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SPECTRAL LUMINESCENCE PROPERTIES OF Eu(III) COMPLEXES WITH TETRACYCLINE ANTIBIOTICS AND HYDROGEN PEROXIDE

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We have studied the spectral luminescence properties of mixed-ligand complexes of Eu(III) ions with tetracyclines and hydrogen peroxide. We have established that incorporation of hydrogen peroxide into the inner sphere of these complexes leads to a significant increase (by a factor of 10–25) in the intensity of luminescence of the Eu(III) ions, redistribution of the intensities of the $^5D_0 \rightarrow {}^7F_j$ *transitions, in particular to an anomalous increase in the intensity of the band for the forbidden transition* $^5D_0 \rightarrow {}^7F_0$.

Key words: *sensitized luminescence, europium, complex, tetracycline, hydrogen peroxide.*

Introduction. We know [1] that antibiotics in the tetracycline series form complex compounds with Eu(III) ions in which, as a result of intramolecular transfer of the excitation energy from the organic ligand molecules to the Eu(III) ion, we observe intense luminescence of the europium ion.

Sensitized luminescence of Eu(III) in these compounds has been suggested [2–5] for determination of tetracycline, doxycycline, chlortetracycline, and oxytetracycline.

At the same time, only scattered information is available on the spectroscopic properties of these complexes. Accordingly, we have studied the spectral luminescence properties of complexes of Eu(III) ions with tetracycline and its derivatives, and also mixed-ligand complexes with hydrogen peroxide.

The Experiment. Analytical grade reagents were used. Standard solutions o? europium and neodymium chlorides (0.1 mol/L) were prepared from the corresponding high-purity oxides. Solutions of tetracycline and its derivatives ($1 \cdot 10^{-2}$ mol/L) were prepared by dissolving exactly weighed amounts of the drugs in 75% methanol. A standard solution of hydrogen peroxide ($1 \cdot 10^{-2}$ mol/L) was prepared from a 3% aqueous solution, the exact concentration of which was determined titrimetrically. The pH of the solutions were established using a 40% aqueous solution of urotropin.

The luminescence spectra of the europium(III) ions were recorded in the 560–650 nm region, $\lambda_{\text{det}} = 612$, 590, and 580 nm (the transitions $^5D_0 \rightarrow {}^7F_2$, $^5D_0 \rightarrow {}^7F_1$ and $^5D_0 \rightarrow {}^7F_0$ respectively) using an ISP-51 spectrometer (luminescence was excited by light from an SVD-120A mercury quartz lamp with UFS-2 light filter isolating radiation with $\lambda_{\max} = 365$ nm). The intensity of luminescence (I_L) was calculated from the peak height. The luminescence decay curves and the integrated intensities of luminescence were recorded on an Aminco Bowman Series 2 spectrofluorimeter (SLM-Aminco, Rochester NY) with a 150 W xenon lamp. The luminescence excitation spectra were measured on an SM2203 spectrofluorimeter (SOLAR, Belarus) with a 150 W xenon lamp.

Results and Discussion. Tetracycline and its derivatives in aqueous alcoholic solutions in the UV region of the spectrum have four (demeclocycline), three (tetracycline, methacycline, chlortetracycline, meclocycline), or two (doxycycline, oxytetracyline, rolitetracycline) absorption bands with high molar absorption coefficients (Table 1), which makes possible efficient absorption of light energy. The energy of the triplet levels of the ligands (which we calculated from the fluorescence spectra of their complexes with gadolinium at 77 K) is higher than the energy of the level for the first excited state of the Eu(III) ion (17,300 cm^{-1}), which is why there is efficient transfer of excitation energy from the organic portion of the molecule in the complex to the lanthanide ion.

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TABLE 1 Some Optical Characteristics of Tetracyclines and Their Complexes with Europium(III) Ions*

Substance	$E_{\max}$, cm^{-1}	λ_{abs}, nm	$\varepsilon \cdot 10^4$	I_L, rel. units	Q
Tetracycline hydrochloride (TC)	19,270	216.1 275.3 357.2	1.45 1.62 1.58	64	0.00260
Doxycycline hydrochloride (DC)	18,830	274.6 345.9	1.43 1.22	141	0.00340
Oxytetracycline (OxTC)	19,160	275.8 353.5	1.34 1.24	106	0.00310
Methacycline (MtC)	18,800	241.4 281.1 344.4	2.22 1.59 1.55	54	0.00074
Chlortetracycline (ChTC)	19,080	229.6 274.8 367.6	2.00 1.62 1.11	19	0.00049
Rolitetracycline (RTC)	19,420	275.6 360.4	1.73 1.62	32	0.00054
Demeclocycline (DmC)	19,010	227.0 248.5 274.6 366.7	2.10 1.65 1.80 1.61	62	0.00087
Meclocycline sulfosalicylate (McC)	19,080	237.8 285.0 344.1	2.77 1.54 1.25	48	0.00068

*$C_{Eu} = C_T = 1 \cdot 10^{-4}$ mol/L, pH 7.0–7.2.

The Eu-doxycycline complex has the highest luminescence intensity I_L; this complex has the smallest difference between the energies of the triplet level of the ligand and the resonance level of the Eu(III) ion. Complexes with chlortetracycline and rolitetracycline, for which this difference is the greatest, have the lowest intensity.

We should note the rather low luminescence quantum yield for the studied complexes, which suggests considerable nonradiative deactivation of the excitation energy in these complex compounds.

Complex compounds of Eu(III) with tetracycline and its derivatives are coordination-unsaturated: the Ln:ligand ratio in them is 1:1, so they can add various electronegative ligands, in particular hydrogen peroxide. Introducing hydrogen peroxide into the Eu-tetracycline system leads to a significant increase (by a factor of ≈15) in I_L for the europium ions in the complex.

This phenomenon was first observed by the authors of [6], who suggested that such enhancement of luminescence is connected with oxidation of tetracycline by hydrogen peroxide in solution, followed by interaction of the oxidation product with the Eu(III) ions. From our standpoint, the increase in I_L for the europium ions in the complexes with tetracyclines in the presence of hydrogen peroxide is connected with the formation of mixed-ligand complexes. In order to confirm this hypothesis, using complexes with methacycline and oxytetracyline as an example, we have studied some optical characteristics of binary (Eu–tetracycline) and ternary (Eu–tetracycline–H_2O_2) complexes.

We have observed that when complexes are formed with europium ions, we see a bathochromic shift by 34.0 nm and 20.5 nm respectively in the absorption spectra of methacycline and oxytetracyline relative to the absorption spectra of the ligands themselves. In the presence of hydrogen peroxide, the optical density of solutions of the complexes increases. The absorption spectra of the antibiotics remain unchanged in the presence of hydrogen peroxide, which indicates there is no oxidation of the reagent by the hydrogen peroxide.

A comparative estimate of I_L for Eu(III) ions in complexes with different tetracyclines in the presence of hydrogen peroxide (Fig. 1) shows that the greatest increase in I_L is observed for oxytetracycline and chlortetracycline.

When mixed-ligand complexes are formed, the luminescence excitation spectra and the luminescence spectra of the Eu(III) ions change. As an example, Fig. 2 shows the luminescence excitation spectra for the Eu–MtC–H_2O_2

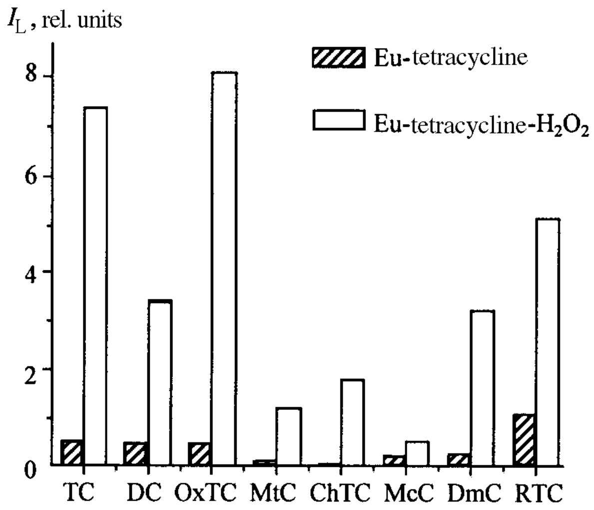

Fig. 1 Effect of hydrogen peroxide on the intensity of luminescence for Eu(III) complexes with tetracyclines (C_{Eu} = 1·10^{-3} mol/L, C_T = 1·10^{-3} mol/L, $C_{H_2O_2}$ = 5·10^{-4} mol/L).

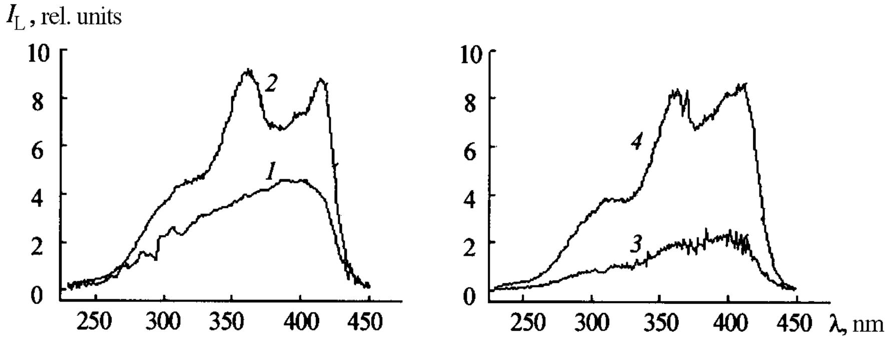

Fig. 2 Luminescence excitation spectra of the complexes (λ_{det} = 612 nm): Eu–MtC (1), Eu–MtC–H_2O_2 (2), Eu–OxTC (3), and Eu–OxTC–H_2O_2 (4) mol/L; C_{Eu} = 1·10^{-3} mol/L, C_{Mtc} = 1·10^{-4} mol/L, $C_{H_2O_2}$ = 5·10^{-4} mol/L, C_{Eu} = 1·10^{-3} mol/L and C_{OxTC} = $C_{H_2O_2}$ = 1·10^{-4} mol/L.

and Eu–OxTC–H_2O_2 complexes; Fig. 3 shows the luminescence spectra for the Eu–OxTC–H_2O_2 complex. As we see, the luminescence excitation spectra of mixed-ligand complexes have higher intensity and are split (in the 350–420 nm region) into two bands with maxima at 362–363 nm and 407–414 nm.

In the luminescence spectra of the mixed-ligand complexes Eu–MtC–H_2O_2 and Eu–OxTC–H_2O_2 (Fig. 3), we observe splitting of the bands for the transitions $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_1$ (612 nm and 590 nm respectively), an increase in the intensities of the bands for the $^5D_0 \rightarrow {}^7F_j$ transitions (612 nm, 590 nm, and 570 nm respectively), which also is indirect confirmation for incorporation of H_2O_2 into the inner sphere of the Eu–tetracycline complexes and coordination to the central ion. We should note the anomalous increase in the intensity of the band for the forbidden transition $^5D_0 \rightarrow {}^7F_0$. This suggests significant distortion of the Eu(III) coordination polyhedron, with lowering of the symmetry of the luminescence centers when H_2O_2 molecules are incorporated into the inner sphere of the complex [7, 8].

From the luminescence decay curves (Fig. 4) for the Eu–OxTC and Eu–OxTC–H_2O_2 complexes, we calculated lifetimes τ of 60 μsec and 87 μsec respectively. This also suggests a decrease in nonradiative deactivation of the excitation energy in the mixed-ligand complex, and leads to an increase in sensitized luminescence of the Eu(III) ions.

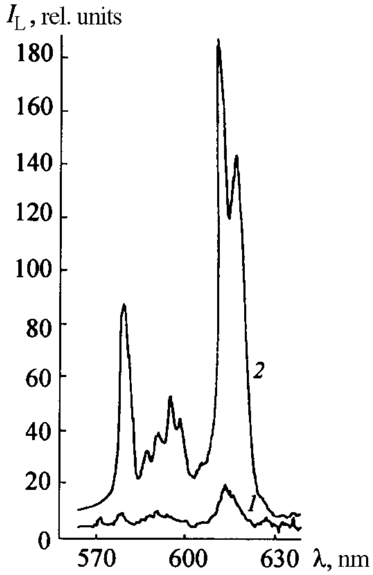

Fig. 3 Luminescence spectra of the complexes Eu–OxTC (1) and Eu–OxTC–H_2O_2 (2); C_{Eu} = $1{\cdot}10^{-3}$ mol/L, C_{OxTC} = $C_{H_2O_2}$ = $1{\cdot}10^{-4}$ mol/L.

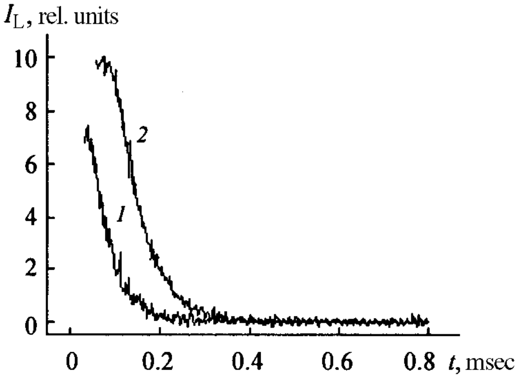

Fig. 4 Sensitized luminescence decay curves of the europium ions in the complexes Eu–OxTC (1) and Eu–OxTC–H_2O_2 (2); C_{Eu} = $1{\cdot}10^{-4}$ mol/L, C_{OxTC} = $1{\cdot}10^{-4}$ mol/L, $C_{H_2O_2}$ = $1{\cdot}10^{-4}$ mol/L, pH 6.5; τ_1 = 60 μsec, τ_2 = 87 μsec.

The ratios of the components in the complexes Eu:MtC:H_2O_2 and Eu:OxTC:H_2O_2, established under optimal conditions by the limiting-logarithm method, is 1:1:2. Hydrogen peroxide probably is incorporated into the inner sphere of the complex as the HOO^- anion and added directly to the central metal atom. In order to confirm this hypothesis, we studied the Stark components of the transitions $^5D_0 \to {}^7F_j$ (j = 0, 1, 2) of the luminescence spectra of the europium(III) ions in binary complexes with tetracyclines and ternary complexes with hydrogen peroxide in an excess of counterions Cl^-, NO_2^-, ClO_4^-, SCN^-. The characteristics of the anions [9, 10] are shown in Table 2. We established a relationship between the Pearson hardness of the studied anions and the corresponding luminescence characteristics of the complexes.

As already noted, in formation of mixed-ligand complexes, the $^5D_0 \to {}^7F_2$ band is split into two components, the $^5D_0 \to {}^7F_1$ band is split into four components, and also the intensity of the forbidden $^5D_0 \to {}^7F_0$ transition increases significantly.

TABLE 2 Intensity of Luminescence for the Eu–OxTC Complex in the Presence of Different Anions and Their Electronic Characteristics*

Anion	η_{Ai}	X_M^E	I_L, rel. units (612 nm)
Cl^-	2.55	2.55	97
NO_3^-	2.85	3.33	80
ClO_4^-	3.25	3.64	70
SCN^-	2.05	2.18	150

*η_{Ai} is the Pearson hardness of the anion, X_M^E is the electronegativity of the anion.

TABLE 3 Ratios of I_L for Europium(III) Bands at 612 nm and 580 nm $I_L(^5D_0 \rightarrow {}^7F_2)/I_L(^5D_0 \rightarrow {}^7F_0)$ in the Spectra of Binary Eu–Tetracycline and Ternary Eu–Tetracycline–H_2O_2 Complexes in the Presence of Different Anions

Anions (0.1 mol/L)	Binary complexes					Ternary complexes				
	OxTC	MtC	DC	TC	ChTC	OxTC	MtC	DC	TC	ChTC
Cl^-	3.13	3.0	4.05	1.82	3.23	1.83	1.82	1.78	1.86	1.85
NO_3^-	3.33	3.08	4.82	2.93	3.69	1.79	1.83	1.86	1.88	1.78
ClO_4^-	3.33	2.06	4.36	2.54	3.80	1.95	1.71	1.79	1.86	1.90
SCN^-	4.68	3.50	5.05	4.28	5.00	1.76	1.88	1.89	1.90	1.79

TABLE 4 Absorption Maxima and Oscillator Strengths for Neodymium Ions and Neodymium Complexes with Methacycline and Hydrogen Peroxide*

Substance	λ_{max}, nm		$\Delta\lambda$, nm		$P \cdot 10^6$
$NdCl_3$	575.2	–	–	–	9.80
Nd–MtC	575.2	582.5	–	7.3	18.89
Nd–MtC–H_2O	575.2	585.6	–	10.4	29.47

*$C_{H_2O_2} = 5 \cdot 10^{-3}$ mol/L.

The nature of the luminescence spectra for the mixed-ligand complexes is preserved in an excess of counterions Cl^-, NO_3^-, ClO_4^-, SCN^-. The difference in the ratio of the intensities of the $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_0$ bands (Table 3), which was observed in the case of binary complexes in the presence of counterions and depended on the Pearson hardness of these anions (Table 3), is evened out in formation of mixed-ligand complexes, suggesting displacement of other inorganic anions from the inner sphere of the complex by the HOO^- anion.

Formation of mixed-ligand complexes was also confirmed using Nd(III) ions. In formation of the binary complex Nd–MtC, a second maximum with $\lambda = 582.5$ nm appears for the absorption band for the ultrasensitive transition of neodymium $^4I_{9/2} \rightarrow {}^{2,4}G_{7/2,5/2}$ (575.2 nm) (Table 4). When hydrogen peroxide is introduced into the system, we observe an increase in the intensity and also a shift by 3.1 nm for the second maximum of the absorption band toward longer wavelengths but no change in the position of this maximum when the hydrogen peroxide concentration increases. This all can be considered indirect confirmation for the formation of mixed-ligand complexes in the system Nd–MtC–H_2O_2. Table 4 also shows the oscillator strengths (P) for $NdCl_3$ and the complexes Nd–MtC, Nd–MtC–H_2O_2.

As we see, in formation of the binary complex, the oscillator strength increases by a factor of 1.9; in the mixed-ligand complex, the oscillator strength increases by a factor of 1.56 compared with the binary complex.

Conclusion. Our studies of the spectral luminescence properties of complexes of Eu(III) ions with tetracyclines and hydrogen peroxide have allowed us to conclude that the hydrogen peroxide molecules are incorporated into the inner sphere of the complex in the form of singly charged anions, which leads to a decrease in nonradiative losses of excitation energy and accordingly to an increase in the luminescence intensity for the Eu(III) ion.

The luminescent properties of the mixed-ligand complexes Eu–OxTC–H_2O_2 and Eu–MtC–H_2O_2 have been used to develop a procedure for determining hydrogen peroxide in wash water in pharmaceutical plants, for determining oxytetracycline in cow's milk and human blood plasma, and also for indirectly determining glucose in human blood plasma and wine.

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