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Co-luminescence of ions and molecules in nanoparticles of metal complexes

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Abstract. The features of co-luminescence (sensitized luminescence) of lanthanide ions and dye molecules inserted in nanoparticles of complexes formed by trivalent metals with β-diketones are discussed. The mechanisms of energy transfer in these nanoparticles that lead to sensitization of Ln^{3+} ion luminescence and dye molecule fluorescence are considered. It is shown that the process of nanoparticle formation and their morphology in aqueous solutions can be studied by analyzing the mechanism of their co-luminescence and co-fluorescence. Examples of chemical, biological and medicinal applications of nanoparticles containing lanthanide ions and dye molecules with enhanced luminescence brightness are given. The bibliography includes 131 references.

I. Introduction

The co-luminescence (co-fluorescence) effect means enhancement of luminescence (fluorescence) of energy acceptor in the presence of metal ions or complexes, organic molecules or conjugated polymers, *i.e.*, energy donors that form micro- or nanocrystals, nanoparticles or nanostructures.[1–8] Traditional luminescent labels represent dye molecules and lanthanide (Ln) complexes. The interest of scientists in Ln^{3+} complexes with organic compounds, which has persisted for several decades, is associated with the fact that the energy transfer from the organic part of the complex to the Ln^{3+} cation produces a system with high absorption coefficient (ε) of organic ligands, narrow luminescence spectra of Ln^{3+} ions, a large Stokes shift and a time of luminescence decay from several microseconds to several milliseconds.[9–18] The last-mentioned fact allows such systems to be used in the time-resolved luminescence analysis which provides additional gain in sensitivity, because by using the time gating, one can separate useful luminescence from Rayleigh and Raman light scattering signals and fluorescence of organic impurities.

Starting from the 1990s, the co-luminescence phenomenon has been used in chemistry, biology and medicine for lowering down the detection limits in the determination of small concentrations of ions[1–7] and ion-labelled antibodies.[9,10] To enhance the sensitivity of luminescence analysis, researchers tend to switch from luminescent labels based on individual molecules or lanthanide complexes to luminescent nanoparticles.[12–17] Particularly, a large number of studies on the synthesis of nanostructures and analysis of their properties deal with particles that consist of inorganic salts or organic complexes of lanthanides or other metals.[17–22]

Depending on the method of synthesis, nanoparticles of lanthanide diketonates measure from 10 to 140 nm.[19–23] Nanoparticles with the predominant size of 10 nm are formed in aqueous solutions[21] and have a molar absorption coefficient (molar absorptivity) of $\sim 10^7$ litre mol^{-1} cm^{-1}. Larger particles are synthesized from polymers and SiO_2 doped with lanthanide complexes or dye molecules.[8,19–24] Such particles exhibit highly bright luminescence in aqueous solutions and can be used to solve a wide circle of analytical problems.

A serious drawback of the luminescence technique based on lanthanide organic complexes is the poor colour selection of such labels. This is associated with the fact that

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among Ln^{3+} only Tb^{3+} and Eu^{3+} (to a lesser extent, also Sm^{3+} and Dy^{3+}) exhibit considerable luminescence efficiency when being surrounded by water molecules and organic ligands containing OH, NH and CH groups, because water molecules and ligands quench almost completely the luminescence of other ions.[25] Hence, this restricts the number of luminescent labels and simultaneous use of several labels with different emission spectra. The ways to attack this problem became clear after the discovery of co-fluorescence of dyes in aqueous solutions upon the self-assembling of nanoparticles of metal complexes.[26,27] It was observed that in aqueous solutions, mixed nanoparticles are formed comprising thousands of trivalent metal organic complexes with inserted molecules of cationic, anionic or neutral fluorescent dyes to which the excitation energy is transferred. A technique has been developed for synthesizing nanoparticles of conjugated polymers doped with other conjugated polymers or neutral dye molecules. For such nanoparticles, co-fluorescence of dopants was also observed and studied.[8]

Nanoparticles become more and more often both research objects and research tools. In particular, they are used for enhancing the sensitivity of various analytical techniques. More than a half of publications in this field are devoted to metal nanoparticles (Ag, Au, Pt, *etc.*),[28] a considerable part of these dealing with the effect of plasmon resonance on the intensity of luminescence of organic and inorganic substances. Polymer nanoparticles are considered in $\sim$15% of publications and $\sim$8% of publications deal with each of quantum dots and SiO_2 nanoparticles doped with dyes or lanthanide organic complexes.[28] A group of scientists from Taiwan, Germany and Italy have published a review[29] on biotechnological applications of quantum dots as the energy donors in the FRET (Förster Resonance Energy Transfer) method.†

Bearing in mind the large number of journal publications and monographs[30] devoted to properties and applications of plasmons[31] and quantum dots,[29] we have restricted our consideration to the studies in which the enhancement of luminescence of ions and dye molecules is achieved due to their sensitization in nanoparticles of organic complexes of metals or π-conjugated polymers.

The structure of this review is as follows. The Introduction is followed by brief history of the discovery of the co-luminescence phenomenon (Section II). Then we consider the studies carried out by the co-luminescence method into the effect of the ion nature and formation conditions on the region of existence and morphology of nanoparticles of metal complexes (Section III). The next section discusses co-luminescence of lanthanide complexes, deactivation of Ln^{3+} ions and energy transfer in nanoparticles. Section V presents data on co-fluorescence of dyes in nanoparticles formed by complexes of fluorescent and non-fluorescent metals and compares them with co-fluorescence data for nanoparticles of conjugated polymers.[32–34] Ultimately, the final Sections (VI, VII and VIII) contain the data on various applications of co-luminescence.

† In this method, to elucidate the structures of biological objects, the regularities of particularly the Förster mechanism of energy transfer are used; hence, the expansion of FRET as Fluorescence Resonance Energy Transfer, which is often encountered in biological publications, is incorrect.

II. Concise history of co-luminescence discovery

The enhancement of luminescence of $Sm(Eu)(TTA)_3$phen complexes (where TTA is 2-thenoyltrifluoroacetonate, phen is 1,10-phenanthroline) upon UV excitation in the presence of $Gd(Tb)(TTA)_3$phen complexes was first observed by Polyektov *et al.*[1] in microcrystalline suspensions of these complexes in aqueous ethanol. Later,[2] similar effects were observed for suspensions of tetraligand lanthanide complexes formed not only with TTA but also with dibenzoylmethane (DBM), benzoylacetone (BA) or acetylacetone (AA) and with bases [2,4,6-trimethylpyridine, 1,3-diphenylguanidine (DPG), *etc.*].

The luminescence intensity‡ of europium complexes remained unchanged in the presence of La, Dy and Yb complexes, was halved in the presence of Ce, Pr, Nd, Sm, Ho, Er and Tm complexes and enhanced 7- and 5-fold in the presence of Gd and Tb complexes, respectively. The luminescence intensity of samarium complexes changed in the same way in the presence of an excess of other lanthanide ions. The effect of enhancement is associated with the absorption of light quanta by gadolinium and terbium complexes followed by the transfer of excitation energy to europium or samarium complexes to enhance their luminescence.

Studies along this line were carried on by the group of Chinese scientists headed by professor Yang.[3,4] They used the same system of europium ions in the presence of an excess of TTA, phen and Tb, Gd, Y, La, Lu complexes. A new point in these studies was the use of a neutral surfactant (Triton X-100) for stabilization of nanoparticles formed in solution. It was noted that the luminescence intensity of europium is enhanced by two orders of magnitude in the presence of the 200-fold excess of terbium complexes. This enhancement was assumed[3,4] to be due to the intermolecular transfer of the triplet energy from ligands of terbium complexes to the ion of the neighbouring europium complex. When comparing the enhancement of luminescence of Eu^{3+} and Sm^{3+} in the $Ln(TTA)_3(TOPO)_2$ and $Ln(TTA)_3$phen systems (where Ln = Eu, Sm; TOPO is tri-n-octylphosphine oxide) in the presence of La^{3+}, Gd^{3+}, Lu^{3+} and Y^{3+}, it was observed[5–7] that the strongest enhancement was provided by those ions whose ion radii were closest to the radius of the sensitized ion. It was also shown that the energy is most probably transferred (migrates) from the triplet level of the gadolinium, terbium, lanthanum, *etc.* complexes to the triplet level of the europium (samarium) complex and further to the luminescent Eu^{3+} (Sm^{3+}) ion.

For this phenomenon, the term 'co-fluorescence effect' was proposed;[5] Yang *et al.*[4] used the term 'co-luminescence' for the same phenomenon. Both terms are actively used in the scientific literature.§

‡ In the studies by Poluektov and co-workers[1,2] the luminescence intensity of europium complex in the presence of a 100-fold excess of lanthanum complexes was taken as unity.

§ In our opinion, the term 'co-fluorescence' is improper when applied to this system, because in the case in question, the luminescence is enhanced for europium and samarium, and emission of these ions does not pertain to fluorescence in accordance with the terminology predominantly adopted in scientific literature. The term 'co-luminescence', which was proposed for this phenomenon by Yang,[4] seems more appropriate.

III. Regularities of formation of nanoparticles of organic metal complexes in aqueous solutions

1. Conditions of nanoparticle formation in solutions

Study of the co-luminescence of nanoparticles formed by lanthanide or other metal chelates in aqueous solutions with and without addition of other solvents makes it possible to elucidate the reasons and conditions for the appearance of nanoparticles and also their formation rate, the compatibility of different metal complexes or complexes and dye molecules in one particle, the particle stability, the effect of surfactants (stabilizers), *etc*. On the addition of organic β-diketones (in concentration of $\geqslant 10^{-6}$ mol litre^{-1}), a synergistic ligand ($\geqslant 3\times10^{-7}$ mol litre^{-1}) and metal salts ($\geqslant 10^{-7}$ mol litre^{-1}) to aqueous solutions, hydrophobic complexes are formed and assembled into nanoparticles, which are pushed out water.

In solution, β-diketones can exist in two forms, *i.e*., ketone and enol forms. The enol form interacts with metal ions. In alcohols, enol form predominates; in water at pH 7, ketone form predominates.

β-Diketones are poorly soluble in water and some of them do not dissolve. Hence, as a rule, β-diketones are preliminarily dissolved in other solvents (ethanol, dimethyl sulfoxide, acetone), and at the final stage, aqueous solutions with small admixtures of these solvents are studied. Insofar as in the studied range of concentrations, nanoparticles are preferentially formed in aqueous solutions, no wonder that nanoparticles of europium complexes were observed [24] to decrease in size as the alcohol concentration in aqueous solution increased. The disappearance of dye co-fluorescence in water – isopropanol solutions containing a Lu^{3+} (or Al^{3+}) + 4-phenylbenzoyltrifluoroacetone (PhBTA) + phen mixture, which we observed for the isopropanol concentration of >18 vol.%, pointed to the absence of nanoparticles of metal complexes at so high isopropanol concentrations. And, the other way round, the addition of a small amount of alcohol to water can stabilize the formed particles.

The mere fact that co-luminescence appears in mixed solutions of non-luminescent metal cations and β-diketones containing small admixtures of luminescent ions or dye molecules suggests that these solutions contain nanoparticles of complexes that contain luminous ions or molecules. This is why analysis of the intensity of co-luminescence (co-fluorescence) simultaneously serves as a method for detecting nanoparticles of metal complexes with β-diketones and also for monitoring their size and concentration in solution. Having determined the concentrations of ions and β-diketone in the aqueous solution at which the co-luminescence of ions (or co-fluorescence of dye molecules) is observed, one can find the solution component concentrations at which the nanoparticles are formed.[35, 36]

Table 1 provides data on the dependences of the intensity of Eu^{3+} co-luminescence and rhodamine 6G (R6G) co-fluorescence on the concentration at a constant DBM concentration. The Table shows the ratios of intensities of the Eu^{3+} co-luminescence to its own luminescence ($I^{Eu^{3+}}_{colum}/I^{Eu^{3+}}_{lum}$) as a function of the Gd^{3+} concentration and also the fluorescence of aluminium complexes ($I^{Al^{3+}}_{fl}$) and the dependences of rhodamine 6G co-fluorescence (I^{R6G}_{cofl}) on the Al^{3+} concentration in aqueous solutions containing the DBM + phen + 1 vol.% ethanol mixture.

Table 1. Concentration dependences of Eu^{3+} co-luminescence, rhodamine 6G co-fluorescence and the fluorescence intensity of the $Al(DBM)_3$.[35, 36]

$[Gd^{3+}]$ (see [a])	$I^{Eu^{3+}}_{colum}/I^{Eu^{3+}}_{lum}$	$[Al^{3+}]$ (see [a])	$I^{Al^{3+}}_{fl}$ (see [b])	I^{R6G}_{cofl} (see [b])
0.0	0.0	0.0	0.0	0.0
0.05	0.0	0.05	0.15	0.0
0.1	0.2	0.1	0.24	0.1
0.2	0.3	0.2	–	–
0.5	1.5	0.5	1.2	0.8
0.8	5.0	0.8	–	–
1.0	7.0	1.0	2.0	1.4
2.0	15.0	2.0	–	–
5.0	–	5.0	6.0	11.0
10.0	19.0	10.0	26.0	54.0
20.0	–	20.0	40.0	62.0

Note. Component concentrations /mol litre^{-1}: $[Eu^{3+}] = 2\times10^{-8}$, $[R6G] = 5\times10^{-8}$, $[DBM] = 3\times10^{-5}$, $[phen] = 10^{-5}$; ethanol, 1 vol.%; exciting light wavelength $\lambda_{exc} = 370$ nm, $T = 293$ K.
[a] Expressed in 10^{-6} mol litre^{-1}. [b] Expressed in rel.u.

In both solutions, co-luminescence of admixtures was observed for the Al^{3+} and Gd^{3+} concentrations in the range from 10^{-7} mol litre^{-1} to the maximum studied concentration (see Table 1). In his review, Buchachenko [37] justly noted that in the initial stage of nanoparticle formation, it is difficult to draw distinction between an associate of several molecules and a nanoparticle; hence, no wonder that the intensity of europium co-luminescence differed little from the intensity of its intrinsic luminescence in solutions with the concentration of added complexes of 10^{-7} mol litre^{-1}. The presence of co-fluorescence proves that complexes of both ions form nanoparticles in solutions containing DBM and phen for concentrations of complexes from 10^{-7} to $(1-3)\times10^{-5}$ mol litre^{-1}, while the more rapid increase in the intensity of dye co-fluorescence as compared with the fluorescence of metal complexes suggests that the size of nanoparticles increases under these conditions.

However, in the general case, the concentration ranges of ions and diketones for which considerable amounts of nanoparticles are present in aqueous solutions differ for different ions and diketones. Thus it was shown [27] that the intensities of Nile Blue (NB) co-fluorescence in nanoparticles of $Eu(NTA)_3$phen (NTA is 2-naphthoyltrifluoroacetonate) and $Eu(MBTA)_3$phen (MBTA is 4-methoxybenzoyltrifluoroacetonate) complexes are comparable and observed only in the Eu^{3+} concentration region from 1 to 5×10^{-5} mol litre^{-1}. For lower concentrations, the intensity of dye co-fluorescence was low. Perhaps, this difference is associated with the fact that in NTA aqueous solutions with pH 7, a considerable fraction of the enol form is present and only starting from a certain concentration of Eu^{3+} ions, they can compete with protons.

The region of existence of metal complexes with β-diketones and their nanoparticles also depends on the ratio of stability constants (k_{stab}) of complexes and hydroxo complexes of these metals. This ratio is different for different ions and β-diketones. This explains the fact that for MBTA solutions for which k_{stab} of MBTA complexes with metals is lower than k_{stab} of DMB metal complexes, the dye co-fluorescence and, hence, the formation of nano-

particles were observed only in Al^{3+} solutions with MBTA concentrations an order of magnitude higher than the Al^{3+} concentration. The above examples show that studying the co-fluorescence of dyes in aqueous solutions containing metal complexes can serve as a method for controlling the conditions needed for the appearance of nanoparticles of these complexes and the regularities of their formation.

In different studies, the following ligands were used for the synthesis of lanthanide complexes and their nanoparticles:

— β-diketones [$RC(O)CH_2C(O)R$]: TTA, DBM, benzoyltrifluoroacetone (BTA), BA, 4-chlorobenzoyltrifluoroacetone (ClBTA), MBTA, PhBTA, NTA pivaloyltrifluoroacetone (PTA), sparfloxacin;

— synergistic ligands, namely, phen and its certain derivatives, 2,2′-bipyridine (bpy), TOPO, triphenylphosphine oxide (TPPO), DPG, 2,4,6-trimethylpyridine, *etc*.;

— organic acids, namely, dipicolinic, trimesinic, phenylphosphonic, phenylphosphinic acid;

— complexing ligand based on polyaminocarboxylate with the attached molecule of 7-amino-4-methyl-2-quinolone, which absorbs energy and transfers it to the lanthanide ion; in the studies of DNA structure, guanosine-5′-monophosphate was taken as the complexing agent for Tb^{3+}; in studying the protein structure, bovine serum albumin (BSA) was used.

2. Nanoparticle size and formation time

Nanoparticles of complexes formed in aqueous solutions of a Eu^{3+} (10^{-5} mol litre^{-1}) + DBM (3×10^{-5} mol litre^{-1}) + TPPO (10^{-5} mol litre^{-1}) mixture were studied[21] by means of an atomic force microscope. It was shown that $Eu(DBM)_3TOPO$ nanoparticles, which were encapsulated by n-octyltrimethoxysilane in an aqueous solution, have a narrow size distribution with the maximum at 10 nm and demonstrate Eu^{3+} luminescence stable in time. The number of complexes per nanoparticle was assessed as ~1000, its molar absorption coefficient at $\lambda = 350$ nm was found to be $\sim5\times10^7$ litre mol^{-1} cm^{-1}, *i.e*., it was higher than that of quantum dots.[38] The quantum yield of Eu^{3+} luminescence was 6%.

Studying the co-luminescence intensity for a given admixture concentration as a function of the solution composition and the time passed from solution preparation allows one to elucidate the duration of nanoparticle formation and the composition of complexes contained in the nanoparticle. It was shown[39] that duration of nanoparticle formation is determined by the rate of appearance of complexes, which is in turn determined by the lability of ions and the solution pH. For pH 7, the nanoparticles of lanthanide complexes were formed in 10 – 15 min and nanoparticles of aluminium complexes were formed within an hour. Acidification of the solution resulted in a decrease in the rates of formation of complexes and nanoparticles and also to attenuation of the observed admixture co-luminescence.

3. Nanoparticles of hydroxo diketonate complexes

In aqueous solution of β-diketones and Al^{3+}, Sc^{3+}, In^{3+} cations (in contrast to Ln^{3+} ions), the fluorescence of admixed dye molecules was observed in the absence of phen and for concentrations of these cations higher or equal to the diketone concentration.[39] Insofar as these metal ions exist in aqueous solutions as hydroxo complexes and hydroxo structures, then under the conditions where the DBM concentration is insufficient (M^{3+} : DBM = 3 : 1) to force out all hydroxo groups from its ionic environment, the presence of co-fluorescence of R6G and Nile Red (NR) molecules proves the presence of nanoparticles of mixed hydroxo diketonate complexes of these ions. The observed co-fluorescence of a neutral NR molecule in solutions of nanoparticles of hydroxodiketonate complexes suggests that not only cationic but also neutral dye molecules can incorporate into such particles.

4. Nanoparticle stability

The choice of the limiting concentrations of diketone and cation is determined by the k_{stab} values for complexes with diketones and with hydroxo groups. By selecting the pH and the ligand and alcohol concentrations, it is possible to obtain a solution of nanoparticles that would exhibit stable fluorescence and co-fluorescence for a week. For instance, invariability of the fluorescence of complexes and the co-fluorescence of R6G was observed in aqueous solutions containing 10% of isopropanol in which R6G-doped $Lu(PhBTA)_3phen$ nanoparticles were formed; neither particle coagulation nor their adsorption on vessel walls were observed although no stabilizer was added. The stability of the fluorescence signal was observed only in solutions of virtually water-insoluble diketones at sufficiency high alcohol concentrations. Perhaps, in this case, the alcohol also served as the stabilizer. More often, stabilization of nanoparticles was achieved by addition of a surfactant to the solution or by coating the nanoparticles with silicate (or polymer). The stability of aqueous solutions of nanoparticles of conjugated polymers with the diameter <10 nm was reported.[8]

Nanoparticles of complexes, like nanoparticles of conjugated polymers, are formed in aqueous solutions due to their higher hydrophobicity as compared with ions and ligands (monomers).

5. The effect of the method of synthesis of nanoparticles on the admixture co-luminescence intensity

The majority of studies devoted to the co-luminescence phenomenon used aqueous solutions of nanoparticles of lanthanide complexes. In the studies carried out by our group,[35, 40] it was discovered that the efficiency of enhancement or quenching of the luminescence of europium (samarium) complexes in a nanoparticle depends not only on the concentration ratio between the luminescent and enhancing (or quenching) ions added to the solution but also on the order and the method of their introduction. In aqueous solutions, the enhancement of dye fluorescence is determined by the time gap between the nanoparticle formation and dye addition to the solution and also between the dye addition and co-fluorescence detection.

For the synthesis of mixed nanoparticles involving complexes of one ion and admixtures of other ions or dye molecules, various methods of the introduction of components were tested.

Method of simultaneous uniform addition of ions. The aqueous solution to be studied was prepared by pouring together and mixing equal volumes of solutions of β-diketone + phen and all ions (or ions and dye molecules) in concentrations twice higher than the studied concentration. This method of nanoparticle formation minimizes the

Table 2. The Eu^{3+} co-luminescence in the presence of Gd^{3+} upon simultaneous (I) and successive (II) introduction of ions into the aqueous solution of β-diketone + phen.[41]

β-Diketone	Diketone concentration /mol litre^{-1}	$I_{colum}^{Eu^{3+}+Gd^{3+}}/I_{lum}^{Eu^{3+}}$	
		I	II
TTA	10^{-4}	30	6
DBM	3×10^{-5}	30	5
MBTA	5×10^{-5}	30	8
NTA	5×10^{-5}	4	1

Note. Component concentrations /mol litre^{-1}: $[Eu^{3+}] = 5\times10^{-8}$, $[Gd^{3+}] = 5\times10^{-6}$; β-diketone : phen = 3 : 1; T = 293 K.

possibility of violation of the random distribution of the admixture.

Method of successive introduction. First, the nanoparticle-forming Ln^{3+} ions and then the admixture ions or dye molecules were added to a solution containing the required concentration of β-diketone. In aqueous solutions, the larger part of nanoparticles of lanthanide complexes was formed over 5–10 min. Hence, this method of introduction afforded nanoparticles of lanthanide complexes non-doped with foreign ions or dye molecules.

It was shown [41,42] that the difference in the nanoparticle morphology for various preparation methods affects the intensity of the admixture co-luminescence, *i.e.*, the ratio $I_{colum}^{Eu^{3+}+Gd^{3+}}/I_{lum}^{Eu^{3+}}$ (Table 2). It can be seen that the co-luminescence effect is much stronger for nanoparticles prepared by simultaneous addition of ions to the solution as compared with their successive addition. This indicates that the simultaneous addition of all components leads to the formation of mixed nanoparticles doped with foreign ion complexes (or dyes). Upon successive addition of components to the solution, the nanoparticle-forming complexes and the nanoparticles are the first to form and the foreign ion complexes and dye molecules are located in the outer layer of the nanoparticles. This structural difference is the reason for the lower intensity of admixture co-luminescence observed in the second method. The effect of the order in which nanoparticle-forming Gd^{3+} and Nd^{3+} ions were added to a solution containing the MBTA + phen and Eu^{3+} admixture was studied.[35] It was shown that the order of introduction of components affects the Eu^{3+} co-luminescence intensity. In a solution stored for a full day, the difference in $I_{colum}^{Eu^{3+}}$ was levelled over, which pointed to the smoothing of the initially non-uniform distribution of different-ion complexes in nanoparticles.

6. The effect of the nature of cation on the composition of mixed nanoparticles

To answer the question whether the composition of mixed nanoparticles formed upon the simultaneous steady addition of ions corresponds to the ratio of concentrations of these ions in solution, the efficiencies of sensitization of Eu^{3+} luminescence were compared for nanoparticles formed by complexes of different metals.[42]

It was noted [9,43,44] that the co-luminescence effect in the Eu(Sm) – Ln(Y) – $(TTA)_3$phen – Triton X-100 systems in water depends on the nature of the sensitizer ion, including the case of ions with similar systems of intrinsic energy levels: La^{3+}, Y^{3+}, Lu^{3+}, Gd^{3+}. The co-luminescence observed upon the addition of different ions depends on the composition of nanoparticles in solution and the ratio of concentrations of admixed ions and nanoparticle-forming ions. The optimal situation is that nanoparticles composed of several hundreds or thousands of complexes of sensitizing ions contain sensitized-ion complexes in a small amount proportional to the added concentrations of each type of ions. However, the k_{stab} values for complexes formed by the first and last lanthanide ions of the series differ by approximately one order of magnitude. Hence, the concentrations of their complexes at the moment of nanoparticle formation

Table 3. Effect of the excess of trivalent ions of nanoparticle-forming elements (M^{3+}) on the luminescence of Eu^{3+}, Tb^{3+}, Sm^{3+} and Dy^{3+} (according to Refs 4–6, 35, 41 and 43).

Ln^{3+} (see [a])	R /Å	Ligand	$I_{colum}^{Ln^{3+}+M^{3+}}/I_{lum}^{Ln^{3+}}$									
			La^{3+} (1.30)	Pr^{3+} (1.27)	Nd^{3+} (1.25)	Gd^{3+} (1.19)	Tb^{3+} (1.18)	Dy^{3+} (1.17)	Yb^{3+} (1.13)	Lu^{3+} (1.12)	Y^{3+} (1.16)	Al^{3+} (0.675)
Eu^{3+}	1.21	TTA[b]	2	1.0	1.0	500	340	–	43	56	–	43
Eu^{3+}	1.21	DBM[b]	2	0.9	1.0	20	22	–	10	9	–	20
Eu^{3+}	1.21	BA	25	–	–	50	100	–	–	40	25	–
Eu^{3+}	1.21	TTA[b,c]	135	–	–	740	493	15	45	504	526	–
Sm^{3+}	1.22	TTA[b,c]	168	–	–	766	757	19	20	729	811	–
Eu^{3+}	1.21	TTA[d]	7.4	–	–	17.8	–	–	–	3.48	7.88	–
Sm^{3+}	1.22	TTA[d]	2.8	–	–	6.23	–	–	–	3.18	2.74	–
Eu^{3+}	1.21	PTA[c]	7.8	–	–	86	–	–	–	57	130	–
Sm^{3+}	1.22	PTA[c]	1.9	–	–	31	–	–	–	31	61	–
Tb^{3+}	1.18	PTA[c]	1.8	–	–	235	–	–	–	295	1078	–
Dy^{3+}	1.17	PTA[c]	1.0	–	–	46	–	–	–	52	102	–

Note. The M^{3+} concentration in solutions was 1–3 orders of magnitude higher than the Ln^{3+} concentration. In parentheses, the ionic radii are given (in Å) corresponding to coordination number of 8 (for Al^{3+}, the coordination number is 6). In the series from La to Lu and then to Al, the stability constants of β-diketonate complexes increase.

[a] Luminescent ion. [b] Synergistic ligand phen. [c] Triton X-100 was added to water. [d] Synergistic ligand TOPO; CTAB (cationic surfactant, trimethylcetylammonium bromide) was added to water.

also substantially differ, which should be reflected in the particle composition.

If the forming nanoparticles resemble nanocrystals as regards their structure, then the efficiency of nanoparticle formation from complexes with the same ligands but different metal ions should be determined by the nature of these ions. The complexes formed by metals with similar ionic radii should be most compatible. Bearing this in mind, one can understand the highly efficient co-luminescence of Sm^{3+}, Eu^{3+}, Tb^{3+} and Dy^{3+} ions with the radius (R) varying from 1.22 (for Sm^{3+}) to 1.17 Å (for Dy^{3+}), which is observed in the presence of Gd^{3+} (R = 1.19 Å). At the same time, the co-luminescence induced by the introduction of a La^{3+} ion with a much higher ionic radius (1.30 Å) is substantially weaker, as follows from the data of Table 3. The luminescence of the listed ions is substantially enhanced in the presence of Lu^{3+}, Y^{3+} and even Al^{3+} ions with the radii much smaller than the radius of sensitized ions (see Table 3). The mentioned facts suggest that β-diketonate complexes of the listed luminescent lanthanide ions more easily form mixed nanoparticles (nanocrystals) from complexes of ions with smaller radii but with higher k_{stab} values as compared with analogous complexes formed by ions with larger radii. Mixed nanoparticles of $Ln(NTA)_3bpy$ (Ln = Tb, Gd, La or Y) complexes with admixed $Eu(NTA)_3bpy$ complexes were studied.[45]

7. Formation of nanoparticles with other complexing agents or in other media

The synthesis of mixed nanoparticles of lanthanide complexes and the investigation of Eu^{3+} co-luminescence were carried out not only in diketone solutions. Co-luminescence of Eu^{3+} in nanosystems formed by Ln^{3+} *o*-aminobenzoate + bipyridine[46] and Y^{3+} complexes with salicylic acid and phen was described in Refs 46 and 47, respectively.

Latva *et al.*[48] synthesized organic complexes of lanthanides with 1,2-bis(2,6-dicarboxypyridin-4-yl)ethane and its derivatives capable of forming polymer structures. The intensity of Eu^{3+} (in concentration of 10^{-7} mol litre^{-1}) luminescence in the presence of an excess of organic ligand sharply increased upon the addition of Y^{3+} (1.3×10^{-5} mol litre^{-1}), which was explained by the energy transfer from yttrium complexes to europium complexes. It was rightly reasoned that in these systems, the triplet–triplet energy transfer along the ligands of yttrium complexes takes place, being followed by intracomplex energy transfer to Eu^{3+}.

The co-luminescence of Eu^{3+} in dipivaloylmethanate complexes, induced by the addition of Gd^{3+} or Y^{3+} complexes, in the Langmuir–Blodgett films formed by arachic acid was studied.[49] In each layer of these films, small nanoparticles were formed in which the energy was transferred and the Eu^{3+} luminescence was enhanced due to photons absorbed by the closely located complexes of Gd^{3+} and Y^{3+}. Gao and co-workers[50] studied the enhancement of $Eu(DBM)_3phen$ luminescence in the presence of $Ln(DBM)_3phen$ (Ln = La, Gd, Dy, Sm, Y) complexes in the polymethyl methacrylate matrix. The highest enhancement (20-fold) was observed for lanthanum in polymethyl methacrylate with the maximum molecular mass. (In the latter publication,[50] the height of the dibenzoylmethane triplet level was mistakenly estimated to be 24000 in place of 20500 cm^{-1}.) Panigrahi *et al.*[51,52] studied the co-luminescence phenomenon for Tb^{3+} and Eu^{3+} complexes with trimesic and phenylphosphonic acids. They concluded that nanoparticles were composed only of complexes with polybasic acids.

IV. Mechanism of Ln^{3+} luminescence enhancement in nanoparticles of organic metal complexes

1. Transfer of excitation energy and inhibition of ion deactivation

Co-luminescence is usually observed in aqueous solutions with an excess of organic ligands and a low concentration of complexes of luminescent ions (Eu^{3+}, Tb^{3+}, Sm^{3+}, Dy^{3+}, *etc.*) upon the addition of considerable concentrations of cations such as Gd^{3+}, La^{3+}, Lu^{3+}, Y^{3+}, Al^{3+}, Sc^{3+}, which do not luminesce in complexes (the resonance level of this cation is higher than the T_1 level of organic ligands). The increase in the co-luminescence intensity is usually explained by incorporation of luminescent ion complexes into nanoparticles, the increase in the total absorption of the system upon formation of nanoparticles and the energy transfer from nanoparticle-forming complexes to complexes of inserted ions.[3,5,9]

This process does occur and in most cases, it makes the major contribution to the enhancement of admixture luminescence; however, this is not the only factor. The luminescence intensity and decay time (τ_{lum}) were studied[53] for europium complexes with TTA, MBTA, DBM, PTA and phen and also for terbium complexes with PTA and phen in aqueous solution as a function of the concentration of these ligands, the concentrations of luminescent and nanoparticle-forming complexes and the degree of solvent deuteration. It was shown that the formation of nanoparticles of europium and terbium complexes and their growth increase τ_{lum} of Eu^{3+} and Tb^{3+} ions. A similar increase in τ_{lum} was observed upon the incorporation of europium complexes into nanoparticles of gadolinium, terbium and lutetium complexes and also upon the insertion of terbium complexes

Table 4. Dependences of $I_{lum}^{Eu^{3+}}$ and $\tau_{lum}^{Eu^{3+}}$ on the size of nanoparticles of europium complexes formed in aqueous solutions containing β-diketone + phen + 1 vol.% EtOH at T = 293 K.

Diketone	10^6[Diketone] /mol litre^{-1}	10^6[Eu^{3+}] /mol litre^{-1}	$I_{lum}^{Eu^{3+}}$ (rel.u.)	$\tau_{lum}^{Eu^{3+}}$ /μs[a]
MBTA	50	0.05	1.0	92
MBTA	50	0.5	11.0	430
MBTA	50	1.0	35.0	520
MBTA	50	2.0	43.0	540
MBTA	50	10	170	720
TTA	200	0.5	1.0	410
TTA	200	5.0	10	500
PTA	500	0.5	1.0	480
PTA	500	5.0	10	810
PTA	500	50	104	830
DBM	30	0.05	1.0	30
DBM	30	0.5	26	90
DBM	30	5.0	490	390

Note. The ratio β-diketone : phen in solution is 3 : 1. The nanoparticle size is regulated by changing the Eu^{3+} concentration at a fixed β-diketone concentration, which exceeded the ion concentration.[53]

[a] The data for the exponential decay of Eu^{3+} luminescence are shown.

into nanoparticles formed by gadolinium complexes. The observed considerable (in certain cases, by an order of magnitude) increase in τ_{lum} points to the suppression of radiationless deactivation upon the insertion into nanoparticles (Table 4).

A rearrangement of the nearest environment of europium complexes upon their incorporation into nanoparticles of gadolinium complexes was indicated by the fact that the luminescence intensity of Eu^{3+} ions (with concentration of 2×10^{-8} mol litre^{-1}) ceased to depend on the degree of solvent deuteration in aqueous alcohol solutions of the MBTA + phen mixture upon the addition of Gd^{3+} (3×10^{-5} mol litre^{-1}).

Thus, the enhancement of the luminescence of the inserted luminescent ion complexes due to accumulation of excitation energy in them is supplemented by inhibition of the photochemical and physical deactivation of these ions upon their incorporation into nanoparticles.[53]

2. Quenching and enhancement of the Eu^{3+}, Sm^{3+} and Tb^{3+} luminescence by complexes of other metals

A more complicated situation arises when the main nanoparticle-forming Ln^{3+} ion is itself capable of luminescence or its electronic levels are situated below the resonance levels of Eu^{3+}, Sm^{3+} and Tb^{3+} and, hence, the latter can be quenched by the mechanism of energy transfer. In this case, the following processes are possible:

— migration of a singlet exciton over organic ligands with the possibility of capture of excitation energy by lanthanide ions or (with higher efficiency) by dye molecules (for details, see the next Section);

— intersystem crossing of the singlet excitation to the lower triplet state of the organic ligand, which may be favoured by a heavy paramagnetic ion in the complex;

— migration over triplet states of the organic ligands, capture of the triplet excitation energy by a lanthanide ion, competition of different ions for the migrating energy of triplet excitation;

— energy transfer from excited ions to acceptor ions.

Back in studies by Poluektov and co-workers.,[1,2] it was stated that in certain cases, the luminescence of Sm^{3+} and Eu^{3+} is enhanced even in the presence of an excess of ions with electronic levels located lower than the levels of Sm^{3+} and Eu^{3+} so that luminescence quenching is expected to occur due to the energy transfer between ions (Table 5). This is associated with the fact that with an excess of ions, nanoparticles are formed and co-luminescence of Eu^{3+} and Sm^{3+} appears and also with the fact that the ion luminescence efficiency is enhanced upon the insertion of the ions into a particle. The degree of enhancement depends on the relative efficiency of capture of the triplet excitation energy by luminescent ions and ions added in excess that simultaneously serve as sensitizers and quenchers. At the same time, for an excess of ions to which the energy of Eu^{3+} and Sm^{3+} can be transferred, the decay time of their co-luminescence is observed to shorten (see the last column in Table 5).

The dependence of I_{colum} of the analyzed ion on acceptors of its excitation energy that are present in the sample was studied. An answer to the question of how I_{colum} and τ_{lum} of europium and terbium complexes change with the formation of mixed nanoparticles containing complexes of lanthanide ions capable of withdrawing the excitation energy of the former ions was reported.[54] The changes in luminescence parameters of Eu^{3+} and Tb^{3+} in mixed nanoparicles containing Pr, Nd, Sm, Dy, Ho, Er, Tm and Yb complexes were studied for different concentration ratios of energy-donating and energy-acceptor ions. This study revealed one interesting relationship, namely, it is only for high and comparable concentrations ($\sim10^{-5}$ mol litre^{-1}) of europium and terbium complexes and complexes formed by energy-acceptor ions that the decrease in τ_{colum} was observed for nanoparticles formed by all combinations of complexes.

Table 5. Variation of co-luminescence intensity of europium complexes (I_{colum}/I^0_{lum}) in the presence of Ln^{3+} complexes, energy acceptors that form nanoparticles in solutions of β-diketones and phen.[54]

10^6[Eu^{3+}] /mol litre^{-1}	10^6[Ln^{3+}] /mol litre^{-1}	Acceptor	I_{colum}/I^0_{lum}	$\tau^{Eu^{3+}}_{lum}$ /μs	$\tau^{Eu^{3+}}_{colum}$ /μs
TTA (10^{-4} mol litre^{-1}) + phen (3.3×10^{-5} mol litre^{-1})					
0.05	5.0	Dy^{3+}	12	333	177
0.05	5.0	Er^{3+}	2.1	333	150
0.05	5.0	Tm^{3+}	1.6	333	100
0.05	5.0	Yb^{3+}	40	333	260
1.0	3.0	Dy^{3+}	1.2	333	177
1.0	3.0	Yb^{3+}	1.9	333	240
MBTA (5×10^{-5} mol litre^{-1}) + phen (1.7×10^{-5} mol litre^{-1})					
1.0	3.0	Sm^{3+}	0.8	615	406
1.0	3.0	Dy^{3+}	2.5	615	425
1.0	3.0	Ho^{3+}	0.6	615	375
1.0	3.0	Er^{3+}	0.6	615	354
1.0	3.0	Tm^{3+}	0.7	615	210
1.0	3.0	Yb^{3+}	4.6	615	550
DBM (3×10^{-5} mol litre^{-1}) + phen (1×10^{-5} mol litre^{-1})					
1.0	3.0	Dy^{3+}	2.2	344	260
1.0	3.0	Yb^{3+}	1.8	344	302

Note. The luminescence decay times are given for europium complexes in nanoparticles formed by europium complexes ($\tau^{Eu^{3+}}_{lum}$) and in nanoparticles formed by complexes of Ln acceptors ($\tau^{Eu^{3+}}_{colum}$). Solvent: H_2O + 1% ethanol-h_6, T = 293 K.

The effect of many energy-acceptor ions changes if their concentration in solution exceeds that of Eu^{3+} ions (see Table 5). A considerable enhancement of Eu^{3+} luminescence in solutions containing TTA, MBTA and DBM + phen was observed in the presence of Dy^{3+} and Yb^{3+} (Ref. 54). The enhancement is more pronounced when the Eu^{3+} concentration is several orders of magnitude lower than the concentration of acceptor ions and nanoparticles of complexes of acceptor ions are formed. Table 5 shows that the introduction of a 100-fold excess of Yb^{3+} and Dy^{3+} ions into an aqueous solution of TTA and Eu^{3+} (5×10^{-8} mol litre^{-1}) results in 40- and 12-fold enhancement of the Eu^{3+} luminescence, respectively. As the triplet energy migrates over the organic moiety of the complexes at a velocity of $\sim10^{11}$ s^{-1} (Ref. 55), different ions are excited in proportion to the rate constants for intracomplex energy transfer to these ions, which are about 10^9 s^{-1} (Refs 55–57). The enhancement of Eu^{3+} luminescence in nanoparticles of complexes formed by energy-acceptor lanthanides suggests that Eu^{3+} is more efficient in capturing the triplet exciton that migrates from ligand to ligand as compared with ions shown in Table 5. At the same time, the

energy transfer from Eu^{3+} to the acceptor ions is not very efficient. This situation is possible when, as the energy migrates over the ligand triplet levels in nanoparticles of dysprosium and ytterbium complexes, the probability of energy transfer to Eu^{3+} is higher than to Dy^{3+} or Yb^{3+}. As for nanoparticles of gadolinium and lutetium, the overall effect of Eu^{3+} co-luminescence is determined by the following two factors: the capture of excitation energy of nanoparticle-forming ions by Eu^{3+} ions and the increase in $\tau_{lum}^{Eu^{3+}}$ upon incorporation into nanoparticles.

A comparison of $\tau_{lum}^{Eu^{3+}}$ in nanoparticles of europium and other metal complexes suggests that the co-luminescence lifetime is shortened due to the energy transfer to the cations of nanoparticle-forming complexes. The degree of shortening depends on the nature of the cation (see Table 5).

The possibility of sensitization or quenching of luminescence of Sm^{3+}, Eu^{3+}, Tb^{3+} and Dy^{3+} ions within nanoparticles of other lanthanide complexes is determined by the relative positions of luminescence and absorption spectra of these ions and also by the molar absorption coefficients of energy-acceptor ions. It was shown [54] that in nanoparticles of neodymium complexes, for all Nd^{3+} and Eu^{3+} concentration ratios considered, τ_{colum} of Eu^{3+} ions decreases and quenching of their luminescence becomes more pronounced. This means that Nd^{3+} can serve as the acceptor of both the triplet energy of ligands and the excitation energy of luminescent Eu^{3+} ions. It was shown [58] that within nanoparticles of gadolinium complexes, the Nd^{3+} ions quench the luminescence of Eu^{3+} ions at equal concentrations of these ions. This study substantiates the high requirements to the purity of components used for obtaining reliable data on the concentrations of luminescent ions in solutions under study.

V. Co-fluorescence of dye molecules in nanoparticles of metal complexes and the relevant energy transfer mechanism

1. Conditions for appearance of co-fluorescence

It was shown [26] that nanoparticles formed in aqueous solutions of β-diketonate lanthanide complexes can capture molecules of fluorescent cationic dyes of the xanthene, oxazine, triphenylmethane and polymethine series. As the system is excited at the absorption band of the complexes, the fluorescence of these dyes (co-fluorescence) sharply increases. Later, nanoparticles of lanthanide and other trivalent metal complexes with inserted molecules of neutral Nile Red (NR) [39, 59] or anionic (eosin, fluorescein) dyes were found to exist. It was shown that, similarly to luminescent Ln^{3+} ion complexes, the I_{cofl} value of a dye depends on the time the dye was introduced into the solution, namely, I_{cofl} is higher when the dye is added in the initial moment of nanoparticle formation and thus gets incorporated into its bulk as compared with the case where the dye is adsorbed on the already formed nanoparticles (Table 6).

Studying the dependence of the existence region of dye co-fluorescence on the concentration of ligands (β-diketones, phen) and metal ions in aqueous solution has confirmed the earlier conclusion that starting from a concentration of complexes of $\sim 1\times10^{-7}$ mol litre^{-1}, the nanoparticles capable of capturing dye molecules from solution are formed. The limiting concentration of nanoparticles at which they do not yet coagulate depends on the particular chosen diketone and the complexing ion and on the concentration of the added alcohol.

Table 6. Concentration dependences of the Eu^{3+} luminescence quenching by NB molecules (I_{lum}^0/I_{lum}) and NB co-fluorescence intensity (I_{cofl}^{NB}) in mixed nanoparticles.[26]

10^7[NB] /mol litre^{-1}	NB on the nanoparticle surface [a]		NB inside a nanoparticle [b]	
	I_{lum}^0/I_{lum}	I_{cofl}^{NB} (rel.u.)	I_{lum}^0/I_{lum}	I_{cofl}^{NB} (rel.u.)
0.05	1.12	1.0	1.4	7.8
0.07	1.17	1.9	1.7	10.6
0.1	1.26	2.9	2.1	12.2
0.15	1.36	3.4	2.6	17.0
0.2	1.50	3.5	3.5	18.2
0.3	1.68	4.0	4.9	19.1
0.5	1.93	6.5	8.9	19.1
1.0	1.80	5.8	25.4	14.6

Note. An aqueous solution containing Eu^{3+} (5×10^{-6} mol litre^{-1}) + MBTA (2×10^{-5} mol litre^{-1}) + phen (7×10^{-6} mol litre^{-1}) was used; T = 293 K.[26]

[a] The dye was added to solution 20 min after the onset of complex formation. [b] The dye was added simultaneously with the onset of complex formation.

The complexes of Al^{3+}, Sc^{3+} and In^{3+} with DBM and NTA fluoresce in aqueous alcohols. Co-fluorescence of admixed dyes and quenching of fluorescence of the mentioned complexes for various metal ion to diketone concentration ratios and at various solution pH were found and investigated.[39] The presence of intense dye co-fluorescence for Al^{3+}, Sc^{3+} and In^{3+} concentrations three times higher than the diketone concentration suggests that there exist not only nanoparticles formed by diketone complexes but also those formed by hydroxo diketone complexes, which also contain inserted molecules of neutral and cationic dyes.

Figure 1 shows the model illustrating the configuration of nanoparticles of metal complexes with an inserted dye. (A spherical nanoparticle shape was chosen because of the thermodynamically most advantageous minimum surface-

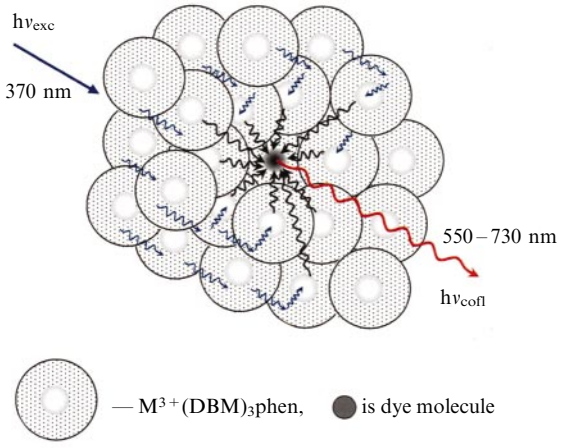


Figure 1. Model of the dye-doped nanoparticles of metal chelates. [60]
Small arrows show the direction of energy migration over the excited complex to dye molecules.

to-volume ratio.) The energy absorbed by organic ligands in nanoparticle-forming complexes migrates over these complexes to be ultimately localized on the inserted dye molecule. The average size of a nanoparticle is ~ 10 nm (Ref. 21) and the size of complex is ~ 1.0 nm; hence, a particle contains ~ 1000 complexes, which accumulate the excitation energy and transfer it to admixture molecules.

Sensitization of dye fluorescence was studied in nanoparticles of $M(BDK)_3$phen complexes, where $M = Eu^{3+}$, Tb^{3+}, Gd^{3+}, Y^{3+}, Lu^{3+}; BDK = MBTA, DBM, PTA and NTA [26,27] and in nanoparticles of hydroxo diketonate complexes $M(BDK)_{3-n}(OH^-)_n$, where $M = Al^{3+}$, Sc^{3+}, In^{3+}; BDK = DBM and NTA. [39,60]

The nanoparticles formed by $Eu(MBTA)_3$phen, $Eu(PTA)_3$phen, $Eu(NTA)_3$phen and $Tb(PTA)_3$phen complexes with the $^5D_0$ and $^5D_4$ electronic levels of lanthanides situated below the triplet levels of diketones exhibit the luminescence of Ln^{3+} ions. Incorporation of dyes into such particles decreases the luminescence intensity of Eu^{3+} or Tb^{3+} ions and gives rise to co-fluorescence of dyes (Fig. 2). Studying the regularities of quenching of Ln^{3+} luminescence and enhancement of I_{cofl} of dye molecules with the increase in their concentration in the initial solution and upon their incorporation into nanoparticles has shown that the dependence of Eu^{3+} luminescence quenching on the dye concentration in aqueous solutions is linear only in the initial segment and superlinear for the high dye concentrations.

The intensity of co-fluorescence of dye molecules incorporated into nanoparticles of europium complexes increases to reach a maximum at their concentration of $\sim 5\times10^{-8}$ mol litre^{-1} and then drops down. It was shown that quenching of the co-fluorescence in nanoparticles formed by complexes of various metals is accompanied by a red shift of the dye fluorescence spectrum. For R6G-doped particles, the maximum in the R6G fluorescence spectrum shifts from 550 nm (for its 5–20 nmol litre^{-1} concentration in the original aqueous solution) to 555 and 562 nm (for concentrations of 50 and 200 nmol litre^{-1}, respectively). This shift points to the concentration quenching [36] and also, probably, to changes in the concentration ratio between the dye molecules incorporated into nanoparticles and those located on the surface. The fluorescence spectra of dyes (*e.g.*, NR) for $>2\times10^{-8}$ mol litre^{-1} concentrations do not coincide for the cases where the excitation occurred *via* complexes or directly in the absorption band.[26,59] This suggests that not all dye molecule are involved in nanoparticles, which is also reflected in the observed dependence of dye I_{cofl} on the concentration.

The maximum I_{cofl} values for NB in nanoparticles of $Eu(MBTA)_3$phen and $Eu(NTA)_3$phen are close to each other (see Fig. 2). However, I_{cofl} of this dye in $Eu(PTA)_3$phen nanoparticles is ~ 50 times lower. Presumably, this is due to the fact that a smaller amount of NB is incorporated into nanoparticles of complexes with aliphatic ligands than into nanoparticles of complexes with aromatic ligands.

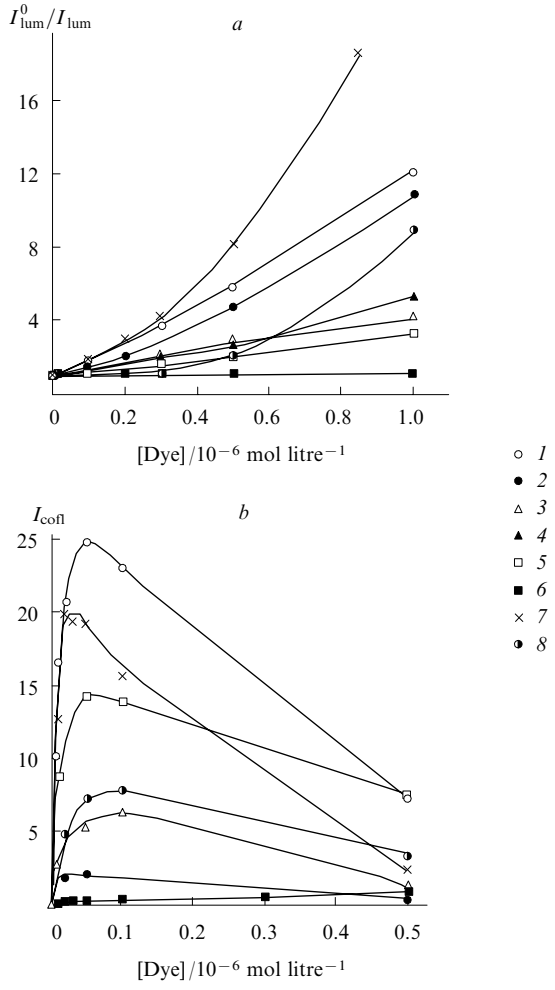


Figure 2. Dependences of Eu^{3+} luminescence quenching (I^0_{lum}/I_{lum}) (*a*) and dye co-fluorescence intensity (*b*) on the dye concentration in solutions of Eu^{3+} (5×10^{-6} mol litre^{-1}) + β-diketone (5×10^{-5} mol litre^{-1}) + phen (1.7×10^{-5} mol litre^{-1}) in H_2O + 1% EtOH ($T = 293$ K, $\lambda_{exc} = 360$ nm for MBTA and NTA, 330 nm for PTA).[60]
(*1*) NB in $Eu(MBTA)_3$phen particles; (*2*) Malachite Green (MG) in $Eu(MBTA)_3$ phen particles; (*3*) cryptocyanine in $Eu(MBTA)_3$phen particles; (*4*) MG in $Eu(PTA)_3$phen particles; (*5*) diethylthiadicarbocyanine iodide in $Eu(MBTA)_3$phen particles; (*6*) NB in $Eu(PTA)_3$phen particles; (*7*) NB in $Eu(NTA)_3$phen particles; (*8*) pinacyanol in $Eu(MBTA)_3$phen particles.

2. Sensitization of dye fluorescence by S_1-S_1 energy migration from one ligand to another

Quenching of metal-complex fluorescence and growth of the dye co-fluorescence in nanoparticles of aluminium, scandium and indium complexes for dye concentrations in the initial solution of $(2-50)\times10^{-9}$ mol litre^{-1} were studied in solutions containing cationic, neutral and anionic dyes, namely, R6G (Fig. 3), NB, NR and sodium fluorescein. The obtained results confirmed the presence of energy migration over singlet excited levels of metal complexes and transfer to the singlet excited levels of dye molecules, *i.e.*, the S_1-S_1 migration.

Despite the difference in the overlap integrals of the fluorescence spectrum of the aluminium complexes and the absorption spectra of R6G and NB, similar dependences for the fluorescence quenching of these complexes on the dye concentration in solution were observed (Table 7) as well as the concentration quenching of R6G and NB co-fluorescence for the dye concentration in solution of $>5\times10^{-8}$ mol litre^{-1}. The fluorescence levels for R6G, NB and NR are below the S_1 and T_1 levels of ligands but, in

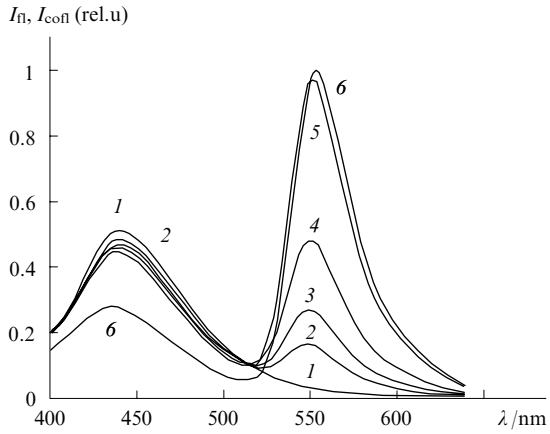


Figure 3. Fluorescence intensity of Al(DBM)$_n$ complexes (λ_{max} = 440 nm) and co-fluorescence of R6G (λ_{max} = 555 nm) inserted into nanoparticles of these complexes formed in an aqueous solution of Al^{3+} (10^{-5} mol litre^{-1}) + DBM (3×10^{-5} mol litre^{-1}) + phen (10^{-5} mol litre^{-1}) + R6G as a function of R6G concentration (λ_{exc} = 340 nm).[36]
[R6G]/mol litre^{-1}: (*1*) 0, (*2*) 5×10^{-9}, (*3*) 1×10^{-8}, (*4*) 2×10^{-8}, (*5*) 5×10^{-8}, (*6*) 1×10^{-7}.

contrast to NB and NR, the S_1 level of R6G lies 1300 cm^{-1} higher than the $^5D_0$ level of Eu^{3+}. This allows one to elucidate how the energy migration from excited levels of complexes to the R6G singlet excited levels affects I_{colum} of Eu(DBM)$_3$phen introduced into aluminium complexes simultaneously with R6G. It was shown that in the presence of R6G molecules in nanoparticles, the I_{colum} value of Eu(DBM)$_3$phen complexes decreases; hence, the S_1-S_1 energy transfer to the dye competes with the intersystem crossing followed by the T_1-T_1 migration of energy to the europium complex. Earlier (see Ref. 55), the energy transfer from the T_1 level of diketone to Eu^{3+} and the inductive – resonance energy transfer from Eu^{3+} to dye molecules[56,61] were observed for non-aqueous solutions of europium complexes containing no nanoparticles.

In nanoparticles of Eu(DBM)$_3$phen complexes, no ligand fluorescence could be observed; nonetheless, for these particles, the R6G co-fluorescence and the R6G-induced quenching of Eu^{3+} luminescence were observed (Table 8). This means that even in nanoparticles of heavy metal complexes, the energy is transferred from singlet excited levels of complexes to singlet excited levels of dye molecules and this process can compete with the S_1-T_1 transitions in the complexes.

Table 7. Concentration dependences of the co-fluorescence intensities of R6G and NB upon their introduction into nanoparticles of aluminium and europium complexes (according to Refs 36 and 60).

10^6[R6G] /mol litre^{-1}	Al(DBM)$_3$phen		Eu(DBM)$_3$phen		10^6[NB] /mol litre^{-1}	Al(DBM)$_3$phen		Eu(DBM)$_3$phen	
	I_{fl} (see [a])	I_{cofl}^{R6G}	$I_{lum}^{Eu^{3+}}$	I_{cofl}^{R6G}		I_{fl} (see [a])	I_{cofl}^{NB}	$I_{lum}^{Eu^{3+}}$	I_{cofl}^{NB}
0	27	0	1050	0	0	29	0	1050	0
0.01	27	13	1000	5.0	–	–	–	–	–
0.02	23	20	980	9.0	0.02	23	1.75	580	23
0.05	18	39	960	18.5	0.05	22	3.2	370	29
0.1	12	42	900	31	0.1	17	3.8	176	32
0.2	8.5	48	780	41	0.2	16	4.7	44	21
0.5	5.0	30	320	30	0.5	7.0	2.2	8.8	9.0

Note. The concentrations of Al(DBM)$_3$phen and Eu(DBM)$_3$phen are equal, 1×10^{-5} mol litre^{-1}; T = 293 K. The luminescence intensities of all solution components are given in relative units for the same excitation conditions. [a] Fluorescence intensity of Al(DBM)$_3$phen.

Table 8. Concentration dependences of co-luminescence intensity of rhodamine 6G and Eu^{3+} ions, fluorescence of aluminium complexes and the same complexes doped with Eu(DBM)$_3$phen and luminescence of Eu(DBM)$_3$phen complexes.[36]

10^9[R6G] /mol litre^{-1}	Al(DBM)$_n$		Al(DBM)$_n$ + Eu(DBM)$_3$phen			Eu(DBM)$_3$phen	
	I_{fl} (see [a])	I_{cofl}^{R6G}	I_{fl} (see [a])	$I_{colum}^{Eu^{3+}}$	I_{cofl}^{R6G}	$I_{lum}^{Eu^{3+}}$	I_{cofl}^{R6G}
0	27	0	33	9.0	0	1050	0
2.5	27	2.0	–	–	–	–	–
5.0	27	7.0	26	8.0	10	–	–
10	25	11.0	23	7.2	16	1000	5.0
20	23	20.0	21	7.0	34	980	9.0
50	18.0	39.0	19	6.0	56	960	18.5
100	12.0	42	13	5.0	61	900	31
200	8.5	48	–	–	–	780	41
500	5.0	30	5.0	2.3	38	320	30
1000	–	–	–	–	–	156	27

Note. Intensity values are given for the maxima of luminescence spectra and are expressed in relative units. Concentrations of complexes in aqueous solutions /mol litre^{-1}: [Al(DBM)$_n$], 10^{-5}; [Eu(DBM)$_3$phen], 5×10^{-7}; [Eu(DBM)$_3$phen], 10^{-5}; excitation in the absorption band of complex λ_{exc} = 370 nm, 293 K. [a] Fluorescence intensity of Al(DBM)$_n$.

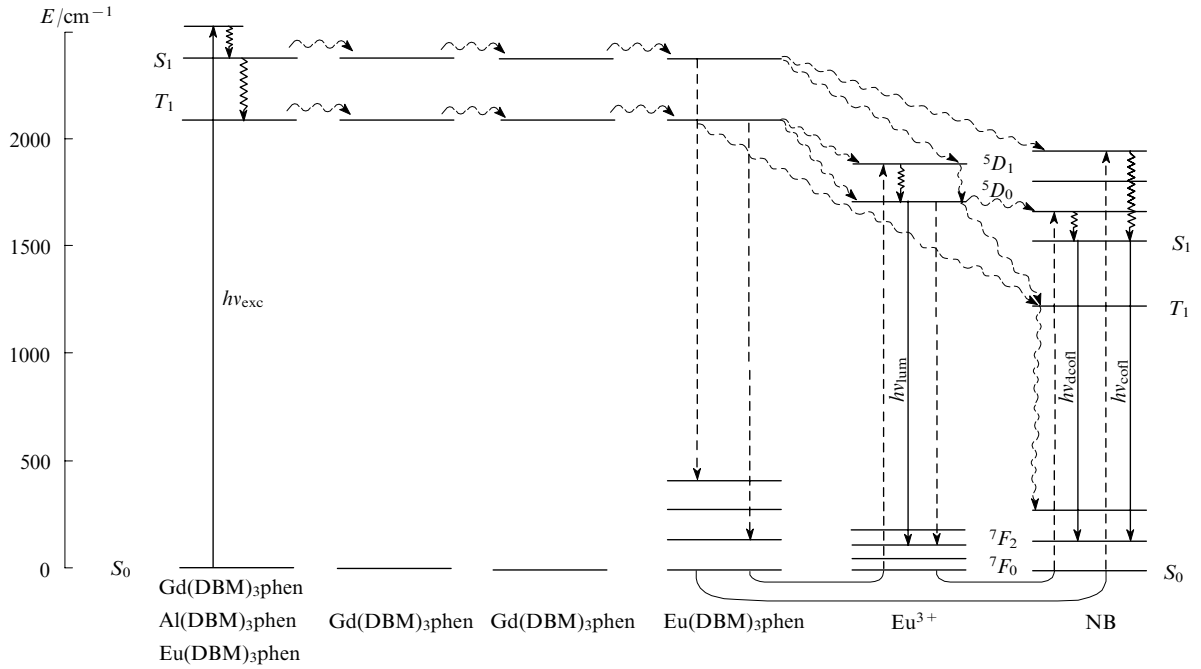


Figure 4. Possible routes of energy transfer in nanoparticles of various metal complexes doped with Eu^{3+} complexes and dye molecules with the first singlet excited level being located below the $^5D_0$ level of Eu^{3+} (Ref. 60).
It is evident that depending on the excitation route, the dye co-fluorescence may be usual and delayed.

3. Sensitization of dye molecules in nanoparticles by energy transfer from luminescent ions. Coexistence of usual and delayed co-fluorescence.

In all nanoparticles under consideration, the excitation of ligands in metal complexes entails the following processes.

1. Migration of excitation energy over the singlet levels of ligands and its transfer to the lower S_1 levels of dyes. This migration induces the co-fluorescence of dyes.

2. In nanoparticles of complexes formed by luminescent lanthanide ions (or incorporating these ions), the energy migrates over T_1 levels of ligands to the Ln^{3+} ion to be subsequently transferred to the S_1 levels of dye molecules, which are located below the levels of these ions (*e.g.*, below the $^5D_0$ level of Eu^{3+}).

The efficiency of these energy transfer processes depends on the nanoparticle size and composition, the impurity concentration, the relative positions of electronic levels for Eu^{3+} ions and dye molecules (Fig. 4).

In nanoparticles of europium complexes with admixed NB whose S_1 level is located below the $^5D_0$ level of Eu^{3+}, τ_{lum} of Eu^{3+} ions decreases, the decrease being dependent on the NB concentration in the initial solution (Fig. 5), and delayed co-fluorescence of NB appears (Fig. 6). This unambiguously proves that in parallel with the S_1-S_1 energy migration to dye molecules, in these nanoparticles, the energy is also transferred to these molecules from the $^5D_0$ level of Eu^{3+}. In nanoparticles of $Eu(MBTA)_3phen$ complexes, the decay time of Eu^{3+} luminescence is 650 μs. Upon the incorporation of NB into these particles, this time considerably decreases even for the 5×10^{-9} mol·litre^{-1} concentration of NB in solution, and at 5×10^{-8} mol litre^{-1} of NB (see Fig. 5), the time decreases more than 5-fold. Thus, the Eu^{3+} luminescence decay curve fails to reach τ_{lum} of unquenched europium complexes even in the late stages. This means that for 5×10^{-8} mol litre^{-1} concentration of NB in solution, almost all excitation energy absorbed in $Eu(MBTA)_3phen$ nanoparticles is transferred from Eu^{3+} ions to the inserted dye molecules.

A comparison of I_{cofl}^{NB} for $Eu(MBTA)_3phen$ and $Lu(MBTA)_3phen$ or $Gd(MBTA)_3phen$ nanoparticles containing admixed $Eu(MBTA)_3phen$ complexes confirms the conclusion concerning high efficiency of the energy transfer from Eu^{3+} ions to dye molecules (Table 9).[27] These studies have shown that for 5×10^{-9} mol litre^{-1} of NB, the effi-

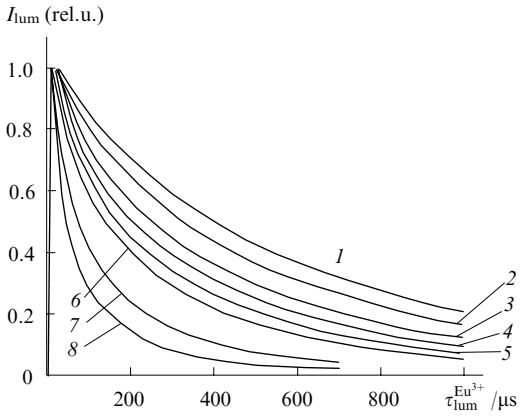


Figure 5. Concentration dependences of the Eu^{3+} luminescence decay in mixed nanoparticles formed by $Eu(MBTA)_3phen$ complexes and NB at 293 K.[27]
The aqueous solution contains MBTA (5×10^{-5} mol litre^{-1}) + phen (1.7×10^{-5} mol litre^{-1}) + Eu^{3+} (5×10^{-6} mol litre^{-1});
10^8[NB]/mol litre^{-1}: (*1*) 0, (*2*) 0.5, (*3*) 1, (*4*) 5, (*5*) 2, (*6*) 3, (*7*) 5, (*8*) 10. Curve *4* pertains to particles with dye molecules on the surface; the other curves characterize the luminescence decay for particles with inserted NB molecules.

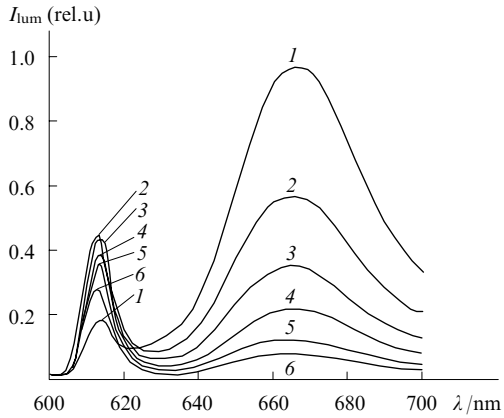


Figure 6. Intensity ratio of Eu^{3+} luminescence (λ = 613 nm) to the sensitized delayed fluorescence of NB (λ = 665 nm) inserted into $Eu(MBTA)_3$ phen nanoparticles as a function of the time gap (t) between the measurement and the excitation pulse.[27]
Aqueous solution contains MBTA (5×10^{-5} mol litre^{-1}) + phen (1.7×10^{-5} mol litre^{-1}) + Eu^{3+} (5×10^{-6} mol litre^{-1}) + NB (5×10^{-8} mol litre^{-1}); t/μs: (*1*) 1, (*2*) 5, (*3*) 10, (*4*) 20, (*5*) 30, (*6*) 50.

ciency of sensitization of dye molecules by such energy transfer is more than one order of magnitude greater than the efficiency of their sensitization by the S_1-S_1 energy transfer in the particles of gadolinium and lutetium complexes. But at 5×10^{-7} mol litre^{-1} of NB in solution, the energy transfer from singlet excited levels of ligands to the dye successfully competes with the population process of the triplet states of ligands and Eu^{3+} ions. This determines the similarity of I^{NB}_{cofl} for nanoparticles formed by $Eu(MBTA)_3$phen, $Lu(MBTA)_3$phen and $Gd(MBTA)_3$-phen.[27]

Comparing the NB-induced quenching of the fluorescence of nanoparticles of aluminium dibenzoylmethanate complexes, the luminescence of Eu^{3+} in nanoparticles of aluminium and gadolinium complexes doped with europium complexes and the I^{NB}_{cofl} values confirms the conclusion that the energy transfer from Eu^{3+} is more efficient than from the ligands provided that the dye S_1 level is located lower than the Eu^{3+} $^5D_0$ level.[60] At the same time, the fact of coexistence of two dye sensitization processes leading to the appearance of delayed and conventional co-fluorescence is beyond question. The intricate dependence of I^0_{lum}/I_{lum} of ions on the dye concentration (see Fig. 2 *a*, curves *7*, *8*) is chiefly explained by the interplay of processes of energy transfer to the dye from the singlet excited states of ligands and the population of the $^5D_0$ level of Eu^{3+} and not only by the violation of the Stern–Volmer dependence as it is superimposed by the Poisson distribution of dye molecules among nanoparticles. From the above analysis of contributions made by different types of energy transfer to the intensity of dye co-fluorescence for various dye concentrations, it follows that the contribution of delayed co-fluorescence would decrease with the increase in the dye concentration The existence of delayed NB co-fluorescence is confirmed by NB fluorescence spectra measured with a time delay of 10 or 50 μs in $Eu(MBTA)_3$phen solutions (see Fig. 6).

4. Energy migration in nanoparticles

In all nanoparticles under consideration, the excitation energy migrates over ligands of complexes to the S_1 levels of dye molecules to be captured by the latter. It was shown [36] that in $Al(DBM)_3$ nanoparticles, the rate constant for the energy transfer (k_{tr}) from singlet excited levels to R6G and NB molecules is independent of the overlap integral of fluorescence spectra of complexes and dye absorption spectra. This can be due to several reasons, namely, the different ability of dyes to be incorporated into nanoparticles, the presence of an additional, photochemical quenching channel, the high migration rate and the long exciton free path exceeding the nanoparticle size.

In our studies, the different capacity of dye molecules for incorporation into nanoparticles formed by different complexes was repeatedly stressed.[26] The appearance of an additional quenching channel is also possible. However, the most probable reason that explains the whole set of data [26, 36] is a mean free path of singlet and triplet excitons (L) exceeding the size of studied nanoparticles (d). For the hopping mechanism of energy migration, the exciton mean free path (the diffusion shift) is described by the formula [55]

$$L = (zD\tau_0)^{1/2}, \qquad (1)$$

where coefficient z is assumed to be 1 to 6 depending on the motion model chosen, D is the exciton diffusion coefficient,

Table 9. Relative co-fluorescence intensities of NB (I^{NB}_{cofl}) and luminescence quenching of Eu^{3+} (I^0_{lum}/I_{lum}) in nanoparticles formed in aqueous solutions of $Eu(MBTA)_3$phen complexes, $Lu(MBTA)_3$phen complexes doped with $Eu(MBTA)_3$phen and $Gd(MBTA)_3$phen complexes doped with $Eu(MBTA)_3$ phen for different NB concentrations in the original solution (λ_{exc} = 370 nm, 293 K).[27]

10^9[NB] /mol litre^{-1}	MBTA (2×10^{-5} mol litre^{-1}) + phen (7×10^{-6} mol litre^{-1})				MBTA (5×10^{-5} mol litre^{-1}) + phen (1.7×10^{-5} mol litre^{-1})			
	Eu^{3+} (5×10^{-6} mol litre^{-1})		Lu^{3+} (5×10^{-6} mol litre^{-1}) + Eu^{3+} (1×10^{-9} mol litre^{-1})		Eu^{3+} (5×10^{-6} mol litre^{-1})		Gd^{3+} (5×10^{-6} mol litre^{-1}) + Eu^{3+} (1×10^{-9} mol litre^{-1})	
	I^0_{Eu}/I_{Eu} (see [a])	I^{NB}_{cofl}	I^0_{Eu}/I_{Eu} (see [b])	I^{NB}_{cofl}	I^0_{Eu}/I_{Eu} (see [c])	I^{NB}_{cofl}	I^0_{Eu}/I_{Eu} (see [d])	I^{NB}_{cofl}
5	1.4	9.9	1.0	0.37	1.4	10.1	1.0	0.45
10	1.8	13.8	1.0	0.72	1.6	16.6	1.0	0.90
20	2.7	22.6	1.3	1.1	2.8	20.5	1.8	1.3
50	7.0	21.8	3.0	2.3	6.0	24.6	3.7	2.5
100	33.0	13.6	10.5	4.0	12.3	22.9	8.6	3.2
500	500	2.3	105.0	3.3	112.0	7.1	82.4	2.8

I^0_{Eu} (rel.u.): [a] 690; [b] 10; [c] 900; [d] 16.

τ_0 is the lifetime of the excited states of molecules over which the energy migrates.

It was found [21] that the size of nanoparticles formed in aqueous solutions is 10 nm. Numerous estimates of the free path for singlet excitation afforded $L = 8-10$ nm in the crystals [55] and nanoparticles [33] in which τ_{fl} of energy donors is $\sim 0.1-1$ ns. Thus, for particles with the diameter of 10 nm, the energy migration is the only factor determining k_{tr}; hence, the lack of correlation between this value and the overlap integrals between the luminescence spectra of complexes and dye absorption spectra is reasonable. According to estimates,[62] τ_{fl} of $Al(DBM)_3$ complexes is 0.1 ns; this is why one can expect the lack of influence of the overlap integral on the coefficient k_{tr} for NB and R6G, which was confirmed experimentally.[36]

The I_{fl} values for hydroxo diketone complexes of aluminium, scandium and indium were compared with I_{cofl} values for R6G molecules inserted into nanoparticles of these complexes.[39] It was shown that I_{cofl}^{R6G} changes insignificantly upon a several-fold decrease in I_{fl} of indium complexes compared to aluminium and scandium complexes. According to data of Table 10, varying the metal ion makes it possible to change τ_{fl} by a factor of several tens and, hence, substantially change the exciton mean free path [see formula (1)].

The effect of variation of τ_{fl} of the nanoparticle-forming complexes on I_{cofl} of the inserted R6G and NR molecules and also on the quenching of fluorescence of the proper complexes in solutions with different dye concentrations has been studied.[62] Table 10 shows that on going from DBM complexes formed by light ions to those formed by heavier ions, τ_{fl} decreases from 0.1 ns (for aluminium complexes) to 3 ps (for gadolinium complexes), *i.e.*, 30-fold. In parallel, according to formula (1), the exciton mean free path should also decrease from ~ 10 nm in nanoparticles of aluminium complexes to ~ 2 nm in particles of gadolinium complexes. In molecular crystals whose size is much larger than L, this decrease leads to substantial reduction of the intensity of sensitized fluorescence of the admixture.

A comparison of fluorescence spectra of R6G [26] and NR [59] in solutions recorded either upon direct excitation into the first absorption band or upon co-fluorescence excitation in nanoparticles has shown that for > 50 nmol litre^{-1} of the dye in solution, only its insignificant part resides in nanoparticles. This suggests that although the absolute number of dye molecules inserted into nanoparticles increases with the increase in the dye concentration in solution, its relative content decreases. For nanoparticles of different metal complexes, the co-fluorescence spectra of inserted R6G molecules shift at the same dye concentration. This suggests that the same number of R6G molecules get incorporated into particles formed by complexes of different metals. For 200 nmol litre^{-1} of R6G in solution when the concentration quenching takes place and, hence, a nanoparticle contains more than one dye molecule, the R6G co-fluorescence intensity is virtually independent of τ_{fl} of the complex. This can be explained by the fact that in nanoparticles, the intensity of impurity co-fluorescence is determined not only by the mean free path but also by the direct energy transfer from the primarily excited complexes to dye molecules.

The difference between I_{cofl} values of dyes in nanoparticles formed by complexes of different metals becomes more noticeable upon decrease in the dye concentration in the original solution and, hence, in the nanoparticle (see Table 10). Taking into account the data [21] on the diameter of nanoparticles of lanthanide complexes with DBM and also the diameters of the complexes in question (~ 1 nm), it can be assumed that the nanoparticle concentration in solution is $\leqslant 10$ nmol litre^{-1}.

For solutions containing 50 and (especially) 10 nmol litre^{-1} of the dye, the I_{cofl}^{R6G} values in nanoparticles of aluminium complexes are higher than in nanoparticles of other complexes, *i.e.*, the effect of τ_{fl} of the complexes on I_{cofl} of the admixture is observable in nanoparticles for which the admixture content can be assumed not to exceed one molecule per particle. However, according to Table 10, a 30-fold change in τ_{fl} of the complexes leads merely to a three-fold difference between dye I_{cofl}. This prompts the following conclusions: the incorporation of several admixture molecules into the nanoparticles in question characterized by τ_{fl} of the complexes of $> (3-5)$ ps is sufficient for I_{cofl} of the impurity to become independent of τ_{fl} of complexes; for impurity concentrations equal to or smaller than the nanoparticle concentration, the effect of τ_{fl} of the complexes on I_{cofl} of the dye is much weaker as compared with large molecular crystals. This means that in the studied nanoparticles measuring ~ 10 nm, the energy migration has

Table 10. Initial fluorescence intensities (I_{fl}^0) and decay times (τ_{fl}) for dibenzoylmethanate metal complexes, fluorescence quenching parameters (I_{fl}^0/I_{fl}) and co-fluorescence intensities (I_{cofl}) of R6G and NR dyes at different concentrations in the original solution.[62]

Ion M^{3+}	$M(DBM)_3$phen (see [a])		R6G (10 nmol litre^{-1})			R6G (50 nmol litre^{-1})			R6G (200 nmol litre^{-1})			NR (5 nmol litre^{-1})	NR (50 nmol litre^{-1})
	I_{fl}^0	τ_{fl}/ns	I_{cofl}	I_{cofl}/I_{fl}^0	I_{fl}^0/I_{fl}	I_{cofl}	I_{cofl}/I_{fl}^0	I_{fl}^0/I_{fl}	I_{cofl}	I_{cofl}/I_{fl}^0	I_{fl}^0/I_{fl}	I_{cofl}	I_{cofl}
–	0.7	–	0	–	1.0	0	–	–	0	–	–	–	–
Al^{3+}	110	0.1	36	0.3	1.0	100	0.9	1.3	77	0.7	2.5	4.0	27
Sc^{3+}	20	0.02	20	1.0	1.0	58	2.9	1.2	66	3.3	2.1	2.3	–
Y^{3+}	7.0	0.007	19	2.7	1.0	56	8.0	1.0	73	10.5	1.0	–	–
Lu^{3+}	5.5	0.005	13	2.3	1.0	47	8.5	1.0	62	10.5	1.0	2.0	7.0
Gd^{3+}	3.5	0.003	11.5	3.3	1.0	38	11	1.0	75	10.0	1.0	–	–

Note. The quantities I_{fl} and I_{cofl} were not corrected for the spectral sensitivity of the setup; I_{fl}^0 values are given for the same absorption of complexes at $\lambda_{exc} = 350$ nm, $T = 293$ K.

[a] The predominant composition of lanthanide and yttrium complexes; aluminium and scandium complexes have the coordination number 6 and do not contain phen.

a substantial effect on the efficiency of energy transfer to the admixture. This explains the fact that the I_{cofl} values for R6G and NR molecules are close in the order of magnitude (but not equal) for nanoparticles formed by complexes of light and heavy metals that have different fluorescence decay times.

5. The use of dye fluorescence in nanoparticles for enhancing the sensitivity of luminescence assay techniques in biology and medicine

At present, the phenomenon of co-luminescence of Ln^{3+} ions is widely used for enhancing the sensitivity of chemical and biological assays. An important object of investigations is to find promising application areas of the co-fluorescence of dye molecules inserted into nanoparticles with the aim of enhancing the sensitivity of determination of fluorescent dyes in solutions. It was observed[60] that the NB fluorescence increases 100-fold as NB passes from an aqueous solution with a concentration of 5×10^{-8} mol litre^{-1} into $Eu(DBM)_3$phen nanoparticles and also that the fluorescence of a solution containing 5×10^{-8} mol litre^{-1} of R6G is enhanced 25-fold upon the formation of particles in an aqueous solution containing 5×10^{-5} mol litre^{-1} of Al^{3+} and NTA. This fluorescence enhancement is due to excitation of complexes and energy transfer from the complexes to the dye. The absorption coefficient of such nanoparticles is $\sim10^7$ litre mol^{-1} cm^{-1}, whereas the absorption coefficient of dyes is $\sim10^5$ litre mol^{-1} cm^{-1}. In the absence of foreign impurities, almost all energy absorbed by a nanoparticle is accumulated on dye molecules. Theoretically, the formation of particles from complexes in dye-containing aqueous solutions makes it possible to decrease the detection limit of the luminescence determination of low dye concentrations in solution by two orders of magnitude. However, the fluorescence spectra of complexes demonstrate a large half-width and the fluorescence yields of aluminium complexes vary from several percent to 10% – 15%;[39] moreover, the presence of the long-wavelength edge of this fluorescence interferes with the determination of small dye concentrations (see Fig. 3).

At the same time, in view of the efficient sensitization of R6G molecules in nanoparticles of weakly fluorescing complexes of heavy metal ions, it can be expected that the presence of a fluorescence 'tail' for complexes in the vicinity of the R6G fluorescence maximum will interfere to a lesser extent with determination of small R6G concentrations in solution that in the case of nanoparticles formed by aluminium complexes.[39] In aqueous solutions with variable R6G concentration, $Lu(DBM)_3$phen nanoparticles that have captured R6G molecules from the solution were synthesized and the fluorescence of these solutions was studied (Fig. 7). By examining the nanoparticles of complexes of these ions on standard spectrofluorimetric instruments, it is possible to reliably determine the R6G concentration in water down to 5×10^{-11} mol litre^{-1}; this is an order of magnitude lower than the R6G concentration that can be determined after direct dye excitation.

For dyes that fluoresce in the red spectral range, the contribution of fluorescence of complexes is less significant; moreover, the fluorescence yields of NB and NR increase upon incorporation of these dyes into nanoparticles.[59, 60] The sensitivity of determination can thus be increased by two orders of magnitude. (For NR, the incorporation into nanoparticles is accompanied by a considerable short-wavelength shift of the fluorescence spectrum.[59]) Studying solutions that contain Malachite Green[26] has shown that triphenylmethane dyes that virtually do not fluoresce in liquid solutions do fluoresce in nanoparticles. Hence, one can monitor their presence in solutions down to concentrations of $(2-3)\times10^{-9}$ mol litre^{-1}. The proposed method of determination of low dye concentrations based on the intensity of their co-fluorescence in nanoparticles can be applied to dyes that fluoresce not only in liquid but also in viscous and solid solutions.

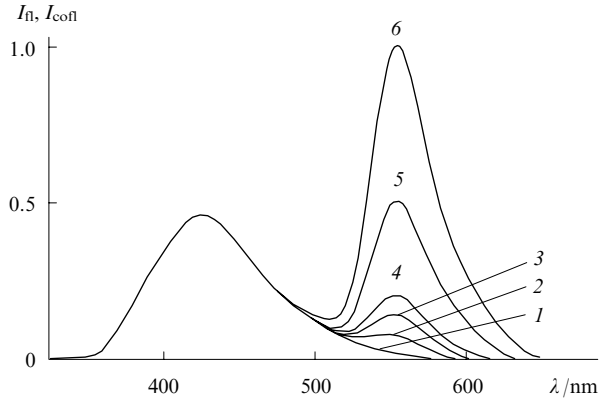


Figure 7. Concentration dependences of the fluorescence intensity of $Lu(DBM)_3$phen (λ_{max} = 420 nm) complexes and co-fluorescence intensity of R6G (λ_{max} = 555 nm).[62]
The original aqueous solution contains Lu^{3+} (10^{-5} mol litre^{-1}) + DBM (3×10^{-5} mol litre^{-1}) + phen (10^{-5} mol litre^{-1}) + R6G; λ_{exc} = 340 nm. [R6G]/mol litre^{-1}: (*1*) 0, (*2*) 5×10^{-11}, (*3*) 1×10^{-10}, (*4*) 2×10^{-10}, (*5*) 5×10^{-10}, (*6*) 1×10^{-9}.

Taking into account the high absorption coefficient of nanoparticles of complexes, it can be assumed that the brightness of their co-fluorescence is commensurate with the brightness of quantum dots. However, it is only for the nanoparticle excitation in the long-wavelength region of the absorption spectrum of the complexes that free ligands do not absorb some of the exciting light energy. Furthermore, even in the nanoparticles of aluminium complexes, the concentration quenching of dyes occurs before all excitation energy has been transferred to the dye (see Table 7). Hence, in nanoparticles of this size, it is impossible to obtain the limiting (theoretical) luminescence brightness.

Stable nanoparticles of a polyfluorene derivative [9,9-dihexenefluorenyl-2,7-diyl (PDHF)] measuring 30 nm with inserted perylene, NR, coumarin-6, tetraphenylporphin (TPP) and 2-methyl-6-(4-dimethylaminostyryl)-4-(dicyanomethylene)-4*H*-pyran (DCM) molecules were formed[33] in aqueous solutions. All dopants were found to quench the PDHF fluorescence, while the sensitized fluorescence of dyes was not observed only for DCM. The appearance of concentration quenching of sensitized fluorescence was noted for coumarin-6 inserted into 30-nm nanoparticles of conjugated polymers. The concentration quenching appeared at higher dye contents as compared with 10-nm nanoparticles of complexes, which is not surprising taking into account the three-times larger size of conjugated polymer nanoparticles. In these coarse nanoparticles, the PDHF fluorescence was successfully quenched in the presence of 2 mass % of perylene and coumarin-6. However, in particles doped with NR and TPP, the PDHF fluorescence

could not be completely quenched even in the presence of 10 mass % of the dyes.

The experimentally observed dependence of the efficiency of energy transfer from nanopaticles to dyes on the dye concentration was compared [33] with the predictions based on the Förster theory. It was shown that just as in the case of particles of complexes, this dependence can be adequately described only with due allowance made for the energy migration over polymers. In another study,[34] octaethylporphyrine was introduced into poly(9,9-dialkylfluorene)platinum nanoparticles and its sensitized phosphorescence was observed. This result also confirms the conclusion that in solutions of nanoparticles, one can observe processes typical of solids. Nanoparticles of PDHF measuring ~30 nm demonstrated the absorption coefficient of 1.5×10^9 mol^{-1} litre cm^{-1}. In particles doped with coumarin-6 and perylene, the total energy transfer from the nanoparticle to the dopant is possible before the appearance of strong concentration quenching. The quantum yield of such doped particles is ~40%; this is why they are much brighter 'beacons' than, say, dye-doped silicate nanoparticles of the same size and nanoparticles of complexes measuring ~10 nm. However, the method of preparation of these particles allows one to insert only hydrophobic molecules from non-aqueous solvents and (in contrast to nanoparticles of complexes) does not allow the extraction of dyes and strengthening of their fluorescence in already existing aqueous solutions of dyes.

The possibility of synthesizing conjugated polymer nanoparticles doped with other conjugated polymers with fluorescence no weaker than the emission of quantum dots existing in a wide spectrum range was demonstrated in the study cited above [33] and in some other studies. A review [8] emphasizes the strict requirements to the purity of used components for obtaining highly bright luminescence of nanoparticles. The co-fluorescence of nanoparticles of conjugated polymers was considered in comprehensive reviews;[8, 63] hence, we do not discuss it here.

Concluding this section, note that nanoparticles of metal complexes with incorporated dye molecules are formed in aqueous solutions of dyes. In these particles, efficient energy migration over singlet and triplet levels of complexes takes place with energy transfer from the singlet excited state and also from the luminescent Ln^{3+} ion to the dye molecule. As a result, in nanoparticles formed by complexes [36, 39, 60] or fluorescent polymers,[8, 33, 34] fluorescence of the dyes is enhanced. This makes it possible to create non-toxic brightly luminescent particles to be used in chemistry, biology and medicine.

VI. The use of co-luminescence in chemical analysis

Back in 1964, Poluektov and co-workers [1] proposed using co-luminescence of $Ln(TTA)_3$phen microprecipitates in the determination of Eu^{3+} concentrations based on the band with a maximum at 612 nm and Sm^{3+} concentrations based on the band at 562 nm. In their next study,[2] they compared luminescence of suspensions of tetraligand lanthanide complexes with TTA, DBM, BA, AA and various organic bases in aqueous solution. The highest intensity was typical of Eu^{3+} complexes with TTA and DBM with collidine and diphenylguanidine. The sensitivity of determination varied from 0.0005% to 0.0001% for Eu_2O_3 and from 0.005% to 0.001% for Sm_2O_3 in Gd, La, Tb, Y, Yb and Lu oxides. A monograph [64] provides the vast bibliography on the luminescence method of lanthanide determination in solutions of salts and complexes. It also describes the methods of enhancing the sensitivity of determination of luminescent lanthanide ions in suspensions of precipitates of their complexes upon the addition of excess Gd^{3+}, La^{3+}, Lu^{3+}, *etc.*; however, the term 'co-luminescence' was not used in this book.

Above (see Section II), we mentioned publications [3, 4] considering the use of $Eu(TTA)_3$phen luminescence enhancement in the presence of Tb^{3+}, Gd^{3+} and other ions for determining europium concentrations. The authors used the same ligands and enhancing ions but added a non-ionic surfactant, Triton X-100, as the stabilizing agent. The highest enhancement (by up to two orders of magnitude) for 10^{-7} mol litre^{-1} of Eu^{3+}, a 200-fold excess of the enhancing ion and pH 5.0–9.0 was found for Tb^{3+} (Ref. 4). Under these conditions, the detection limit of europium was 10^{-13} mol litre^{-1}. When studying the luminescence enhancement in aqueous solutions of $Eu(BA)_3$phen, the strongest effect was also observed for the addition of Tb^{3+}. The fluorescence intensity in the Eu–Tb–BA–phen system depended linearly on the europium concentration in the range of 10^{-9}–2×10^{-7} mol litre^{-1}. However, the detection limit of europium complexes with BA was much lower (2×10^{-11} mol litre^{-1}) as compared with those with TTA. The system Eu–Gd–BA – diphenylguanidine–Triton X-100 proved to be more sensitive.[65] The detection limit of europium lowered down to 4×10^{-13} mol litre^{-1}, while the linear dependence between the luminescence intensity and the europium concentration could be observed in a range from 2.0×10^{-10} to 2.0×10^{-7} mol litre^{-1}.

To enhance the luminescence of Eu^{3+}, Yang *et al.* [66] used the Eu–Tb–DBM–NH_3 combination, which is similar to the Eu–Gd(Y, La, Lu)–DBM–diethylamine system studied earlier by Poluektov and co-workers.[67] It was shown [65, 66] that the co-luminescence effect increases upon the addition of 10%–20% of acetone or methanol to the aqueous solution. The optimal content of enhancing ions (Tb^{3+}) was 1.5×10^{-5} mol litre^{-1} for Eu^{3+} concentration varying from 10^{-8} to 10^{-7} mol litre^{-1}, the DMB concentration of 4×10^{-4} mol litre^{-1} and solution pH of 8.0–10.0. The detection limit of europium was 4×10^{-11} mol litre^{-1}. A system completely identical to that considered by Poluektov and co-workers [67] was also studied by other authors.[68] The differences consisted in the simultaneous determination of samarium and europium as analytes, the addition of 30% acetone to water and the new detection limits found for europium (down to 8×10^{-14} mol litre^{-1}) and samarium (down to 5×10^{-13} mol litre^{-1}). In their next work,[69] researchers of this group studied co-luminescence in the system Eu–Tb(Lu)–TA–$CTAB^+$; the europium detection limit was found to be 1×10^{-13} mol litre^{-1}.

The use of co-luminescence in the Eu(Sm)–(TTA)–TOPO–$CTAB^+$ and Sm–TTA–phen–Triton X-100 systems containing La^{3+}, Gd^{3+}, Tb^{3+}, Lu^{3+} and Y^{3+} as the enhancing ions for determination of europium and samarium concentrations was considered.[5–7] The highest enhancing effect was observed for Gd^{3+}, the detection limits were found to be 5×10^{-12} mol litre^{-1} for europium and 8×10^{-11} (Ref. 5) and 8×10^{-12} mol litre^{-1} (Ref. 7) for

samarium. Co-luminescence was observed in the Eu–Tb–TTA–dibenzo-18-crown-6-ether–Triton X-100 system.[70] The possibility of determining down to 10^{-13} mol litre^{-1} of europium impurities in lanthanum, praseodymium and dysprosium oxides was demonstrated.

In a series of studies,[71–73] a new ligand, 1,6-bis-(3-methyl-1-phenyl-1,5-dihydropyrazol-4-on-5-yl)hexane-1,6-dione (BPMPHD), was proposed for the formation of complexes to be used in the co-luminescence chemical analysis. The new ligand exhibits intrinsic fluorescence with the maximum at 446 nm, which is enhanced by two orders of magnitude in the presence of Y^{3+} and $CTAB^+$ ions. This was used to develop a luminescence method with Y^{3+} detection limit of 2 ng ml^{-1} (Ref. 71). Apparently, this method is not selective because the possibility of considerable enhancement of ligand fluorescence in the presence of Be^{2+}, Al^{3+} or Sc^{3+} cannot be ruled out.[39] It was also shown[71] that Tb^{3+} luminescence is sensitized in $[Tb–Y–(BPMPHD)_2]^-(CTAB)^+$ complexes. This was utilized to develop a method for Tb^{3+} determination in solutions in the concentration range of 2.0×10^{-11}–8.0×10^{-6} mol litre^{-1}, the detection limit being 2.0×10^{-12} mol litre^{-1} (Ref. 72), which is impossible for other ligands (because the energy of their T_1-state is lower than the energy of the resonance $^5D_4$ level of Tb^{3+}). In a later study,[73] a much higher value was found for the detection limit of terbium, namely, 2.7×10^{-9} mol litre^{-1}. For the same ligand and surfactant combination, the co-luminescence of Sm^{3+} ions upon the addition of Lu^{3+}, Y^{3+}, Gd^{3+} was studied.[74] The detection limit of samarium was found to be 2.0×10^{-8} mol litre^{-1} and a linear dependence of co-luminescence intensity on the concentration of this element was observed up to 8.0×10^{-6} mol litre^{-1}. A similar detection limit of Dy^{3+} equal to 3×10^{-8} mol litre^{-1} for the same ligands, surfactants and enhancing Gd^{3+} ions (1.5×10^{-5} mol litre^{-1}) was observed by Tong *et al.*[75]

The co-luminescence of Tb^{3+} complexes with phenyl salicylates was observed in water containing ethanol (2.5%) and Triton X-100 (0.025%) with pH of 9.0.[76] The strongest enhancement was observed upon the addition of a 30-fold excess of La^{3+} relative to Tb^{3+}, the addition of Lu^{3+}, Gd^{3+} or Y^{3+} proved to be less efficient. The detection limit of terbium was 3×10^{-9} mol litre^{-1}.

For determination of Tb^{3+} in water, the (3,4-dihydroxyphenyl)-L-alanine (L-DOPA) complexing agent, Triton X-100 and Lu^{3+} were used.[77] The detection limit of terbium was found to be 2×10^{-8} mol litre^{-1} for the concentration ratio Tb : Lu = 1 : 1; the co-luminescence *vs.* concentration dependence was linear in the range of 10^{-7}–10^{-4} mol litre^{-1}.

In several publications[78–82] dealing with europium determination by the intensity of its co-luminescence, it was proposed to use Eu^{3+} complexes with fluoroquinolone and quinolone antibiotics (sparfloxacin,[78,79] gatifloxacin[81] and ofloxacin[82]) with addition of an excess of La^{3+} (Ref. 81), Lu^{3+} (Ref. 78), Y^{3+} (Ref. 79) or Gd^{3+} (Refs 79, 80 and 82). The detection limit of europium in the presence of cationic surfactants was 2×10^{-13} mol litre^{-1} with sparfloxacin, 1.5×10^{-10} mol litre^{-1} with ofloxacin, 7×10^{-14} mol litre^{-1} with gatifloxacin and 5×10^{-12} mol litre^{-1} with oxolinic acid.

Luminescence of the Tb^{3+} complex with levofloxalin was used[83] to quantify antibiotics in tablets, urine and blood serum. The sensitization of luminescence of Tb^{3+} and Eu^{3+} ions by drugs (pyrocatechol, naphthyridine) and also by derivatives of aromatic heterocyclic acids was studied.[84] As a result, a sensitive method was proposed for the determination of these drugs. Studies devoted to applications of luminescent analytical techniques based on the use of lanthanide complexes for the determination of drugs were surveyed in a review.[85]

Luminescence of the Eu^{3+} complex with tetracycline in the presence of citric acid salts was studied.[86] Co-luminescence was observed with excess Tb^{3+} ions, which allowed Eu^{3+} ions to be determined in lanthanide mixtures in the Eu^{3+} concentration range from 6×10^{-9} to 4×10^{-6} mol litre^{-1} with the detection limit of 1.4×10^{-9} mol litre^{-1}.

In a series of publications,[51,52,87,88] the range of ligands that allow for co-luminescence observation was extended. Trimesinic, pyromellitic, mellitic and phenylphosphonic acids were used as the ligands for Eu^{3+}, Tb^{3+} and Dy^{3+} complexes. The limits of detection of the mentioned ions by co-luminescence in the presence of La^{3+}, Gd^{3+} or Y^{3+} varied from 0.025 (for Tb^{3+}) to 0.5 ng ml^{-1} (for Dy^{3+}). Similar results were obtained for Tb^{3+} complexes with trimesinic acid in the presence of La^{3+} and Gd^{3+} ions.[89]

A technique for determination of uranyl, terbium and europium ions in water was developed using 2,6-dipicolinic acid (DPA) as the sensitizer.[90] The detection limits for the simultaneous determination of these ions were 2.2×10^{-7}, 1×10^{-8} and 5×10^{-9} mol litre^{-1} for UO_2^{2+}, Tb^{3+} and Eu^{3+}, respectively. The DPA complexes were also used[91] in the co-luminescence method to decrease the detection limit of Eu^{3+} down to 100 ppq and the detection limit of Tb^{3+} down to 60 ppq. However, the proposed mechanism of enhancement is still unclear, because the addition of yttrium (10^{-7} mol litre^{-1}) to a $Eu(DPA)_3^{3-}$ solution resulted in a more than 100-fold decrease in the detection limit of the latter, although the excitation takes place in the absorption band of Eu^{3+} rather than of DPA and, hence, the migration over ligand triplet levels is ruled out.

VII. The use of co-luminescence in immunoassay

The luminescence durations for complexes of samarium, dysprosium, europium and terbium (from several tens to several thousands of microseconds) and the possibility of synthesizing nanoparticles of metal complexes in aqueous solutions are the factors that make it possible to use co-luminescence for extending the possibilities of immunoassay, first of all, the time-resolved luminescence analysis.[92,93] The immunoassay is carried out in two stages. In the first stage, labelled antibodies that have participated in a specific reaction are separated from unreacted antibodies, and Ln^{3+} ions are separated from the antibodies. Lanthanide chelates and their nanoparticles are prepared by adding an enhancing solution that consists of two separately stored components to a solution of label ions. Examples of compositions of such components can be found elsewhere.[9] In the determination of Eu^{3+} and/or Sm^{3+} labels, the first solution contained TTA or BTA ligands (30–70 μmol litre^{-1}), Triton X-100 (0.02%–0.075%), Y^{3+} ions (1.5–8.5 μmol litre^{-1}) and acetic acid (0.98 cm^3 litre^{-1}). For the simultaneous determination of all ions involved in this assay (Eu^{3+}, Sm^{3+}, Tb^{3+} and Dy^{3+}) or only of Tb^{3+} and Dy^{3+} ions, the PTA ligand was used, in place of TTA and BTA, as the triplet state energy of PTA is higher than the resonance level energies of all mentioned lanthanide

ions. In this case, the first solution included PTA (70–100 μmol litre^{-1}), Y^{3+} (3–7.5 μmol litre^{-1}), Triton X-100 (0.0006%–0.06%) and acetic acid (1–2 cm^3 litre^{-1}) and also 20% to 30% of ethanol. In the majority of cases, the second solution included a synergistic agent phen (0.4–1.75 mmol litre^{-1}), 1,3-diphenylguanine (5 mmol litre^{-1}) and an alkaline buffer component, namely, tri(hydroxymethyl)methylamine (0.2–0.375 mmol litre^{-1}). 1,3-Diphenylguanidine was dissolved in ethanol. The co-luminescence signal was recorded 10–20 min after mixing of the solution components. The excitation light wavelength was varied from 365 to 314 nm depending on the diketone used. The co-luminescence of lanthanide complexes was measured at the following wavelengths:

Ion	Eu^{3+}	Sm^{3+}	Tb^{3+}	Dy^{3+}
λ/nm	612	648	545	574
τ_{lum}/μs	730–1060	48–96	240–320	11–27

The luminescence duration depended on the particular ligand used. As was mentioned,[9] by combining co-luminescence measurements with the time-resolved technique, the detection limits could be decreased 15-fold (down to 2.5×10^{-15} mol litre^{-1}) for Eu^{3+} and 37-fold (down to 9×10^{-14} mol litre^{-1}) for Sm^{3+}, if the enhancing metal ions were of ultrahigh purity. For example, yttrium of 99.9999% purity was used.[9]

The applicability of the proposed method was demonstrated[9] for the follicle-stimulating hormone (FSH) assay. The co-luminescence technique made it possible to increase the luminescence signal level, but its sensitivity proved to be close to the sensitivity of the commercial technique DELFIA (Delayed Enhanced Lanthanide Fluorescence Immuno Assay), which was attributed to the more pronounced dependence of the sensitivity on the non-specific binding of labelled antibodies than on the label detection sensitivity. The co-luminescence technique was used in the simultaneous determination of two hormones, namely, FSH and luteinizing hormone.[94,95] The sensitivity of this method turned out to be 5–50-times higher than the sensitivity of the radioimmunoassay or spectrofluorimetry. By synthesizing nanoparticles with an aliphatic diketone capable of sensitizing all four Ln^{3+} ions that possess sufficiently high co-luminescence intensity (Eu^{3+},Tb^{3+}, Sm^{3+} and Dy^{3+}), the researchers[9] demonstrated the possibility of simultaneous determination of all four labels for the four substances which are determined during the new-born screening in order to reveal hereditary diseases: thyroid-stimulating hormone (TSH), immunoreactive trypsin (IRT), creatine kinase MM (CK-MM) and 17-α-oxyprogesterone (17α-OHP).

A procedure of preparation and properties of silicate-coated nanoparticles consisting of terbium complexes with *N*,*N*,*N*′,*N*′-[2,6-bis(3-aminomethylpyrazol-1-yl)phenylpyridine] tetraacetate were described.[22] The synthesized stable nanoparticles of 42 ± 3 nm size exhibited bright luminescence with the decay time of 1.5 ms. The prospects for modifying the surface of nanoparticles towards protein immobilization were demonstrated. The authors cited[22] used time-resolved luminescence analysis to achieve the limiting sensitivity of 7×10^{-9} ng litre^{-1} for the human prostate-specific antigen.

The authors of another publication[96] used styrene–acrylic acid co-polymerization to prepare nanospheres 45 nm in diameter with negatively charged surfaces. These nanoparticles contained europium and samarium complexes with NTA and TOPO and also terbium and dysprosium complexes with 4-(2,4,6-tri-n-decyloxyphenyl)pyridine-2,6-dicarboxylic acid. For synthesized nanoparticles, the extremely low detection limits were found: 1 fmol litre^{-1} for nanoparticles of europium and terbium complexes and even considerably lower, 1 pmol litre^{-1}, for samarium and dysprosium complexes.

It was shown[97] that the luminescence intensity of Tb complexes with BSA and HSA (HSA is human serum albumin) is enhanced upon the addition of cetylpyridinium bromide (CPB) from which the energy is transferred to BSA (HSA). Further enhancement of Tb^{3+} luminescence occurs upon the addition of non-luminescent ions Gd^{3+}, La^{3+}, Lu^{3+}, Y^{3+}. This method is suitable for determining the concentrations of these proteins of about several nanogram per millilitre.

It was proposed[98] to use europium chelate nanoparticles as the energy donors that transfer energy to a dye that fluoresces with a maximum at 680 nm. Due to the longer luminescence of the donor (Eu^{3+}), the acceptor (dye) co-fluorescence also decays for a sufficiency long time, which allows one to use the time-resolved technique for its detection. The potential of this technique was demonstrated by one-step immunoassay for estradiol. Europium chelate nanoparticles used had a size of 92 nm and were coated by Fab-fragments of the 17β-estradiol-specific recombinant antibody, which in turn was bound to the infrared dye Alexa Fluor 680. The immunoassay sensitivity depended on the density of Fab-fragments on the nanoparticle surface. The optimal amount of binding fragments was reached when the radius of the sphere surrounding the fragment on the nanoparticle surface approximately corresponded to the Förster radius of energy transfer from europium chelate to the dye and these regions did not overlap. Using this method, estradiol in concentration of 70 pmol litre^{-1} was determined.[98]

Co-luminescence of the Tb^{3+} complex with adrenaline was studied.[99] It was shown that this phenomenon can be used to determine adrenaline concentration with a detection limit of 4.5×10^{-9} mol litre^{-1} . It was found[100] that luminescence of this terbium complex is enhanced in the presence of bovine blood serum and sodium dodecyl sulfate (SDS), which allowed a fluorescence technique to be developed for determination of this protein with the detection limit of 1.3×10^{-9} g ml^{-1}.

A simplified method of synthesis of luminescent $Eu(NTA)_3TOPO-SDS$ nanoparticles with the diameter of 62 nm was developed.[23] Assuming the diameter of the complex to be 1.5 nm, the authors assessed the number of complexes per nanoparticle as 70000. They have also developed a synthetic route to $Eu(DBM)_3phen$ and $Eu(DBM)_3aphen$ nanoparticles, where aphen is the amino derivative of phenanthroline.¶ Prostate-specific monoclonal antibodies were immobilized on a nanoparticle by physical adsorption from a carbonate buffer. The limit of detection

¶ The ions and ligands are added to water followed by addition of 3-thiocyanopropyltriethoxysilane and a small amount of tetraethoxysilane in ethanol; nanoparticles are isolated by centrifugation and ultrafiltration. Nanoparticles with diameters of 92 nm are formed, the number of complexes in each particle is ~250 000.

of these antibodies by time-resolved fluorimetry was 28 ng litre^{-1}.

VIII. The use of Ln^{3+} labels and co-luminescence of lanthanide complexes in the nucleic acid assays

The luminescence techniques and the radiationless energy transfer are widely used in biology and medicine.[12] Luminescence of lanthanides upon complexation with nucleic acids is actively used in the DNA and RNA assays.[101]

In 1973, it was discovered that luminescence of Tb^{3+} ions is enhanced upon their binding to certain nucleotides and yeast RNA,[102] the energy being transferred from the guanosine residue. Later, it was shown [103–105] that sensitization of Tb^{3+} is observed only if guanosine is located in a single nucleic acid strand.

The coordination of Eu^{3+} ions to transport RNA was studied.[106] It was shown that in solutions, this ion enters into specific interaction with two sites of this molecule. Europium and terbium complexes with diethylenetriaminepentaacetic acid with an attached aromatic ring that absorbs in the UV range were synthesized.[107] The complexes that contained a particular base, cytosine, demonstrated the highest luminescence yields; however, their luminescence was unstable. In another work, the ways for enhancing the Tb^{3+} luminescence in the presence of nucleic acids and phen were studied and a method of determination of thermally denatured DNA and RNA in the range of 0.4–20 mg ml^{-1} with the detection limit of 0.1–0.2 mg ml^{-1} was proposed.[108]

Klakamp and Horrocks [109] studied the interaction of Eu^{3+} ions with various guanosine-based oligonucleotides [oligo$(dG)_n$] at pH 6.0. Insofar as europium complex with bases or proper nucleic acids, in contrast to terbium complexes, does not luminesce on excitation in the nucleic acid absorption band, the laser pulse excitation of Eu^{3+} luminescence was carried out in the absorption band corresponding to the $^5D_0 \leftarrow {}^7F_0$ transition. When studying the decay curves of Eu^{3+} luminescence in H_2O and D_2O, the researchers observed the presence of two types of bonds formed by ions with oligo$(dG)_n$, where $n = 4-10$:

— the stronger bonded Eu^{3+} ion has, in its first coordination sphere, two water molecules and six or seven oligomer molecules coordinated to europium *via* phosphate groups and base fragments;

— the weaker bound Eu^{3+} ion has six or seven water molecules in its first coordination sphere with the oligomer and is bound to one or two atoms of the oligomer phosphate group.

In their next study,[110] the researchers extended these conclusions to oligomers of other nucleic bases: oligo$(dA)_8$, oligo$(dT)_8$, oligo$(dC)_8$ and also their dihydroxymonophosphates dAMP, dCMP and dTMP.

Selvin *et al.*[111] studied the radiationless transfer of energy from an europium complex attached to one 5′-amino group of DNA to the fluorescent cyanine dye CY-5 attached to another 5′-amino group of DNA. To increase the useful signal-to-background luminescence ratio, a time-resolved spectrofluorimeter was used. From analysis of the decrease in the luminescence decay time for europium complex in the presence of a dye by means of the Förster equation, the distance (4.6 nm) between the sites of attachment of the europium complex and CY-5 to DNA was determined. Yang *et al.*[112] have observed that for pH 6.9–7.5, deoxyguanosine diphosphate (dGMP) forms a complex with Tb^{3+} and sensitizes its luminescence. They proposed a method for determination of dGMP in the presence of other nucleotides with the detection limit of 3×10^{-8} mol litre^{-1} and with linear dependence of the luminescence signal on the concentration in the range of 2×10^{-7} to 1×10^{-5} mol litre^{-1}.

The method of determination of DNA and RNA by quenching luminescence of the Tb–Tyrone complex (Tyrone is disodium salt of 4,5-dihydroxybenzene-1,3-disulfonic acid) was proposed.[113] For the ratio Tb : Tyrone = 3 : 10 and solution pH of 6.9, the addition of nucleic acids quenched the Tb^{3+} luminescence (the terbium complex with nucleic acid does not luminesce). The detection limit was $\sim$1 ng ml^{-1}. The formation of a luminescent Tb^{3+} complex with DNA bases was used to study high-order sequences in the DNA double helix.[114] Fu and Turro [115] studied the Tb^{3+} luminescence enhancement in the presence of nucleic bases. It was found that in aqueous solutions, only cytosine (C) is capable of enhancing the Tb^{3+} luminescence; at the same time, among the dihydroxymonophosphate bases, the Tb^{3+} luminescence can be enhanced only by the guanidine derivative dCMP. It was also shown that double helices of oligonucleotides fail to enhance the Tb^{3+} luminescence, while their single strands and breaks of bonds of the bases in the double strand do enhance the Tb^{3+} luminescence. Based on the obtained results, a luminescence technique for damage detection in the DNA double strand was proposed.[115]

Nanoparticles of metal complexes, in particular co-luminescence effect, find their application in the DNA and RNA assays. Leif *et al.*[116–118] proposed an original method of enhancing the signal from a complex formed by europium coordinated to the ligand *via* six nitrogen atoms or the terbium complex with dipicolinic acid. This method was named the resonance energy transfer enhanced luminescence (RETEL). To the adsorbed luminescent complexes of europium with nitrogen macrocycle or terbium complex with dipicolinic acid, an ethanol solution that contained either an excess of gadolinium complex with TTA or an excess of dipicolinic acid without Gd^{3+} was added, and the resulting solutions were dried. After drying, the desired signal intensity in the dry layer increases 6-fold due to the energy transfer from the complexes or ligands added. This method was proposed [118] for solving problems of cytometry. A CCD camera was used for taking images of cells labelled with luminescence markers (Eu^{3+}); taking into account the enhancement of luminescence of these markers, the possibility of separate determination of DNA-synthesizing and dying cells was demonstarted.

The enhancement of luminescence of terbium complexes with DNA in the presence of Gd^{3+}, Lu^{3+}, Y^{3+}, La^{3+} and Sc^{3+} ions was studied.[119, 120] The most pronounced enhancement of luminescence of terbium complexes with fish sperm DNA and yeast RNA at pH 9.0 was observed in the presence of Gd^{3+} and Lu^{3+} complexes. Based on the retrieved data, a luminescence method for determination of nucleic acid concentrations was proposed. The detection limit was found to be 4×10^{-9} g ml^{-1} for DNA and 6×10^{-9} g ml^{-1} for RNA. The region of the linear relationship between the DNA and RNA concentrations and the luminescence intensity was greater than four orders of magnitude. Later, it was found [121, 122] that the co-luminescence effect of terbium complexes with DNA and RNA

induced by Gd^{3+} becomes much stronger in the presence of cetylpyridinium bromide. It was assumed that Tb^{3+} and Gd^{3+} ions are attached to nucleic acids *via* phosphate groups. In our opinion, these results are not quite consistent with the statement [115] that undamaged DNA fails to sensitize the luminescence of Tb^{3+} ions. The effect of co-luminescence was observed [123] for the complex of Tb^{3+} with guanosine 5′-monophosphate (GMP) upon the addition of Gd^{3+}, La^{3+}, Y^{3+}, Sc^{3+} and Lu^{3+}. On the basis of this effect, a technique of GMP determination in the concentration range from 3×10^{-8} to 3×10^{-5} mol litre^{-1} with the detection limit of 4×10^{-9} mol litre^{-1} was proposed. However, the hypothesized structure of polynuclear complexes responsible for co-luminescence seems to be unlikely, because the free vacancies in the first coordination sphere should most probably be occupied by water rather than ammonia molecules as was claimed by the authors of the above study.[123]

For DNA assay, a complex of Tb^{3+} (1×10^{-6} mol litre^{-1}) with 4-hydroxy-2-oxoquinoline-3-carboxylic acid (1×10^{-6} mol litre^{-1}) was proposed.[124] The luminescence intensity of the complex increases several-fold in the presence of fish sperm DNA and calf thymus DNA in concentrations of 0.05–1.5 mg ml^{-1} at pH 9.0. The detection limit for the mentioned DNAs was $\sim$10 ng ml^{-1}. Further increase in sensitivity is possible in time-resolved luminescence measurements. Chen *et al.*[125] obtained a new Tb^{3+} complex with polyaminocarboxylate with attached 7-amino-4-methyl-2-quinolone (carbostyryl-124) as an efficient sensitizer of Tb^{3+} luminescence. Nanoparticles of SiO_2 measuring $\sim$50 nm with inserted $\sim$350 terbium complexes were synthesized. By time-resolved luminescence assay, DNA labelled with these nanoparticles could be detected down to concentration of $\sim10^{-10}$ mol litre^{-1}.

IX. Conclusion

The demands of chemistry, biology and medicine in enhancing the sensitivity and extending the spectral range of luminescence methods and also methods for determining the protein structure and conformation dictate the necessity of synthesizing new luminescent nanoparticles and studying their properties. The phenomenon of co-luminescence of europium, samarium, terbium and dysprosium complexes inserted into nanoparticles formed by complexes of other lanthanides is already used to enhance the sensitivity of the immuno-fluorescence assay where these ions serve as the labels.[92, 93] In the present review, we discussed the results of studies on the effect of the chosen complexing ion, the parameters of its spectra and the size of nanoparticles on the co-luminescence and luminescence decay time of the lanthanide ions inserted into the nanoparticles. These studies shed light on the difference between the limits of detection of various ions admixed to the Eu^{3+}, Sm^{3+}, Tb^{3+} and Dy^{3+} ions under study by monitoring the co-luminescence of the latter ions during the formation of nanoparticles of their complexes.

The methods of synthesis of nanoparticles of trivalent metal complexes doped with ionic or neutral molecules of fluorescent dyes and also nanoparticles of conjugated polymers containing admixtures of neutral molecules or other conjugated polymers are described. The singlet–singlet energy migration to the admixture is shown to be responsible for the co-fluorescence of dyes observed in the nanoparticles formed by complexes of non-luminescent metals and nanoparticles of fluorescent conjugated polymers. In nanoparticles formed by complexes of fluorescent Ln^{3+} ions, the S_1-S_1 migration over complexes to the dye and the T_1-T_1 migration over complexes to Ln^{3+}, and, if possible, also energy transfer from the ions to the dye coexist (and sensitize the dye molecules).

It was shown that the use of co-fluorescence in nanoparticles of metal ion complexes allows increasing the sensitivity of determination of dye concentrations in aqueous solutions by one to three orders of magnitude (depending on the particular dye and material purity). At the same time, analysis of changes in the dye co-fluorescence intensity and spectra during the formation of nanoparticles of the complexes serves for monitoring of this process and the particle morphology. The methods of synthesis of nanoparticles of metal ion complexes doped with luminescent ions or fluorescent molecules and also nanoparticles of fluorescent conjugated polymers with inserted admixtures of fluorescent organic molecules that are described in this review are used to prepare particles with large absorption cross-sections and bright luminescence throughout the visible and near-IR ranges. The work on the synthesis of conjugated polymers with incorporated molecules of fluorescent dyes and investigation of their co-fluorescence has been started recently (have been performed for four years). Non-toxic organic nanoparticles show promise for medicinal and biological applications.[126]

The following potential applications of dye co-fluorescence in nanoparticles of metal complexes in aqueous and mixed water – alcohol solvents can be proposed:

— detection of very low concentrations of fluorescent molecules in aqueous solutions;

— fabrication of highly bright fluorescent nanoparticles to be used as the labels in biology and medicine.

Whereas the former problem is by and large solved (see Section V), the production of highly bright fluorescent nanoparticles is held up due to the concentration quenching of co-fluorescence observed for dye concentrations at which the complete energy transfer from these complexes to the dye has not yet occurred but the concentration quenching of their fluorescence has already begun (see Fig. 2). This problem was solved by using coumarin-30 in nanoparticles formed by β-diketonate complexes of metal ions with coordination number 8–9 (Ln^{3+}, Y^{3+}). It was found [127–129] that coumarin-30 is involved in nanoparticles in an amount of up to one molecule per each metal complex with three β-diketonate ligands. In this case, coumarin serves as a synergistic agent that stabilizes the complex and may substitute 1,10-phenanthroline usually taken for this purpose. At the same time, due to absorption of coumarin-30, the co-fluorescence excitation spectrum extends to the visible range up to 440 nm and its concentration quenching is totally absent.

The first publications on the use of co-fluorescence of conjugated polymer nanoparticles doped with dyes for enhancing the sensitivity of the luminescence assay of biological reactions [130] and insvestigation processes *in vivo* [131] have already appeared.

Numerous studies carried out in recent years (see references in review [8]) have demonstrated that cells of living organisms uptake and accumulate conjugated polymer nanoparticles of different types, which allows studying them under a microscope with the resolution power of

5 – 20 nm. However, to study the DNA structure and conformations, it is necessary that nanoparticles contain functional groups with the specific binding properties.

Currently, studies on the nature of stability of dye-doped nanoparticles formed by complexes and polymers with the size of $\leqslant 10$ nm in water and aqueous alcohols containing no surfactants are in progress. To use these nanoparticles *in vivo*, it is necessary to examine the stability of the co-fluorescence signal of these nanoparticles as a function of the nature of dye and nanoparticle-forming components in pure aqueous solutions (containing no admixtures of other solvents).

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