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E. A. Bozhevolnov, S. U. Kreingold, R. P. Lastovskii,

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Abstract

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**E. A. Bozhevolnov, S. U. Kreingold, R. P. Lastovskii,
V. V. Sidorenko**

On the Use of Luminescent Reagents in the Kinetic Method of Analysis

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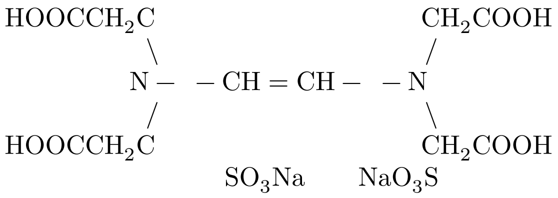
The most sensitive luminescent reactions for the determination of cations are those accompanied by the elimination of internal radiationless transitions in the reagent molecule as a result of the formation of an intracomplex compound with the cations [1,2]. However, these reactions cannot be used in analytical chemistry practice for the determination of cations with unfilled electron shells, since the latter are quenchers of fluorescence [3]. It is known that cations with unfilled electron shells often prove to be catalysts of homogeneous reactions between inorganic substances [4], or between inorganic and organic substances [4,5]. When these catalytic reactions are used in analysis, in both cases their progress is judged by the absorption or scattering of light. Kinetic reactions involving fluorescent substances have not yet been described.

The use of fluorescent reagents in kinetic methods of analysis has the advantage over colored reagents that it makes it possible to reduce the amount of reagent down to $1 \cdot 10^{-6}$–$1 \cdot 10^{-8}$ M. This increases the sensitivity of the reactions, shortens the time required for analysis, and in a number of cases makes it possible to dispense with thermostating, since there is no need to carry out the reaction at elevated temperature. In addition, recording the effect of a kinetic reaction by the change in the luminescence of its products expands the range of objects in the search for new reactions, since many transformations of organic substances are not accompanied by a change in color, but are accompanied by a change in luminescence.

We have for the first time found new kinetic reactions with a luminescent end point for the determination of iron and copper.

The reagent for the determination of iron ions (Fe^{3+}) is 4,4′-diamino-(N,N,N′,N′-tetracarboxymethyl)-stilbene-2,2′-disulfonic acid:



$$\begin{array}{ccc} \mathrm{HOOCCH_2C} & & \mathrm{CH_2COOH} \\ \backslash & & / \\ \mathrm{N}- & -\mathrm{CH}=\mathrm{CH}- & -\mathrm{N} \\ / & & \backslash \\ \mathrm{HOOCCH_2C} & & \mathrm{CH_2COOH} \\ & \mathrm{SO_3Na} \qquad \mathrm{NaO_3S} & \end{array}$$

Aqueous solutions of the reagent fluoresce blue in an acidic medium. The fluorescence spectrum is a structureless band in the wavelength range 400–480 mμ. In an acidic medium, in the presence of iron ions and hydrogen peroxide, the luminescence gradually weakens as a result of oxidation of the reagent, which proves to be a weak complexing agent in an acidic medium. This is evidenced by the values of the third and fourth ionization constants of the reagent, determined by us from potentiometric titration data and equal to: $pK_3 = 3.10$, $pK_4 = 4.94$.

Complex formation plays an essential role in the catalytic reaction; therefore, in the presence of complexon III, sodium fluoride, *o*-phenanthroline, and other complexing agents, the reaction does not proceed or proceeds very slowly.

In the absorption spectrum of the product of the catalytic transformation of the reagent there is no band corresponding to electronic transitions along the conjugation chain of the molecule, whereas in the absorption spectra of 4,4′-diaminostilbene-2,2′-disulfonic acid and of the reagent this band is present.

Since the reagent is a weak complexing agent, it is very probable that the iron ion in the complex has free coordination sites and, owing to this, a complex is formed–reagent (Fe^{3+}—H_2O_2). This complex is thermodynamically unstable; oxidation of the reagent occurs at the site of the double bond, and the fluorescence of the solution disappears. In the presence of hydrogen peroxide the reaction proceeds according to a first-order equation with respect to the reagent

$$-\frac{dI}{d\tau} = \chi[\mathrm{Fe}]\,I, \tag{1}$$

where I is the fluorescence intensity of the reagent solutions, proportional to its concentration up to $2 \cdot 10^{-5}\ M$; τ is the reaction time (min); [Fe] is the catalyst concentration (M); χ is the catalytic coefficient (l/mol • min).

Table 1

Dependence of the reaction rate and fluorescence intensity on the pH of the solution

Hydrochloric acid	Hydrochloric acid	Hydrochloric acid	Acetate buffer solutions	Acetate buffer solutions	Acetate buffer solutions
pH	I_0	K	pH	I_0	K
1.0	36	0.006	2.5	80	0.029
1.5	52	0.010	3.5	92	0.025
2.0	60	0.013	4.1	100	0.019
2.5	72	0.020	5.5	166	0.007
3.0	82	0.020	6.0	180	0.000

To clarify the role of complex formation in the catalytic process, we studied the dependence of the reaction rate on the pH of the solutions. Table 1 presents the values of K, calculated from the formula $K = \Delta \lg \frac{I_0}{I} \cdot \Delta\tau^{-1}$, with each value of K corresponding to a straight line in the coordinates $\lg \frac{I_0}{I} - \tau$, in accordance with equation (1). The slowing of the reaction on going from pH 2.5 to pH 6.0 can be explained by the fact that the complex of the iron ion with the reagent becomes coordinatively saturated as a result of hydrolysis and therefore loses its catalytic properties. At pH 1–2 the stability of the complex of iron with the reagent is so low that the reaction rate decreases.

A study of the effect of hydrogen peroxide on the reaction rate showed that the latter is proportional to the concentration of hydrogen peroxide up to $8 \cdot 10^{-5}$ M; with an excess of peroxide relative to the reagent, the reaction rate increases, but only slightly. It was shown that, in the ionic-strength range of the solutions 0.035–0.135, its change does not affect the reaction rate. This fact indicates that the process determining the reaction rate is a process involving uncharged molecules.

Figure 1 presents data on the effect of temperature on the rate of the catalytic reaction, which made it possible to calculate the reaction-rate constants at temperatures of 308 and 322°: $K_{308} = 0.009 \pm 0.001$; $K_{322} = 0.021 \pm 0.002$. Consequently, $E_{\text{act}} = 11.9$ kcal/mol. The value obtained for the activation energy of the reaction is considerably lower than the energy required for the rupture of any bond between atoms in the reagent. At the same time it is known that in some cases complex formation lowers the activation energy of a process leading to the rupture of bonds between atoms ([6]).

The optimal conditions for carrying out the reaction are as follows: the reaction is carried out in a 0.03–0.17 N solution of acetic acid; the concentration of hydrogen peroxide is 0.006%, and that of the reagent $2 \cdot 10^{-5}$ M. Using twice-distilled water and "chemically pure" glacial acetic acid, one can determine 0.0005 μg of iron in 1 ml of solution. Under the experimental conditions, up to 100 μg of the following ions do not exhibit catalytic activity: Na^+, Li^+, K^+, Rb^+, Cs^+, Ag^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Be^{2+}, Al^{3+}, Sc^{3+}, Ga^{3+}, In^{3+}, La^{3+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Cr^{3+}, Sb^{5+}, Mn^{2+}, Ni^{2+}, Re^{7+}, Ru^{6+}. They do not interfere with the determination

Fig. 1. Dependence of lg $\frac{I_0}{I}$ on reaction time at different temperatures. Reagent concentration $2 \cdot 10^{-5}$ M, hydrogen peroxide $1 \cdot 10^{-4}$ M, iron $2 \cdot 10^{-7}$ M.

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Fig. 2. Development of luminescence of a $2 \cdot 10^{-5}$ M reagent solution in the presence of copper in 10 ml of 0.002 M $NaHCO_3$ solution: 1—0.02 μg, 2—0.05 μg, 3—0.10 μg, 4—0.20 μg.

Figure 2: Fig. 2. Development of luminescence of a $2 \cdot 10^{-5}$ M reagent solution in the presence of copper in 10 ml of 0.002 M $NaHCO_3$ solution: 1—0.02 μg, 2—0.05 μg, 3—0.10 μg, 4—0.20 μg.

iron down to 10 μg, Sn^{4+}, Th^{4+}, Te^{6+}, Cu^{2+}, Pt^{6+}, Co^{2+}, Mo^{6+}, Zr^{4+}. Thus, the reaction is distinguished by high sensitivity and specificity and may find broad application in analysis. In its use, one may employ either the tangent method or the fixed-time method (7). The duration of the determination of iron in solution is 15-20 minutes.

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For the determination of copper we used *n*-dimethylaminobenzylidenebenzoylaminoacetic acid.

Its aqueous solutions are colorless and do not fluoresce. In the presence of traces of copper, weakly alkaline solutions of the reagent begin to fluoresce green; moreover, with time the luminescence increases and reaches a constant intensity.

The results of studying the kinetics of the development of fluorescence are shown in Fig. 2. Since the fluorescence intensity of the solutions I_ϕ is proportional to the concentration of the luminescent reaction products C_ϕ up to $4 \cdot 10^{-5}$ M, in Fig. 2, in addition to the ordinate I_ϕ, the ordinate C_ϕ is given. Processing of the data presented in Fig. 2 showed that the luminescence develops according

Structural formula of *n*-dimethylaminobenzylidenebenzoylaminoacetic acid.

Figure 3: Structural formula of *n*-dimethylaminobenzylidenebenzoylaminoacetic acid.

to a second-order equation with respect to the reagent:

$$\frac{C_\phi}{C_\phi^0(C_\phi^0 - C_\phi)} = \chi' [\mathrm{Cu}]\, \tau, \tag{2}$$

where C_ϕ^0 is the concentration of the reagent (M); χ is the catalytic coefficient, $[\mathrm{Cu}]$ is the concentration of copper (M).

The development of luminescence over time at different pH values of the solution showed that, in weakly acidic and acidic solutions, the luminescence of the reagent in the presence of copper develops much more slowly than in an alkaline medium. The brightnesses of the solutions at pH values of 7.7–8.0 are maximal; moreover, the pH interval in which complete quenching occurs corresponds to the acid ionization constant $pK = 4.75$. At optimal pH values the luminescence of the solutions develops according to a second-order equation; at pH 8.6–9.5 the reaction-rate constant decreases, which is apparently connected with hydrolysis of copper as the reaction proceeds. The reaction-rate constants in the pH range from 7.0 to 8.35 remain practically constant, which indicates the absence, in the formal kinetic equation of the reaction, of the concentration of hydrogen ions. With increasing ionic strength of the solutions, the rate of formation of the luminescent form of the reagent decreases.

Taking all this into account, as well as the data of the IR absorption spectra, which showed the presence of hydrogen bonds in the reaction product differing from the hydrogen bonds in the reagent, it may be assumed that formation of the luminescent form of the reagent proceeds through a stage of interaction of a divalent ion with a singly charged anion of the reagent:

$$\mathrm{Cu}^{2+} + \mathrm{R}^- = \mathrm{CuR}^+. \tag{3}$$

The intermediate product CuR^+ reacts with the anion R^-

$$\mathrm{CuR}^+ + \mathrm{R}^- = \mathrm{CuR}_2. \tag{4}$$

In this connection, the formal reaction equation is of second order with respect to the reagent. The product CuR_2 may decompose, regenerating copper and the fluorescent dimer:

$$\mathrm{CuR}_2 = \mathrm{Cu}^{2+} + \mathrm{R}_2^{2-}. \tag{5}$$

From the data presented in Fig. 2, the values of the rate constant of the noncatalytic reaction were calculated: $K \approx 1 \cdot 10^2$ l/mole • min, and the catalytic coefficient $\chi = 1.3 \cdot 10^{13}$ $\mathrm{l}^2/\mathrm{mole}^2 \cdot \mathrm{min}$. Since the factor determining the sensitivity of the reaction is the rate of the noncatalytic reaction (^{9}), then, at a maximum determination error of 30%, $[\mathrm{Cu}]_{\min}\chi = 0.3K$, or $[\mathrm{Cu}]_{\min} = \dfrac{0.3K}{\chi}$, and the minimum determinable concentration of copper will be $2.4 \cdot 10^{-9}$ M, or 0.0007 μg in 5 ml of solution. Under actual analytical conditions, the sensitivity of the reaction is limited by contamination of the solutions and reagents with copper, and we have achieved 0.005 μg in 5 ml.

On the basis of the study of the reaction, the optimal conditions for carrying it out were found: reagent concentration $2 \cdot 10^{-5}$ M, concentration of acetate-ammonia buffer 0.02 M, pH 10.0; for the development of luminescence, the solutions are heated on a boiling water bath for 10 minutes and cooled. The solutions are fluorimetrically measured at wavelength $\lambda = 520$ mμ, with excitation by the light of a PRK-2 or PRK-4 lamp with a UFS-3 light filter. The determination of copper is not interfered with by 10 μg of ions Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Al^{3+}, Zn^{2+}, Cd^{2+}, Ag^{+}, La^{3+}, Sn^{2+}, Pb^{2+}, Hg^{2+}, or by larger amounts of alkali metals.

The examples given of kinetic reactions with luminescent reagents open up new ways of finding sensitive luminescent reactions for cations with unfilled electron shells.

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CITED LITERATURE

1. E. A. Bozhevol' nov, *Izv. AN SSSR, ser. fiz.*, **24**, 762 (1960).

2. E. A. Bozhevol' nov, G. V. Serebryakova et al., *Acta chim. Hung.*, **32**, 199 (1962).

3. Z. Holzbbecher, *Chem. listy*, **52**, 249 (1962).

4. K. B. Yatsimirskii, *Chem. listy*, **54**, 795 (1960).

5. V. I. Kuznetsov, *Tr. Komissii po analiticheskoi khimii*, **8**(11), 52 (1958).

6. L. S. Kichenko, L. A. Nikolaev, in: *Kinetics and Catalysis*, Publishing House of the Academy of Sciences of the USSR, p. 239, 1962.

7. K. B. Yatsimirskii, *Tr. Komissii po analiticheskoi khimii*, **8**(11), 82 (1958).

8. G. M. Panchenkov, V. P. Lebedev, *Chemical Kinetics and Catalysis*, Moscow, 1961.

9. K. B. Yatsimirskii, *ZhAKh*, **10**, 339 (1955).

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