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Study of photochemical fluorescence enhancement of the terbium-lomefloxacin complex

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Abstract

Lomefloxacin (LMFX) and terbium ion can form a complex and the sensitized fluorescence of the terbium ion can be observed. It was found that the sensitized fluorescence intensity can be notably enhanced when the terbium complex is exposed to 365 nm ultraviolet light. By the fluorescence spectra, phosphorescence spectra, fluorescence quantum yield and fluorescence lifetime of the system, it was proved that irradiation of the complex made it undergo a photochemical reaction and a new terbium complex which is more favorable to intramolecular energy transfer was formed. This is why the sensitized fluorescence enhancement can be observed.

Keywords: Terbium complex; Lomefloxacin; Photochemical reaction; Fluorescence

1. Introduction

Lomefloxacin (LMFX) is one of the synthetic antibacterial fluoroquinolone agents of the third generation (its chemical structure shown in Fig. 7(A)), which exhibits high activity against a broad spectrum of gram-negative and gram-positive bacteria. It has a good effect in clinical treatment [1,2]. In consequence, it is of great importance to determine its contents in various biological fluids (blood, urine and tissues).

For the time being, the detection of the LMFX for its pharmacokinetical study is usually performed by liquid chromatography method and microbiological assay method [3,4]. We attempted to explore the chelate formation between LMFX and lanthanides because of the narrow and intense fluorescence of the lanthanide ions [5,6] with the aim of improving the sensitivity and specificity by fluorimetric method. On investigating the sensitized fluorescence of Tb^{3+}-LMFX complex, we were surprised to find that the sensitized fluorescence of the terbium ion was enhanced gradually with the excitation time going on. This was of great interest to us. So it was tried that Tb^{3+}-LMFX complex was pre-irradiated for 30 min under a 365 nm UV lamp, and observed that the sensitized fluorescence of the terbium ion of the irradiated system was enhanced notably and the fluorescence intensity can keep stable for at least 24 h. The mechanism of the fluorescence enhancement is investigated in this work. To our knowledge, no report on the photochemical fluorescence enhancement of the lanthanide complex has been found.

2. Experimental

2.1. Apparatus

Fluorescence spectra were recorded with a Hitachi-850 spectrofluorimeter (Japan) equipped with a 150 W-xenon lamp light source. Absorption spectra were recorded on a Shimadzu-UV250 spectrophotometer (Japan). The pH measurements were made with a pHS-2 meter (Shanghai). A ZF-I Ultraviolet (UV) analytical meter (Shanghai) was used as the light source for the photochemical reaction. Fluorescence lifetime measurements were made with a SLM48000s multi-frequency lifetime fluorimeter (America). Phosphorescence spectra were measured on a FL-ZTZ spectrofluorimeter equipped with a 193D phosphorimeter (SPEX Co., America). Fluoride measurements were made with a fluoride electrode (model PF-1, Shanghai, China) as the indicator electrode and a saturated calomel electrode as the reference electrode (model 801, Jiangsu, China). The electromotive

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force of the electrode was measured with a pH meter (model pHs-3C, shanghai, China).

2.2. Reagents

All of the reagents used in the experiment were of analytical grade and distilled, deionized water was used throughout this study.

Stock standard solution of LMFX (Institute of Medical Biotechnology, Beijing; 1.00×10^{-3} mol l^{-1}) was prepared by dissolving 38.8 mg LMFX in an appropriate amount of water containing three drops of 0.1 mol l^{-1} NaOH and diluting to 100 ml with water, stored at 4°C and protected from light, and diluted to the desired concentration when used.

Stock standard solution of the Tb^{3+} ion (1.00×10^{-2} mol l^{-1}) was prepared by dissolving 186.9 mg Tb_4O_7 (purity, 99.99%) in hot (75°C) 1 : 1 HCl and evaporating the solution to be almost dry before diluting to 100 ml with water, stored in a plastic bottle, kept at 4°C, and diluted to the desired concentration when necessary.

A 0.2 mol l^{-1} ammonium acetate–ammonia buffer solution (pH = 7.0) was obtained by adjusting 0.2 mol l^{-1} ammonium acetate with 1 : 1 ammonia.

The 1.00×10^{-2} mol l^{-1} sodium fluoride solution was used.

2.3. Procedure

2.3.1. Spectral recording

A proper volume of 1.00×10^{-4} mol l^{-1} standard LMFX solution, 1.00×10^{-2} mol l^{-1} standard Tb^{3+} ion solution and 5.00 ml of pH 7.0 NH_4Ac–NH_3 buffer solution were added in a 10 ml calibrated tube with a stopper, then it was diluted up to the mark with water before shaking. After the sample was irradiated under a UV lamp at 365 nm for 30 min (the irradiation intensity was 30 mW cm^{-2}), its absorption spectrum, fluorescence spectrum and room temperature phosphorescence spectrum were recorded. As a contrast, the same experiment was made with the unirradiated sample.

2.3.2. Fluoride measurement

A 1.00 ml of 1.00×10^{-3} mol l^{-1} standard LMFX solution, 1.00 ml of 1.00×10^{-2} mol l^{-1} standard Tb^{3+} ion solution, 5.00 ml of pH 7.0 NH_4Ac–NH_3 buffer solution and 3.00 ml water were added in a 20 ml beaker (the final volume of the sample solution was 10 ml). Then the beaker was placed under the UV lamp and the sample solution was irradiated by 365 nm light for 30 min before adding 0.5 g of NaCl to it to adjust the total ionic strength of the solution to be constant. A fluoride electrode was taken as the indicator electrode and a saturated calomel electrode as the reference electrode to measure the electromotive force of the cell. The concentration of the fluoride was determined by Gran's plot [7]. The same experiment was made with the unirradiated sample.

3. Results and discussion

3.1. Spectroscopic characteristics

The absorption spectra of LMFX and the Tb^{3+}-LMFX systems with and without irradiation are shown in Fig. 1. From the curves 1 and 2, it can be seen that there are two absorption bands for each of them, but the absorption peak at 280 nm for LMFX has a slight red shift to 283 nm for Tb^{3+}-LMFX, and the peak intensity of the latter increases. This suggests that the complexation of the Tb^{3+} ion and LMFX has occurred. From curve 3, we can see that two absorption peak bands at 273 nm and 324 nm for the irradiated Tb^{3+}-LMFX system are observed, which are different from the unirradiated Tb^{3+}-LMFX system. It implies that the absorption property of the Tb^{3+}-LMFX complex has been changed after it was irradiated by 365 nm UV light, i.e., the complex may have undergone a photochemical reaction and some new substances may have been produced in the process.

Fig. 2 shows the fluorescence excitation and emission spectra of LMFX and the Tb^{3+}-LMFX systems with and without irradiation. From the excitation spectra, it can be seen that the maximum excitation wavelengths for LMFX, Tb-LMFX (unirradiated) and Tb-LMFX (irradiated) systems are at 278, 273 and 280 nm, respectively. The changes between the excitation spectra and the absorption spectra of the two Tb^{3+} complex systems may arise from the correlation between excitation wavelengths and the fluorescence quantum yields of the species observed and the instrumental factors. In order to avoid the direct excitation of the terbium ion and the disturbance of the scattered excitation light at these wavelengths, the excitation was performed at the shoulder (320 nm) of the excitation spectrum. From the emission spectra, we can see that the native fluorescence emission wavelength of LMFX is 420 nm and after the Tb^{3+}-LMFX complex is formed, the 430 nm broad band arising from the ligand decreases in intensity greatly while

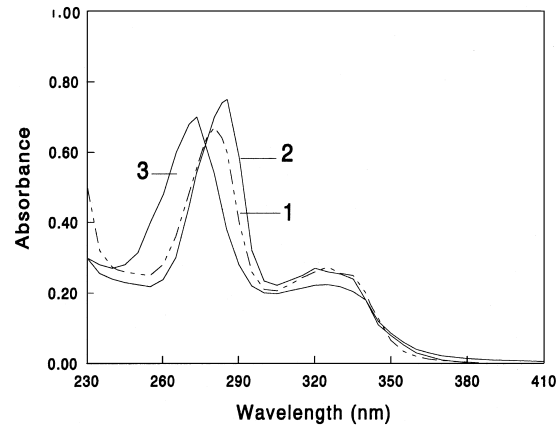

Fig. 1. Absorption spectra of LMFX and Tb^{3+}-LMFX systems in the UV region. $C_{Tb^{3+}} = 2.00 \times 10^{-3}$ mol l^{-1}; $C_{LMFX} = 2.00 \times 10^{-5}$ mol $^{-1}$; pH = 7.0; 1: LMFX; 2: Tb^{3+}-LMFX (unirradiated); 3: Tb^{3+}-LMFX (irradiated).

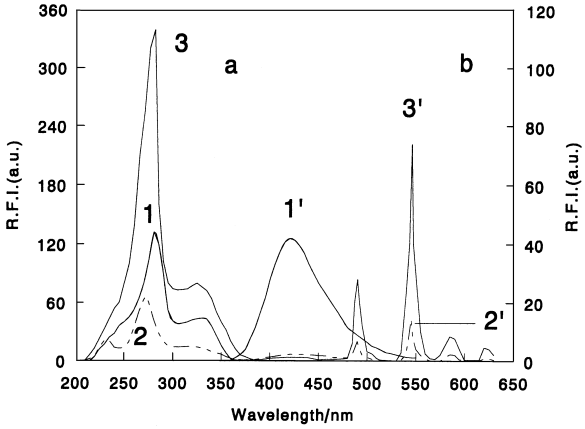

Fig. 2. Fluorescence (a) excitation and (b) emission spectra of LMFX and the Tb^{3+}-LMFX systems. $C_{LMFX} = 5.00 \times 10^{-6}$ mol l^{-1}; pH = 7.0; 1, 2, 3: λ_{em} = 420, 545 and 545 nm, respectively; 1′, 2′, 3′: λ_{ex} = 320 nm; 1′, 2′, 3′: right coordinate; 1, 1′: LMFX; 2, 2′: Tb^{3+}-LMFX (unirradiated); 3, 3′: Tb^{3+}-LMFX (irradiated).

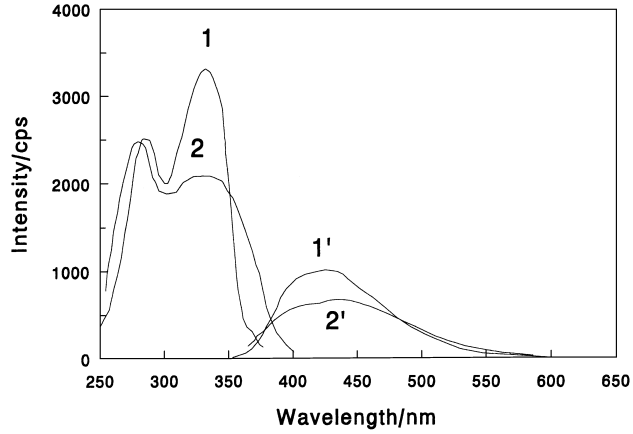

Fig. 3. Phosphorescence excitation (1, 2) and emission (1′, 2′) spectra of LMFX with and without irradiation. $C_{LMFX} = 1.00 \times 10^{-5}$ mol $^{-1}$; pH = 7.0; 1,1′: unirradiated; 2, 2′: irradiated; 1: λ_{em} = 429 nm; 2; λ_{em} = 433 nm; 1′: λ_{ex} = 331 nm; 2′: λ_{ex} = 334 nm.

the narrow emission bands characteristic of the terbium ion appear at 490, 545, 585 and 620 nm, corresponding to the transitions of the Tb^{3+} ion $^5D_4 \rightarrow ^7F_6$,$^5D_4 \rightarrow ^7F_5$ $^5D_4 \rightarrow ^7F_4$ and $^5D_4 \rightarrow ^7F_3$, respectively. Under the same conditions, the emissions of the non-coordinated Tb^{3+} ion itself are very weak. Therefore, it can be concluded that the intramolecular energy transfer has occurred between LMFX and the terbium ion. From the curve 3′, it can be seen that in the irradiated Tb^{3+}-LMFX system the broad band emission at 420 nm arising from the ligand is further decreased and the sensitized fluorescence emission of the terbium ion is enhanced notably. This suggests that the new Tb^{3+} ion complex, the photoproduct, has a higher energy transfer efficiency than the Tb^{3+}-LMFX complex, i.e., the photoproduct is more favorable to intramolecular energy transfer.

Fig. 3. shows the phosphorescence spectra of LMFX systems with and without irradiation. As we can see, the two phosphorescence excitation bands at 282 nm and 331 nm of LMFX shift to 277 nm and 334 nm of the irradiated LMFX system. The phosphorescence emission peak has a red shift from 429 nm to 433 nm after irradiation. It implies that the structure of the irradiated LMFX is different from that of LMFX, and the former has a lower triplet energy.

The phosphorescence spectra of the Tb^{3+}-LMFX systems with and without irradiation are shown in Fig. 4. The two excitation peaks at 292 nm and 331 nm of the Tb^{3+}-LMFX complex shift to 281 nm and 334 nm, respectively, after irradiation. The phosphorescence emission shifts from 425 nm of the Tb^{3+}-LMFX complex to 430 nm, which corresponds to the phosphorescence emission of the ligands. When the phosphorescence spectra were recorded, the delayed time was set to 0.001 ms. In the emission spectra the short-lived fluorescence emissions of the ligands were filtered, but the long-lived fluorescence emissions of the terbium ion appeared at 490, 545, 590 and 620 nm, respectively. It can be seen from Fig. 4. that the sensitized fluorescence of the terbium ion was enhanced markedly after the complex was irradiated.

The fluorescence lifetimes of the terbium ion in the Tb^{3+}-LMFX systems in ammonium acetate–ammonia buffer (pH = 7.0) with and without UV irradiation were measured by pulse fluorescence method. The values obtained were 0.42 ms and 0.04 ms, respectively. The lifetime in the unirradiated system is not very accurate because of its low fluorescence intensity and the instrument limitation for measuring short lifetime. But the results can show that the irradiated system has a longer lifetime than the unirradiated one. This demonstrates further that a new fluorescent substance is produced on irradiation of the Tb^{3+}-LMFX complex. At the same time, the result provides a foundation for the time-resolved fluorescence method.

At room temperature (20°C), the relative fluorescence quantum yields (ϕ_F) of the terbium ion were measured according to Park and Rees' method [8], using quinine

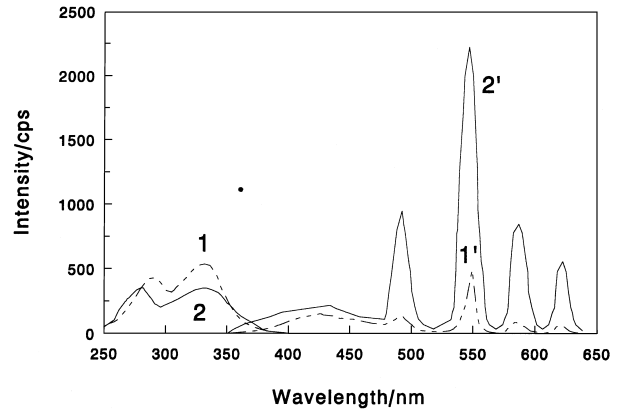

Fig. 4. Phosphorescence excitation (1, 2) and emission (1′, 2′) spectra of Tb^{3+}-LMFX with and without irradiation. $C_{LMFX} = 1.00 \times 10^{-5}$ mol l^{-1}; $C_{Tb^{3+}} = 2.00 \times 10^{-3}$ mol l^{-1}; pH = 7.0; 1, 1′: unirradiated; 2, 2′: irradiated; 1: λ_{em} = 425 nm; 2: λ_{em} = 430 nm; 1′: λ_{ex} = 331 nm; 2′: λ_{ex} = 334 nm.

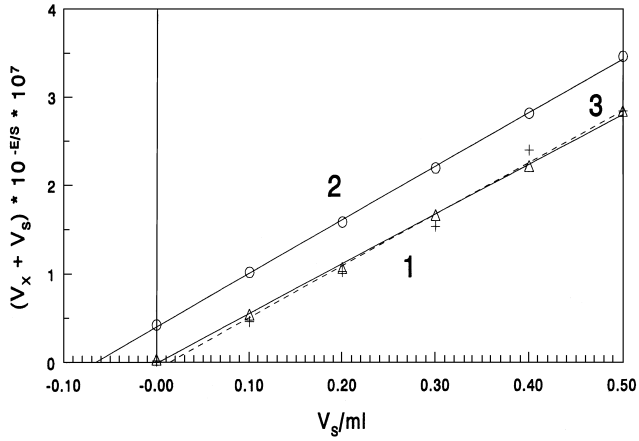

Fig. 5. Graph of fluoride determination. $C_{LMFX} = 1.00 \times 10^{-4}$mol l^{-1}; $C_{Tb^{3+}} = 1.00 \times$ mol l^{-1}; pH = 7.0; 1: unirradiated; 2: irraditated; 3: blank graph – dotted line; V_x: initial volume of sample solution; V_s: volume of standard fluoride solution added; E: potential of fluoride electrode (mV); S: electrode slope (57 mV).

hydrogen sulphate in 0.5 mol l^{-1} sulfuric acid as a standard ($\phi_F = 0.55$), the values measured were 0.0223 and 0.0783, respectively. It is obvious that the value of the irradiated system is much higher than that of the non-irradiated one. This indicates that the new terbium complex facilitates the energy transfer from the ligand to the terbium ion.

3.2. The mechanism of the photochemical fluorescence enhancement of the terbium-LMFX complex

According to the spectral characteristics of the system investigated, it was shown that irradiation of the terbium-LMFX complex changed its molecular structure and a new fluorescent photoproduct was obtained. The characteristics of the photoproduct were investigated.

The first contrast experiment was that under identical conditions the Tb^{3+}-LMFX complex and LMFX itself were irradiated, the Tb^{3+} ion was added to the irradiated LMFX system and the fluorescence excitation and emission spectra of the two systems were recorded. It was found that their spectra were the same. The result indicates that the same structural change for the ligand may have taken place for the two irradiated systems, the irradiated Tb^{3+}-LMFX system and the irradiated LMFX.

As described above in the fluoride measurement, the fluoride contents in the irradiated and unirradiated Tb^{3+}-LMFX systems were measured. The results obtained are shown in Fig. 5.

The results show that there is only a little fluoride ion in the non-irradiated solution (produced by the sunlight irradiation), but about 80% of the F_8 which is selective defluorination from position eight exists in the irradiated solution. This result substantiates that a photochemical reaction occurs indeed to the Tb^{3+}-LMFX complex upon UV light irradiation. This is identical with the photolysis of LMFX itself [9,10]. On this basis, the following contrast experiments were made. An air-equilibrated Tb^{3+}-LMFX solution (in the presence of oxygen) and a nitrogen-flushed solution (in the absence of oxygen) were irradiated and their fluorescence excitation and emission spectra were recorded, as shown in Fig. 6. It can be seen that their excitation spectra are different from each other in shape and intensity, and a new excitation peak appears in the nitrogen-flushed irradiated system at 232 nm. From the emission spectra we can see that the emission intensity at 545 nm is much higher for the air-equilibrated irradiated system. It suggests that the photoproducts may be different from each other for the systems irradiated in the presence of air and under nitrogen atmosphere. From the above findings, it can be deduced that the photoproduct is of the structure of a phenol form in the absence of oxygen, and a quinone form in the presence of oxygen [9].

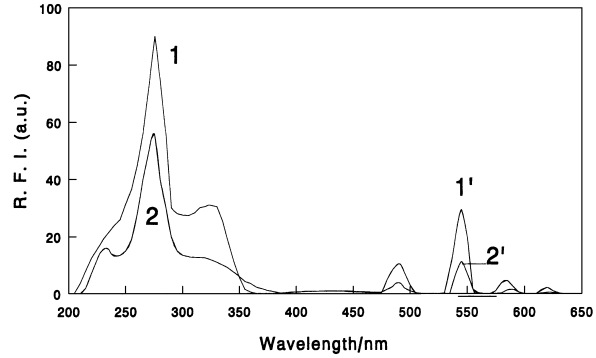

Fig. 6. Fluorescence excitation and emission spectra of Tb^{3+}-LMFX systems irradiated under air-equilibrated (1, 1′) and nitrogen-flushed (2, 2′) conditions. 1, 2: excitation spectra ($\lambda_{em} = 545$ nm); 1′, 2′ emission spectra ($\lambda_{ex} = 320$ nm).

The experimental results have confirmed that the photolysis of the Tb^{3+}-LMFX complex occurs to the ligand, which has a structural change on irradiation ($\lambda = 365$ nm) of the Tb^{3+}-LMFX system when taking terbium chloride as the starting material and using ammonium acetate–ammonia (pH = 7.0) as buffer solution. The photochemical changes of the ligand part are shown schematically in Fig. 7. The ligand part in the photoproducts may have five forms, A, B, C, D and E [9,10]. Among them E has the highest conjugation and a relatively low triplet energy matching the lowest emission energy level $^5D_4$ of the terbium ion. The terbium complex with E contributes to the high efficiency of the intramolecular energy transfer, the increase of the fluorescence quantum yield of the terbium ion and the sensitized fluorescence enhancement.

4. Conclusions

This paper reports the photochemical fluorescence enhancement of the Tb^{3+}-LMFX complex. The Tb^{3+}-LMFX complex irradiated by 365 nm light undergoes the same structural changes as the ligand LMFX itself on irradiation. The terbium complex with the photoproduct

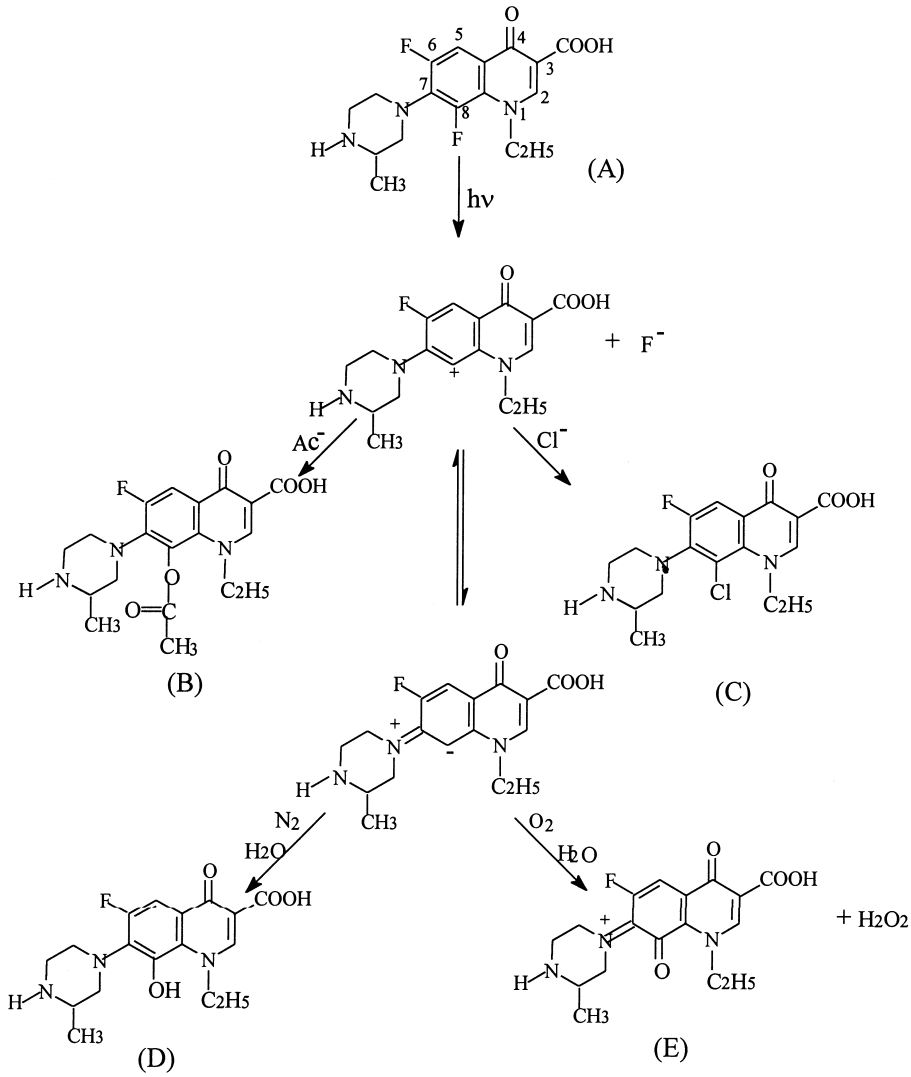

Fig. 7. Photochemical changes of the ligand in the Tb^{3+}-LMFX complex.

can give a good explanation for the fluorescence enhancement of the Tb^{3+}-LMFX system irradiated. This paper provides a simple photochemical method for increasing the intensity of sensitized fluorescence of the lanthanides and a probability for the time-resolved fluorescence technique owing to the long-lived fluorescence of the photoproduct. A high sensitive and selective fluorimetric method for LMFX determination may be established on the photochemical reaction of the Tb^{3+}-LMFX complex.

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