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Short communication

Use of sensitized luminescence of lanthanides in analysis of drugs[1]

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Abstract

The sensitization of the Tb(III) and Eu(III) ions luminescence by drugs, the pyrocatechol, naphthyridine and heterocyclic aromatic acid derivatives, were studied. It was shown that a result of intramolecular energy transfer from the ligand molecule to the lanthanide ion increases the luminescence intensity of the latter by 10^8–10^{10} times. The luminescence properties of the complexes in solutions were investigated. The highly sensitive methods for luminescence determination of dopegyt, levodopum, dophaminum, nevigramon, furosemidum and cinchophenum were developed. The detection limits of the drugs are 0.0005, 0.02, 0.5, 0.01, 0.05 and 0.1, respectively.

Keywords: Drugs; Luminescence determination; Terbium and europium ions

1. Introduction

The lanthanide ions in solutions of simple salts and complex compounds possess the luminescence properties due to, mainly, the transitions within 4f-shell [1–3]. The excitation of lanthanide ion in complex solutions occurs at the expense of the intramolecular energy transfer from excited organic molecule to lanthanide ion [4,5]. The sensitization of luminescence of lanthanide ions in complexes with organic ligands, including the bioactive substances, allows their application as the luminescence probes to establish the structure and properties of biological objects—phospholipids, nucleotides, proteins, ionophores, biological membranes, vesicules [6,7]. The determination of theophylline in the presence of caffeine [8] and ciprofloxacin [9] by sensitized luminescence of europium(III) and terbium(III) ions was also described.

We have studied the possibility of application of luminescence sensitization of lanthanide ions in complexes with organic ligands relating to the

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drugs with the purpose of determination the latter. The possibility of determination some of pyrocatechol derivatives used in medical practice as the hypotensive means and some acids of aromatic and heterocyclic series with the antibacterial, antiinflammatory and diuretic action was investigated (Table 1). The Tb(III) and Eu(III) ions possessing the highest luminescence intensity (I_{lum}) in solution of complex compounds were chosen.

2. Experimental

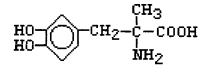
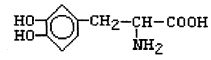
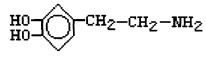
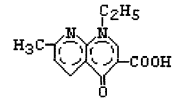
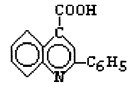
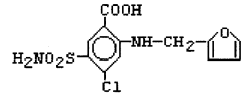
2.1. Apparatus

The luminescence measurements were obtained on an SDL-2 spectrophotometer (Leningrad Opto-mechanical Association, St. Petersburg, Russia). A xenon lamp was used as an excitation source. The Arcus 1230 fluorimeter (LKBWellac) was also used. The pH values of solutions were measured using an OP-211/1 laboratory digital pH-meter (Radelkis, Budapest, Hungary). Reagents. The terbium and europium chloride solutions of 0.1 mol l^{-1} and 1 mg ml^{-1} concentrations were prepared by dissolving their oxides (99.99%) in HCl followed by evaporation of HCl excess and dissolution with distillated water to required volume. Metal concentration were determined by complexometric titration with Arsenazo I as the indicator. The solutions of drugs were obtained by dissolving of accurately weighed preparation in water (*m*-DOPA, DOPA and dophaminum), ethanol (furosemidum) and upon alkalinizing with NaOH (or KOH) solution (nalidixic acid and cinchophenum). To prevent the oxidation of solutions of *m*-DOPA and DOPA the freshly prepared 1% aqueous solution of sodium borohydride was used.

2.2. Methods

The procedure for studying the complexation and performance the determination of drugs was

Table 1
Studied drugs and their triplet levels

№	Drugs	Formula	T_1, cm^{-1}
1	L,α-methyl-β-(3,4-dioxyphenyl)alanine (Dopegyt), (m-DOPA)		20500
2	β-(3,4-Dioxyphenyl)alanine (Levodopum), (DOPA)		21505
3	β-(3,4-Dioxyphenyl)ethylamine (Dophaminum)		20800
4	1-Ehyl-7-methyl-1,8-naphyridine-4-on-3-carboxylic (nalidixic) acid (Nevigramon)		21500
5	4-Chloro-N-(2-furylmethyl)-5-sulfamoylanthranilic acid (Furosemidum)		21600
6	2-Phenylquinoline-4-carboxylic acid (Cinchophenum)		17700

the following: the terbium (europium) chloride solution was added to the solution to be analyzed, the required medium was created by introduction of sodium borohydride or urothropine, solvent (to $V = 10$ ml) and the luminescence intensity of Tb(III) and Eu(III) was measured at $\lambda = 546$ and 612 nm, respectively. The drug content was determined either from calibration plot or by the method of additives. In the last instance the content of the preparations was calculated by the formula: $X = (C \times h_x)/(h_{x+g} - h_x)$, where h_x and h_{x+g} are the luminescence intensity of the assay and assay with additive (height of the pike, mm), and C is the content of additive, mg.

The triplet levels of the ligands were obtained from phosphorescence spectra of yttrium complexes at 77 K.

3. Results and discussion

Optical characteristics of the ligands and complexes. According to the existing ideas [4,5], effective excitation energy transfer from ligand to the lanthanide ion takes place when the triplet state energy of the ligand molecule is equal or higher than the energy of resonance level of the metal ion. As it is seen (Table 1), triplet levels of the ligand studied (nos. 1–4, 6), except cinchophenum, are equal or higher than 20500 cm^{-1}, i.e. these ligands are effectively able to transfer the excitation energy to Tb(III) ion, its first excited level $^5D_4$ lies at 20500 cm^{-1}. Indeed, the compounds with the mentioned substances show the intensive luminescence of Tb(III) ion. The Eu(III) ion in complex of nalidixic acid demonstrates the intensive luminescence that is, evidently, caused by the excitation energy transfer from the ligand to the europium energy level $^5D_2$ (21500 cm^{-1}) and non-radiative deactivation to the first Eu(III) excited level $^5D_0$ from which the emitting luminescence takes place.

The triplet level of cinchophenum (Table 1, reagent No5) is fairly low and in this instance the excitation energy transfer occurs to Eu(III) ion (17300 cm^{-1}) only that shows the intensive luminescence in this compound, Tb(III) ion demonstrates no luminescence in complex with cinchophenum.

All the ligands studied possess the absorption bands with high extinction coefficients 21000–140000 in near ultraviolet spectral region rendering the possibility of effective light energy absorption and its transfer to the lanthanide ion. Owing to this the luminescence intensity of Eu(III) and Tb(III) ions in solution increases by 10^8–10^{10} times.

3.1. Conditions of the complexation

All the drugs considered form the complex compounds with the components ratio Me:L = 1:3 with Tb(III) and Eu(III) ions. In the case of *m*-DOPA, DOPA and dophaminum the complexation occurs through dioxyphenyl group (Table 1). For the rest of the ligands the coordination is realized via carboxylic group that is confirmed by an IR spectroscopy method.

The highest luminescence intensity for the solutions of Tb(III) complexes with pyrocatechol derivatives was observed in alkaline medium at pH 10.0 (DOPA), 10.5 (*m*-DOPA) and 9.0 (dophaminum). Since the Tb(III) complexes of *m*-DOPA and DOPA decompose rapidly in air to prevent their oxidation the sodium borohydride was used. For example, the dependence of I_{lum} of Tb(III) in complex with *m*-DOPA on sodium borohydride amount in solution is shown in Fig. 1. As can be seen (Fig. 1), the highest I_{lum} is achieved with 7–10 mg of $NaBH_4$ content in solution, upon that, pH is 9.8–10.0.

The heterocyclic acids and furosemidum form the complex compounds with lanthanide ions in neutral solutions. The maximum I_{lum} for Tb(III) complexes of nalidixic acid and furosemidum and Eu(III) ones of cinchophenum is observed at pH 6.3–7.0. At the values of $pH < 6$ the complete complex formation is not achieved, at $pH > 7$ the hydrolytic decomposition of the complex in solution is began and the luminescence intensity decreases. In the case of nalidixic acid, furosemidum and cinchophenum the necessary pH values were obtained through the addition of urothropine solution.

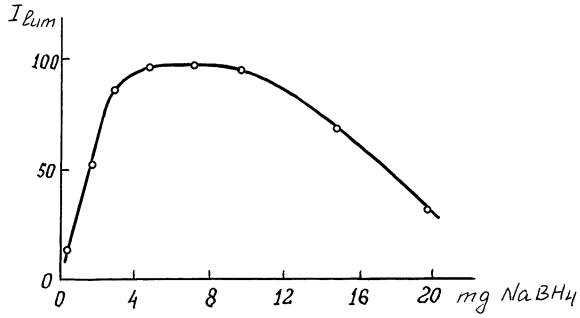


Fig. 1. Dependence of Tb(III) I_{lum} in complex with *m*-DOPA on the sodium borohydride amount. $C_{Tb} = 1 \times 10^{-5}$ mol l^{-1}.

3.2. Optimization of analytical signal

In certain cases the luminescence intensity of the lanthanide ion can be increased when the mix-ligand complexes are formed as well as upon the introduction of surfactants or replacing of the solvent. Thus, the luminescence intensity of Eu(III) ion in complex with cinchophenun is low and I_{lum} increases significantly (by 20 times) in the presence of 1,10-phenanthroline (Phen) (Fig. 2). Since the suspension of complex is forms as a result of reaction, then surfactant OS-20 was used for its stabilization. The OS-20 preparation is the polyethyleneglycole monoalkylated ethers based on the primary fat alcohols, C_nH_{2n+1}–O–

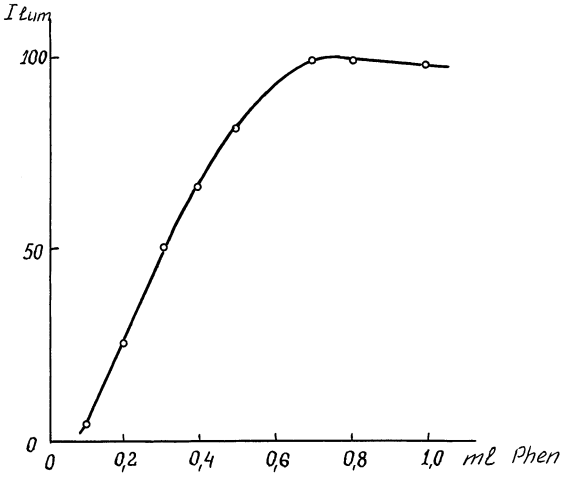


Fig. 2. Dependence of Eu(III) I_{lum} in complex with cinchophenum on amount of the 1,10-phenanthroline added $C_{Eu} = 200$ μg, $C_{Phen} = 1\%$.

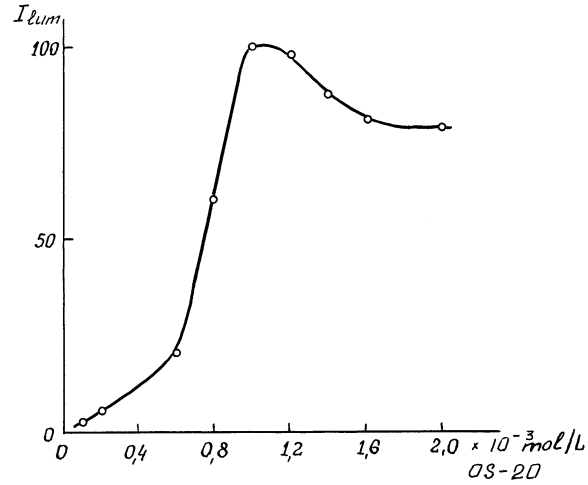


Fig. 3. Dependence of Eu(III) I_{lum} on the OS-20 amount in complex with cinchophenur and Phen. $C_{Eu} = 200$ μg.

$(C_2H_4O)_xH$, where $x = 20$. OS-20 contains a long hydrocarbon hydrophobic radical that reduces essentially the hydration of particles in solution that decrease significantly the nonradiative deactivation degree of the Eu(III) ion due to the vibrations of hydrogen atom of O–H bond in water molecules and lead, respectively, to the substantial increase of the luminescence intensity (Fig. 3).

Investigation of the dependence on the solvent nature (20% v.v.) has been demonstrated that I_{lum} of Tb(III) ion for the complexes with *m*-DOPA and DOPA is the highest in aqueous-iso-propanolic solutions, for complex with dophaminum in aqueous and with furosemidum in aqueous-ethanolic ones (Table 2).

For the Eu(III) cinchophenum complexes the influence of the solvents was not studied because the reaction was performed in micellar solutions. In complexes with nalidixic acid the introduction of organic solvents in solution decreases the luminescence intensity of Tb(III) ion. In each instance the influence of the solvent amount on luminescence intensity of Tb(III) ion was studied.

3.3. Analytical performance

The optimum conditions for performing the analysis and the detection limits of the drugs by

Table 2
Solvent influence on the I_{lum} of Tb(III) in complex solutions

Solvent	Tb(III) I_{lum} (%)			
	m-DOPA	DOPA	Dophaminum	Furosemidum
Water	0.7	—	100.0	8.0
Methanol	1.5	23.0	44.0	80.0
Ethanol	9.0	66.0	24.0	100.0
iso-Propanol	100.0	100.0	34.0	50.0
Acetone	—	0.6	3.0	2.0
Dimethylsulfoxide	2.3	12.3	30.0	65.0
Dimethylformamide	—	16.0	64.0	6.5

Table 3
Optimum conditions for the deter∼nination of some drugs by the sensitization of Tb(III) and Eu(III) ions

Drug	pH of solution	Solvent	Detection limit (μg ml^{-1})
Dopegyt	9.8	iso-Propanol, 90% v.v.	0.0005
Levodopum	10.5	iso-Propanol, 90% v.v.	0.02
Dophaminun1	9.0	Water	0.5
Nevigramon	6.5	Water	0.01
Furosemidum	6.5	Ethanol, 90% v.v.	0.05
Cinchophenum	7.0	Water	0.1

proposed method are given in Table 3. As can be seen (Table 3), the detection limits for proposed methods are a great lower than for the known means of their determination.

As an example, the method for determination of furosemidum in the drug 'furosemidum' is given below. The tablet of 'furosemidum' preparation is carefully pounded, dissolved in 100 ml of ethanol and filtered. To perform the determination by the additives method, 0.1 ml of solution to be analyzed is placed into the each of three test-tubes, 0.1 and 0.2 ml of the standard furosemidum solution is introduced into two of them. A total of 0.2 ml of terbium chloride solution, 0.3 ml of 40% urothropine solution is added in all test-tubes and diluted with ethanol up to 10 ml. Simultaneously the blank solution containing all components excepting furosemidum is prepared. The luminescence intensity of Tb(III) ion is measured at $\lambda = 546$ nm. The content of furosemidum (x, mg) in the assay is calculated by the above formula. The content of furosemidum in the tablet is equal to $1000x$, mg.

Table 4
Results of the determination of furosemidum in substance ($n = 10$)

Added (μg)	Found (μg)	S_r
0.5	0.48	0.014
1.0	1.04	0.013
3.0	2.94	0.021
6.0	5.92	0.019
10.0	10.10	0.03
20.0	19.85	0.025

Accuracy and precision of the method were checked in the laboratory conditions by the 'added-found' method and by the statistic treatment of data of the furosemidum determination in the substance (Table 4).

The statistic treatment of determination data was similarly performed for all the studied drugs. The relative standard deviations (S_r) do not exceed 0.03–0.04.

4. Conclusions

Highly sensitive methods for luminescence determination of dopegyt, levodopum, dophaminum, nalidixic acid, furosemidum, cinchophenum, based on the sensitization of Tb(III) and Eu(III) of luminescent ions in solutions, have been developed. The proposed methods posses more low detection limits as compared with the known ones and are simple in performing and sufficiently reliable.

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