matrix of the starting concentrations of substances to be added to the system. For the simplicity sake, depending on the context, the same symbol **c** must be understood both as the matrix of concentrations of optically active components and as the matrix of simply all the components. Then (in the approximation of ideal solutions) the c matrix can be obtained by numerically solving nonlinear system of equations (5), for example, by Newton's method [11] [from here on $f(\mathbf{x})$ means $\|f(x_{ij})\|$].

$$\begin{cases} \mathbf{S} \times \ln \mathbf{c} = \ln \mathbf{K} \times (1, 1, ..., 1). \\ \mathbf{M} \times \mathbf{c} = \mathbf{M} \times \mathbf{c}_0. \end{cases} \tag{5}$$

Here **S** is the matrix of stoichiometric coefficients; **K**, the vector-column of the equilibrium constants; (1,1, ..., 1), the vector-raw of length n; and **M**, matrix of material balance found from orthogonality relationship (6).

$$\mathbf{S} \times \mathbf{M}^{\mathrm{T}} = 0. \tag{6}$$

Here $\mathbf{M}^{\mathbf{T}}$ is the transposed matrix **M**.

According to the Gibbs rule, the number of basis components involved in the balance, summed up with the number of linearly independent stoichiometric equations, must be equal to the total number of substances in the system [12]. This fact should be taken into consideration when constructing system of equations (5).

Therefore, functional (1) will depend on the optimized **f**, **a**, and **K** parameters [expression (7)].

$$\sum_{i=1}^{l}\sum_{j=1}^{n}[D_{ij}(\mathbf{f},\ \mathbf{a},\ \mathbf{K}) - D^{e}_{ij}]^2 \longrightarrow \min. \qquad (7)$$

If it is necessary to take into account the effect of temperature on the chemical equilibrium, we can use Eq. (8), which is valid in the approximation that the functions of state are constant within a narrow temperature range.

$$\ln K = -\Delta H/RT + \Delta S/R. \qquad (8)$$

Here K is the equilibrium constant of a chemical reac= tion, and ΔH and ΔS are the enthalpy and entropy of the reaction. With regard to relationship (8), functional (7) will be written as (9).

$$\sum_{i=1}^{l}\sum_{j=1}^{n}[D_{ij}(\mathbf{f},\ \mathbf{a},\ \Delta\mathbf{H},\ \Delta\mathbf{S}) - D^{e}_{ij}]^2 \longrightarrow \min. \qquad (9)$$

In principle, functional (7) as well as (9) can be minimized by any method of optimizing functions of many variables, from zero to the second order inclusive [9, 13]. However, owing to nonlinearity of these functionals with respect to a_{ij} and f_{ij} and especially owing to a great number of these elements (from several hundreds to several tens of thousands), there is good reason to single out their determination as a separate auxiliary problem. This problem must be solved at every step of iterative search for the elements of **K**, Δ**H**, and Δ**S** vectors. This problem can be solved in a finite number of steps either by the linear least-squares method [11], or, in the case of nonnegativeness condition being imposed on the solution, by one of the methods of quadratic programming [14].

In the case of photostationary (in the absence of oscillatory reactions), system of equations (5) is supplemented with the steady-state conditions, and with regard to them it will be written as expression (10).

$$\begin{cases} \mathbf{S}^{T} \times \exp[\ln \mathbf{k} \times (1, 1, ..., 1) - \mathbf{S}_{R} \times \ln \mathbf{c}] = 0, \\ \mathbf{S} \times \ln \mathbf{c} = \ln \mathbf{K} \times (1, 1, ..., 1), \\ \mathbf{M} \times \mathbf{c} = \mathbf{M} \times \mathbf{c}_0. \end{cases} \qquad (10)$$

Here **k** is the vector-column of the reaction rate constants, and $\mathbf{S}_R$, matrix of the subset of the reagents of the **S** matrix.

When practically solving system (10), we must divide substances into two groups on the basis of the fact whether their concentrations are found in the equilibrium or steady-state approximations. We must also exclude the linearly dependent equations from the subsystems of the law of mass action and the conditions of steady state. The total number of equations must be equal to the number of unknowns, i.e., to the number of substances in the system whose steady-state (and quasiequilibrium) concentrations should be determined. System (10) is conveniently solved with respect to the logarithms of constants and concentrations rather than to these very values. In such a way, some scaling of these values is achieved and the condition of their nonnegativeness is met automatically.

We also emphasize that the linear dependence of the absorption intensity on the concentration of a pigment is realized only for low optical densities. In the general case, the intensity of the light absorption, W, is proportional to the fraction of absorbed radiation [relationship (11)].

$$W = W_0(1 - 10^{-\varepsilon cl}). \qquad (11)$$

Here W_0 is the intensity of the incident radiation, and ε, molar extinction coefficient.

At low optical densities, Eq. (11) transforms into Eq. (12), which is a first-order kinetic equation.

$$W = 2.303 W_0 \varepsilon cl. \qquad (12)$$

The temperature dependence of the reaction rate constants is usually taken into account by Arrhenius equation [9] [relationship (13)].

$$\ln k = \ln k_0 - E_a/RT. \qquad (13)$$

Here E_a is the activation energy.

For the matrix of fluorescence intensities **I**, we can write expressions (14) and (15) similar to relationships (3) and (4).

$$\mathbf{I} = \mathbf{A} \times \mathbf{c}, \qquad (14)$$

$$\mathbf{I} = \mathbf{f} \times \mathbf{a} \times \mathbf{c}. \qquad (15)$$

Here **A** is the matrix of Einstein's coefficients for spontaneous radiative transitions corresponding to various emission wavelengths. Then the problem of searching for optimal parameters will be reduced to the minimization of functional (16).

$$\sum_{i}\sum_{j}(I_{ij} - I^{e}_{ij})^2 \longrightarrow \min. \qquad (16)$$

In the practically important specific representation, expression (15) can be given as relationships (17) and (18).

$$\mathbf{I} = \varphi \times \tau^{-1} \times \mathbf{c} = \varphi \times \mathbf{c}', \tag{17}$$

$$\mathbf{I} = \mathbf{f}' \times \mathbf{a}' \times \mathbf{c}'. \tag{18}$$

Here φ is the matrix of differential quantum yields of fluorescence of the emitting components depending on the wavelength; τ, diagonal matrix of lifetimes of the emitting components; and $\mathbf{f}'$, matrix of fluorescence spectra of pure components (in relative units, if the diagonal elements of the $\mathbf{a}'$ matrix are equal to each other). Let us denote elements of the $\mathbf{c}'$ matrix, which, by their physical sense, are the intrinsic rates of the decay of excited molecules in the absence of other processes, as active photostationary concentrations. The active photostationary concentrations have such a remarkable property that, when the optical density of a multicomponent system is invariable and the photochemical reactions occurring in it do not involve changes in the number of excited molecules, their sum in a series of solutions will apparently remain constant. Thus, the current photo- steady-state concentrations can be considered as the numbers of peculiar photostationary equivalents in a unit volume.

When determining the kinetic and equilibrium constants from the spectrofluorimetric data, a number of additional circumstances should be taken into account. The concentration of excited molecules is usually very low (several orders of magnitude lower than the fluorophore concentration). In such a case, the precise intensity of the incident light, W_0, is unknown as a rule. If we exclude the concentrations of excited molecules from the subsystem of the material balance of system (10), then, for the most typical case of first-order reactions with respect to excited molecules, the arbitrary choice of W_0 is equivalent to simple scaling of the concentrations of excited particles or to multiplication of the steady-state conditions by a constant coefficient. Such a scaling corrected by the matrix of the spectral amplitudes will have no effect on the optimized constants. The knowledge of individual lifetimes of the emitting components is not necessary for the successful resolution of the fluorescence spectra, since system (10) (on condition that excited molecules are excluded from the equation of material balance) can be solved relative to active photostationary concentrations. However, in this case the dimensionalities and/or the optimized constants will be, generally speaking, different.

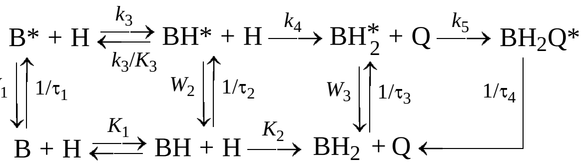
In practice, the range of applicability of linear law (12) can be broader than the range inferred from expression (11). This follows from the fact that expression (12) is idealized and does not take into account effects of the inner filter of the first and second kinds depending on the geometric parameters of the cell and on the overlap of the absorption and fluorescence spectra [15]. The presence of distinct isoemission points in the fluorescence spectra can strongly support linear law (12).

As expressions (4) and (15) formally coincide, when searching for the constants we can combine the absorption and fluorescence spectra into a common (usually two-dimensional) matrix of experimental spectra and treat them simultaneously.

To check the applicability of our approach to the determination of the composition of multicomponent systems, to the resolution of the spectra of pure components, and to the determination of equilibrium and kinetic constants, we solved a model problem of resolving the fluorescence spectra of a multicomponent system and also a number of problems of analyzing binary systems on the basis of experimental absorption and fluorescence spectra. We carried out the calculations with a Pentium II computer (300 MHz) using MatLab 6.0 program package.

The processes in a model multicomponent system are shown in Scheme 1.

Scheme 1.



Here W_1, W_2, and W_3 are intensities of light absorption by the species B, BH, and BH_2, respectively; τ_1, τ_2, τ_3, and τ_4, intrinsic lifetimes of B*, BH*, BH_2^*, and BH_2Q^*, respectively; K_1, K_2, and K_3, equilibrium constants; and k_3, k_4, and k_5, rate constants of forward adiabatic photoreactions. The system contains nine species; four of them, which are in excited states, are capable of emitting light. The fluorescence spectra shown in Fig. 1 were assigned to these species. The stoichiometric matrix of the system contains 13 rows corresponding to 13 chemical processes. Only six of them are linearly independent. Therefore, generalized system (10) will contain six plus three, i.e., nine equations: four conditions of the steady state for the rates of consumption of B*, BH*, BH_2^*, and BH_2Q^*; two equations relating quasiequilibrium concentrations of H, B, BH, and BH_2; and three equations of material balance of the basis components (for ex-

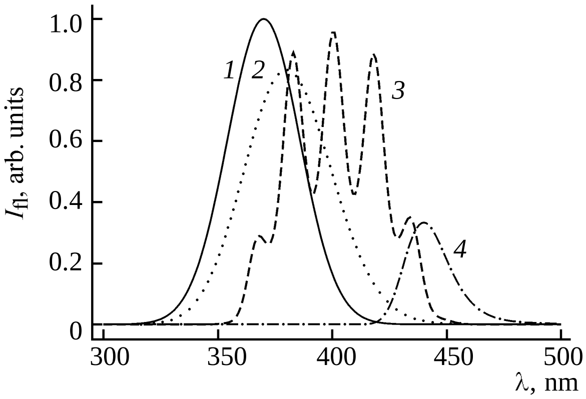


Fig. 1. Fluorescence spectra of pure components of the model system (Scheme 1). (*1*) B*, (*2*) BH*, (*3*) BH_2^*, and (*4*) BH_2Q^*.

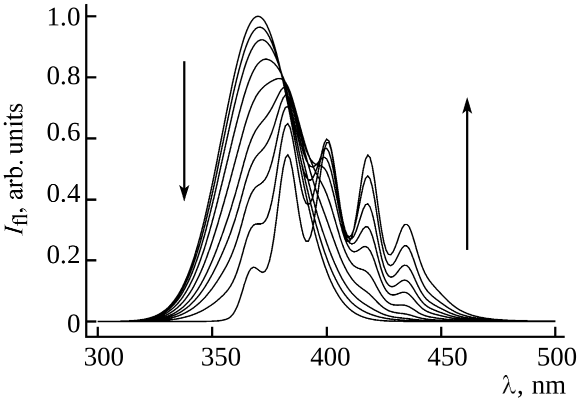


Fig. 2. Fluorescence spectra of a multicomponent model system at various concentrations of H and Q.

ample, B, H, and Q). To solve system (10) and to find steady-state (and quasiequilibrium) concentrations of all the nine substances, we specified certain values of constants and the matrix of initial concentrations of the dimensionality 9 × 10. After numerical solution of system (10), the fluorescence spectra of a series of ten solutions were calculated at 201 wavelengths by expression (15) (Fig. 2). The elements of the matrix of amplitudes were taken proportional to $1/\tau_i$. In such a manner, the direct model problem was solved.

Then we set an inverse problem of searching for spectra of pure components BH*, BH_2^*, and BH_2Q^*, and also for constants K_1, K_2, k_3, k_4, and k_5. The spectrum of B* and also eight other parameters were considered as known and were not subjected to optimization. We minimized functional (16) by Newton's method with approximation of inverse Hessian after Gill and Murray [13] with singling out the linear problem of searching for the spectra.

We estimated the rms deviation of the nonlinear (linear) parameters by the Gauss–Markov formula [4] [relationship (19)].

$$\sigma_i^2 = F\cdot H_{ii}^{-1}/r. \qquad (19)$$

Here σ_i is the standard deviation of the ith parameter; F, sum of the deviations squared; H_{ij}^{-1}, diagonal element of the inverse Hessian of the nonlinear (linear) problem; and r, number of the degrees of freedom.

The relative rms deviations of the resulting constants and of the intensities of the spectra of pure components depending on the degree of perturbation, ξ, of the starting $\mathbf{I}^\mathbf{e}$ matrix are given in Table 1. The perturbation was carried out by multiplying the elements of the $\mathbf{I}^\mathbf{e}$ matrix by $(1 + \xi x)$ (x is a random variable evenly distributed over the [–1, 1] interval). As seen from Table 1, small variations in the solution correspond to small variations in the input data of the problem. This circumstance is one of the necessary criteria of a correctly formulated problem [9]. A comparatively low accuracy of determining k_3 and k_4 results from a substantial linear correlation between them, which follows from considering the variance matrix (see below). By and large, the solution of the inverse model problem can be considered successful and stable to perturbations.

$$\begin{array}{rrrrr}
1.000 & & & & \\
0.506 & 1.000 & & & \\
0.027 & 0.661 & 1.000 & & \\
-0.028 & -0.671 & -0.987 & 1.000 & \\
0.640 & 0.433 & -0.045 & 0.043 & 1.000
\end{array}$$

Aside from considering the model system, we compared the equilibrium and kinetic constants obtained by treating the data of the absorption and fluorescence spectra at a single wavelength with constants determined by a global numerical analysis of the absorption and fluorescence spectra [i.e., by minimizing functionals (1) and (16)].

The protonation of 2-(3-quinolyl)-1,3-benzothiazole (**I**) with trichloroacetic, trifluoroacetic, and sulfuric acids in ethanol in the ground and excited states was studied previously [16]. Typical dependences of the absorption and fluorescence spectra of **I** on the acid concentration are shown in Fig. 3. It was found that the dynamic luminescence quenching of **I** with acids, resulting from adiabatic transfer of a proton, occurs irreversibly, below the diffusion limit, and is not accompanied by a noticeable induced nonradiative

Table 1. Relative standard deviations of intensities of the obtained spectra of pure components and of the constants of the model problem as functions of the degree of perturbation ξ[a]

ξ	I(BH*)	I(BH$_2^*$)	I(BH$_2$Q*)	K_1	K_2	k_3	k_4	k_5
–	7.0	6.1	5.9	5.4	5.4	2.7	3.6	4.7
5	5.7	4.9	4.6	5.3	5.2	2.6	3.5	4.5
4	4.7	3.9	3.6	4.4	4.4	1.7	2.6	3.7
3	3.7	2.9	2.6	3.4	3.4	0.7	1.6	2.7

[a] Decimal logarithms of reciprocal values are given.

deactivation. With regard to this fact, the reactions of benzothiazole (**I**) with acids can be described by the following simplest scheme.

Scheme 2.

$$\begin{tikzcd}
\mathrm{B^* + H} \arrow[r, "k_q"] \arrow[d, shift right, "W"'] & \mathrm{BH^{*+}\cdots A^-} \arrow[d, shift right, "W'"'] \\
\mathrm{B + HA} \arrow[u, shift right, "1/\tau_0"'] \arrow[r, shift left, "K"] & \mathrm{BH^{+}\cdots A^-} \arrow[l, shift left] \arrow[u, shift right, "1/\tau'_0"']
\end{tikzcd}$$

Here W and W' are the intensities of the light absorption by the starting B form and by the protonated $\mathrm{BH^+\cdots A^-}$ form, respectively; τ_0 and τ'_0, intrinsic lifetimes of the starting B* form and protonated $\mathrm{BH^{*+}\cdots A^-}$ forms, respectively, in the electronically excited state; K, constant of the acid–base equilibrium; and k_q, rate constant of the luminescence quenching of the starting form. In the case of sulfuric acid, the protonating agent HA is actually a protonated solvent molecule, i.e., $\mathrm{EtOH_2^+}$. The lifetimes of excited molecules **I** and **IH$^+$**, measured in the absence of sulfuric acid and in the presence of its excess, are 0.3 and 1.4 ns. These values were taken as τ_0 and τ'_0, respectively, for all the three systems (the knowledge of the last value is not necessary). By treating the spectra at a single wavelength, we found K and k_q as parameters of regressions (20) and (21).

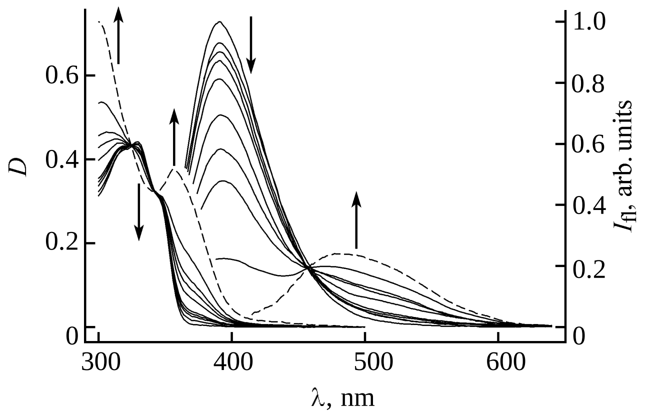


Fig. 3. Absorption spectra and uncorrected fluorescence spectra of benzothiazole **I** in the presence of $\mathrm{CCl_3COOH}$ (c_{HA} 0, 0.025, 0.05, 0.075, 0.1, 0.25, 0.375, 0.5, and 1 M). The arrows show how the spectra change as the acid concentration increases. The calculated spectra of pure protonated form are shown by the dashed lines.

$$(D - D_0)^{-1} = (K\Delta D[\mathrm{HA}])^{-1} + \Delta D^{-1}, \qquad (20)$$

$$I_0/I = (1 + \mathrm{K}[\mathrm{HA}])(1 + k_q\tau_0[\mathrm{HA}]). \qquad (21)$$

In expressions (20) and (21), D and I are the optical density and fluorescence intensity at specified wavelengths; D_0 and I_0, optical density and fluorescence intensity of the starting form; and ΔD, difference between the optical densities of the protonated and starting forms (for the same wavelength).

When determining K by Eq. (20), it is appropriate to choose a wavelength at which the change in the optical density is as maximal as possible, and when determining k_q by Eq. (21), the wavelength at which it is still possible to neglect the contribution of the protonated species to the fluorescence intensity. Such optimal wavelengths are termed analytical. We used optical densities at λ 360 nm and fluorescence intensities at λ 380 nm.

When determining the K and k_q by the method of global numerical analysis, we used the $\mathbf{D^e}$ and $\mathbf{I^e}$ matrices of dimensionality 201 × 9 and 431 × 9, respectively. The analysis of these matrices by Wallace–Katz method showed the presence of two absorbing and two emitting components. Thus, the diagonal elements of the $\mathbf{D^e}$ and $\mathbf{I^e}$ matrices for the system **I** + $\mathrm{CCl_3COOH}$, after reducing them to a trapezoidal form by Wallace–Katz method and referring to diagonal elements of error matrices, are 0.1790, 0.0458, 0.0016, 0.0004, 0.0002, 0.0002, 0.0002, 0.0002, 0.0002 and 0.3333, 0.0581, 0.0099, 0.0057, 0.0040, 0.0037, 0.0015, 0.0012, 0.0003, respectively (the measurement errors of the spectrophotometer and spectrofluorimeter used in [16] are 0.002 and 0.03, respectively). As seen from these data for the third and higher diagonal elements, the accuracies of the experimental absorption and fluorescence spectra are essentially different; hence, their combined treatment using the least-squares method of equal accuracy is not appropriate. Therefore, we determined K separately by the global numerical analysis of the absorption spectra and used the obtained value in the determination of k_q by the numerical analysis of the fluorescence spectra. We determined these constants in the same sequence by Eqs. (20) and (21). Simultaneous determination of K and k_q solely from the fluorescence spectra by any of these methods is not appropriate because of a very strong correlation between them.

Table 2 gives the K and k_q constants determined both by treating the data at a single wavelength (local procedure) and by the method of global numerical analysis (global procedure), and also the weighted difference t_s between the constants found by different methods (Student's test). For the system **I**+ $CF_3 \cdot COOH$, the plot of I_0/I vs. [HA] shows no deviations from a straight line. This suggests that the fluorescence quenching in this system occurs by the static mechanism exclusively. For the other systems, the photoprotonation is manifested quite distinctly. The dependence of the degree of protonation of benzothiazole **I** in the ground and excited electronic states on the concentration of trichloroacetic acid is shown in Fig. 4. As a consequence of an increase in the basicity of a molecule of **I** upon excitation, its protonation in the excited state is much more efficient than in the ground state.

At the same time, in these systems the complete conversion of the neutral form into the protonated form is impossible at reasonable concentrations of not very strong organic acids; hence, the problem of calculating the absorption and fluorescence spectra of the individual protonated species is urgent. A simple further increase in the acid concentration is undesirable, as it can result (and results) in a significant distortion of the spectra owing to changes in the solvent parameters.

Table 2. Constants (K, l mol^{-1}; k_q, l mol^{-1} s^{-1}) of reactions between benzothiazole **I** and acids, as determined by the treatment at analytical wavelengths (local procedure) and by the method of global numerical analysis (global procedure), and Student's test (t_s) for the constants determined by different methods

Acid	Constant	Procedure		t_s
		local	global	
CCl_3COOH[a]	K	1.2 ± 0.3	1.148 ± 0.009	0.3
CCl_3COOH[b]	$k_q \times 10^{-9}$	2.98 ± 0.09	2.91 ± 0.02	0.8
CF_3COOH[a]	K	1.9 ± 0.4	1.72 ± 0.02	0.5
CF_3COOH[b]	K	1.80 ± 0.05	1.768 ± 0.004	0.6
H_2SO_4[a]	$K \times 10^{-1}$	2.07 ± 0.11	2.037 ± 0.010	0.3
H_2SO_4[b]	$k_q \times 10^{-10}$	1.18 ± 0.13	1.046 ± 0.010	1.1

[a] Absorption spectra. [b] Fluorescence spectra.

As seen from Table 2, the constants determined by two different methods are in good agreement and almost everywhere differ by less than one standard deviation. At the same time, the method of global analysis has a number of significant advantages (perceptible, of course, only with powerful calculating tools at hand). Owing to a great number of degrees of freedom, the unknown parameters are determined with a higher accuracy. As expressions (4) and (15) are very general, the method does not require any special points in the spectra, the regions of nonoverlapping spectra, smooth peaks, etc. There is no problem of choosing analytical wavelengths. There is no need in analytical computations, which can be very complicated or even impossible for no less than three- and four-component systems. Almost the only thing that is required is to specify a scheme of occurring processes as a matrix of stoichiometric coefficients. The method allows the spectra of individual components to be determined along with the equilibrium and kinetic constants. In this case, in contrast to the spectra determined by expressions of type (20), they are at once obtained compatible with the optimal values of the constants.

Thus, we constructed a mathematical model capable of describing the experimental series of absorption and fluorescence spectra of multicomponent systems via steady-state and (quasi)equilibrium concentrations of the system components, including those in the electronically excited state. The latter fact makes it possible to take into account changes in the fluorescence spectra resulting from photochemical reactions. The equilibrium and kinetic parameters of the model and also the spectra of pure components can be found by the nonlinear least-squares method. The parameters of the model (except the amplitude matrix having an auxiliary significance) are invariant with respect to the intensity of the exciting light of

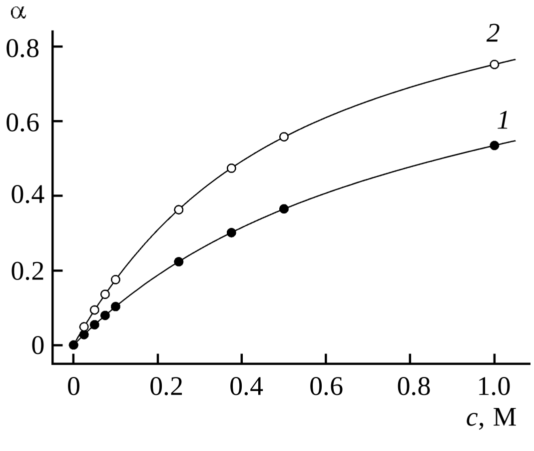


Fig. 4. Degree of protonation (α) of benzothiazole (**I**) in the (*1*) ground and (*2*) excited electronic states vs. concentration (*c*) of CCl_3COOH.

the spectrofluorimeter, which is unknown as a rule. Moreover, the fluorescence spectra of the pure components are invariant with respect to the lifetimes of these components. Therefore, although kinetic equations do appear in the mathematical model of the method, it is not required to know any kinetic parameters of the instrument or of the pure components for solving the problem of resolving the photostationary fluorescence spectra. The program implementation of the method was carried out; it was shown that the method is applicable to the determination of the composition of multicomponent systems, resolution of the spectra of pure components, and determination of the equilibrium and kinetic constants.

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REFERENCES

1. Aragoni, M.C., Arca, M., Crisponi, G., and Nurchi, V.M., *Anal. Chim. Acta*, 1995, vol. 316, no. 2, p. 195.
2. Suryani, S., Theraulaz, F., and Thomas, O., *Trends Anal. Chem.*, 1995, vol. 14, no. 9, p. 457.
3. Toptygin, D., Packard, B.Z., and Brand, L., *Chem. Phys. Lett.*, 1997, vol. 277, nos. 5–6, p. 430.
4. Liwo, A., Skurski, P., Oldziej, S., Lankiewicz, L., Malicka, J., and Wiczk, W., *Comput. Chem.*, 1997, vol. 21, no. 2, p. 89.
5. McIntyre, N.S., Pratt, A.R., Piao, H., Maybury, D., and Splinter, S.J., *Appl. Surf. Sci.*, 1999, vols. 144–145, no. 4, p. 156.
6. Neumann, B., *Dyes Pigments*, 2002, vol. 52, no. 1, p. 47.
7. Shcherbakova, E.S., Gol'dshtein, I.P., and Gur'yanova, E.N., *Usp. Khim.*, 1978, vol. 47, no. 12, p. 2134.
8. Bershtein, I.Ya. and Kaminskii, Yu.L., *Spektrofotometricheskii analiz v organicheskoi khimii* (Spectrophotometric Analysis in Organic Chemistry), Leningrad: Khimiya, 1975.
9. Bezdenezhnykh, A.A., *Inzhenernye metody sostavleniya uravnenii skorostei reaktsii i rascheta kineticheskikh konstant* (Engineering Methods for Constructing Equations of Reaction Rates and for Calculating Kinetic Constants), Leningrad: Khimiya, 1973.
10. Wallace, R.M. and Katz, S.M., *J. Phys. Chem.*, 1964, vol. 68, no. 12, p. 3890.
11. Turchak, L.I. and Plotnikov, P.V., *Osnovy chislennykh metodov* (Principles of Numerical Methods), Moscow: Izd. Fiz.-Mat. Lit., 2002.
12. Evseev, A.M. and Nikolaeva, L.S., *Matematicheskoe modelirovanie khimicheskikh ravnovesii* (Mathematical Simulation of Chemical Equilibria), Moscow: Mosk. Gos. Univ., 1988.
13. Gill, Ph.E., Murray, W., and Write, M.H., *Practical Optimization*, London: Academic, 1981.
14. Abramov, L.M. and Kapustin, V.F., *Matematicheskoe programmirovanie* (Mathematical Programming), Leningrad: Leningr. Gos. Univ., 1981.
15. Levshin, L.V. and Saletskii, A.M., *Opticheskie metody issledovaniya molekulyarnykh sistem* (Optical Methods of Studying Molecular Systems), Moscow: Mosk. Gos. Univ., 1994, part 1.
16. Volchkov, V.V., Gromov, S.P., Uzhinov, B.M., and Alfimov, M.V., *Izv. Ross. Akad. Nauk, Ser. Khim.*, 2001, no. 7, p. 1130.