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ORIGINAL PAPER

Optical Properties of Metacycline, Oxytetracycline and Chlortetracycline Europium Complexes in the Presence of Hydrogen Peroxide

Andrea Nastri Grasso · Luciane dos Santos Teixeira · Nilson Dias Vieira Jr. · Lilia Coronato Courrol

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Abstract Tetracycline possesses a great tendency to form complexes with a number of chemical species, particularly with Eu^{3+} ions. In this work we investigate the europium optical properties of three tetracyclines europium complexes: Metacycline (MTc), Oxytetracycline (OTc), and Chlortetracycline (CTc), in the presence and absence of hydrogen peroxide (HP). The results show that the emission band of EuOTc have enhancement in the presence of hydrogen peroxide. A calibration curve was shown for this complex with the best molar ratio obtained.

Keywords Spectroscopy · Fluorescence · Lanthanides complexes · Hydrogen peroxide

Introduction

Tetracyclines were discovered in 1948 as natural fermentation products of a soil bacterium, *Streptomyces aureofaciens*. The first chemically purified tetracycline was Cchlortetracycline, obtained in 1954 [1]. There are three groups of tetracyclines: tetracycline natural products, tetracycline semi-synthetic compounds, and chemically modified tetracyclines [2]. Besides acting as antibiotics, tetracyclines may also affect inflammation, immunomodulation, cell proliferation, and angiogenesis [3–5].

The tetracycline molecular structure is shown in Fig. 1. Among the tetracycline drugs, Oxytetracycline (OTc), Chlortetracycline (CTc) and Doxycycline (DTc) are the most widely used and commercially available. The ring structure of tetracyclines, called the DCBA rings, is surrounded by upper and lower peripheral zones. These contain various chemical functional groups and substituents [6, 7].

Tetracyclines form complexes with multivalent cations [7]. Studies have indicated that chelation can occur at the A ring (tricarbonyl) or BCD ring (phenolic β-diketone) systems [8–10]. In 1982 Richardson [11] verified changes in tetracycline absorption bands in the presence of europium, indicating the ion complexation. Hirschy et al [12] reported in 1984 that when complexed with Eu^{3+}, tetracycline displays the broad absorption band characteristic of an organic ligand. The excited ligand undergoes an intersystem crossing to a triplet state, and then transfers its energy to a 4*f* level within the Eu^{3+} ion, resulting narrow, line-like, luminescence of the Eu^{3+} ion that has a peak intensity ten times greater than the peak intensity of the uncomplexed form.

Rakicioglu et al. [13] observed in 1999 a significant increase in the luminescence of Europium-Tetracycline (EuTc) complex in the presence of hydrogen peroxide (HP). The europium tetracycline complex EuTc was known to show only weak fluorescence with an emission maximum at 617 nm. With the addition of hydrogen peroxide (HP), a strongly fluorescent EuTc:HP complex was formed, displaying a 15-fold increase in the luminescence intensity.

A. N. Grasso · L. d. S. Teixeira · N. D. Vieira Jr.
Centro de Lasers e Aplicações, IPEN/CNEN-SP,
Av. Lineu Prestes 2242 - Cidade Universitária,
São Paulo, SP, Brazil

L. C. Courrol (✉)
Departamento de Ciências Exatas e da Terra,
Universidade Federal de São Paulo—UNIFESP,
Campus Diadema, Rua Prof. Artur Riedel, 275, Bairro Jd,
Eldorado—Diadema, São Paulo, Brazil 09972-270
e-mail: lcourrol@gmail.com

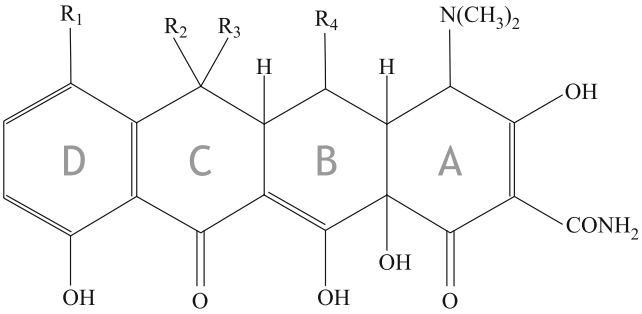

Fig. 1 Chemical structure of tetracycline and table of commonly used tetracycline derivatives

This effect enabled determination and imaging of HP [13–32].

A molecular sensor for hydrogen peroxide is important in the environmental and bioanalytical sciences for a number of reasons. HP is present in small but significant concentrations in the atmosphere [33] and in marine environment [34], and it is widely used in industry [35] and released to the environment in large quantities [36]. H_2O_2 is one of the products of the activity of almost all oxidases [29].

In this work we investigate the europium optical properties in three tetracyclines europium complexes: Metacycline, Oxytetracycline, and Chlortetracycline, in the absence and in the presence of the same concentrations of hydrogen peroxide (HP) and to determine which complex is suitable to be applied as a HP biosensor.

Table 1 Tetracyclines optical properties

Compound	*Tcs* (nm)	*EuTcs* (nm)	*Shift* (nm)
Oxyetracycline (Tc)	249	233	−16
	275	275	0
	355	392	+37
Chlortetracycline (CTc)	351	401	+50
Metacycline (MTc)	348	392	+44

Materials and methods

Materials All inorganic salts used have analytical purity and were obtained from Sigma Aldrich and Molecular Probe. All solutions were prepared in 10 mmol L^{-1} of 3-(N-morpholino) propanesulfonic acid (MOPs from Serva) buffer (pH 3–5). Tetracyclines hydrochloride were obtained from Sigma Aldrich.

Solution I MOPs buffer, 2.09 g of MOPs salt in 1,000 mL of distillated water (the pH was adjust to 6.9).

Solution II 63 μmol L^{-1} solution of Eu^{3+}, 2.29 mg of $EuCl_3.6H_2O$ in 10 mL of solution I(3:1). For molar ratio 1:1 the solution was prepared with 21 μmol L^{-1} solution of Eu^{3+}.

Solution III 21 μmol L^{-1} solution of tetracyclines in 10 mL of solution I.

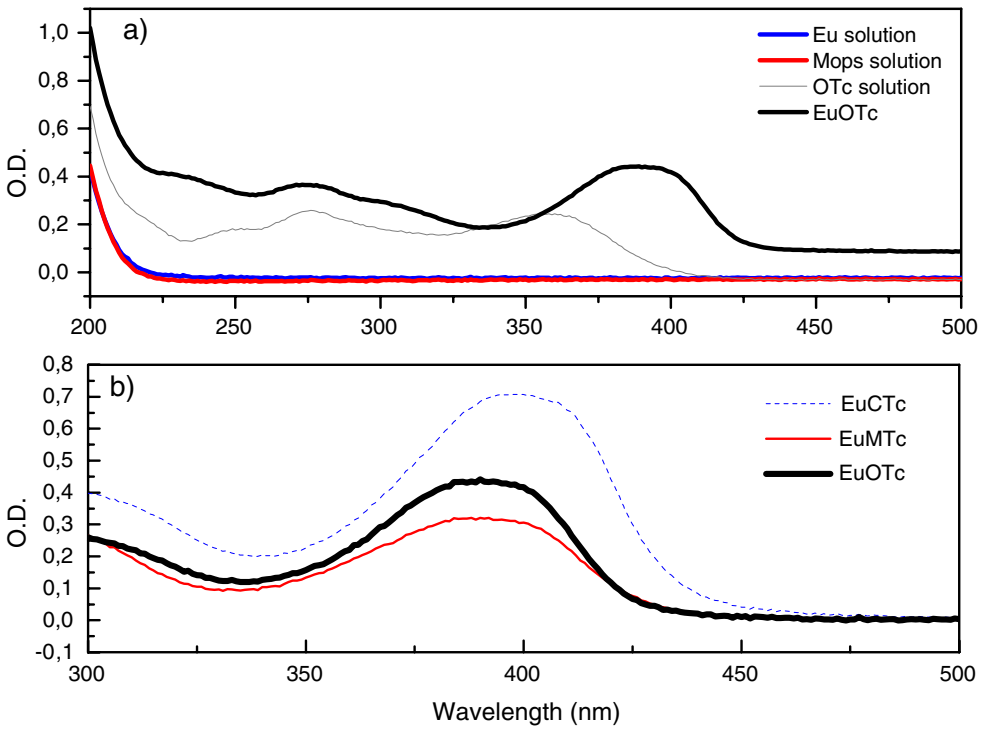

Fig. 2 **a** Optical absorption of Tc, OTc, EuTc, EuOTc, Mops buffer and europium solution; **b** Main absorption band of EuTcs solutions

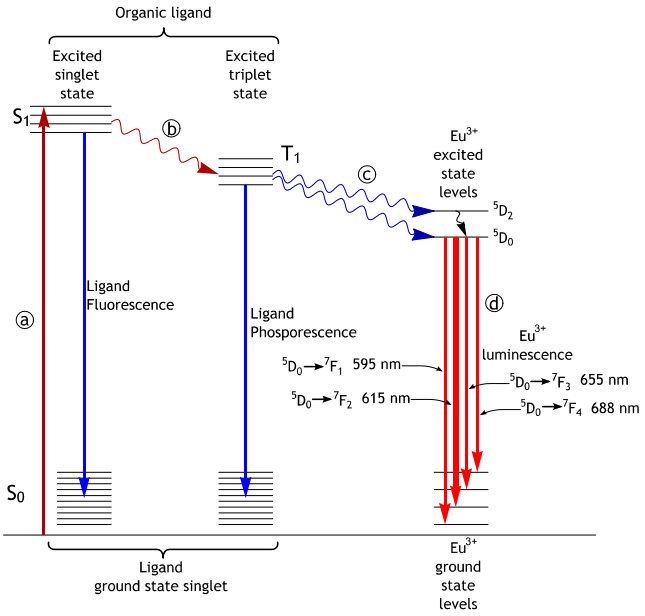

Fig. 3 Fluorescence emission process of europium tetracyclines complexes

Solution IV EuTcs solutions: Mix 10 mL of solution II, 10 mL of solution III and completed with MOPs to 100 mL.

HP Solution 1 mL of 30% HP was dissolved in 10 mL of distilled water. 50 μL of this solution was dissolved to 100 mL with distilled water to obtain solution HP.

EuTcs:HP=1 mL of solution IV was mixed to 500 μL solution HP and 500 μL distilled water to obtain a EuTcs: HP solutions.

To obtaining HP calibration curve it was prepared the EuOTc complex with molar ratio 3.5:1 and the HP concentrations shown in Table 2.

Spectroscopic characterization The absorption spectra at room temperature of the samples in the range 300–460 nm were measured in a Varian Cary 17D spectrometer. The emission spectra were obtained with a modular, fully automated spectrofluorometer Jobin Yvon Fluorolog 3 exciting samples at 400 nm. All measurements were carrying out at room temperature.

Results

Optical characterization of EuTcs complexes

Figure 2 a presents the optical absorption spectra of europium, OTc, and EuOTc solutions prepared in MOPS buffer. In this figure, it can be seen that neither the europium nor the buffer solutions exhibit absorptions from 230 to 500 nm, and that the OTc solution presents absorption bands centered around 249, 275 and 355 nm. When Eu^{3+} is added to the OTc, the 355 nm band is red-shifted to 390 nm, the 249 nm band is blue shifted and the

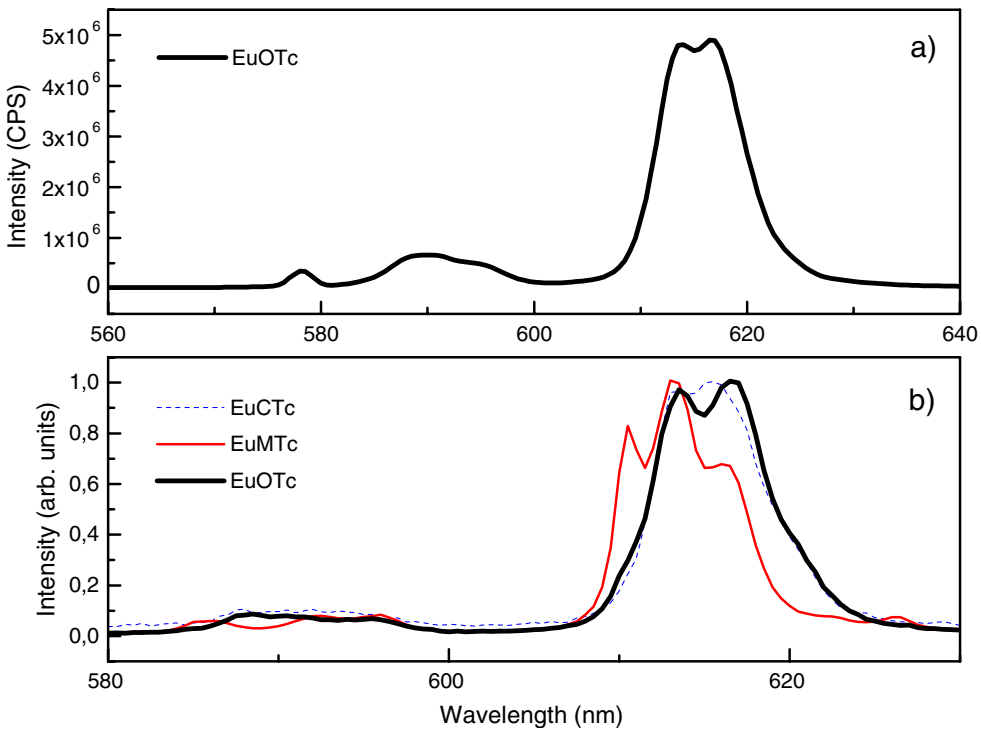

Fig. 4 **a** Europium emission spectra obtained exciting EuTc and EuOTc complexes at 390 nm; **b** Normalized emission spectra obtained for the tetracyclines complexes excited at 390 nm

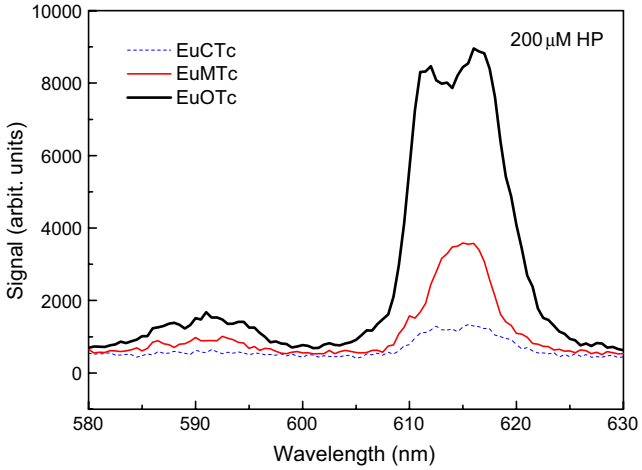

Fig. 5 Emission spectra obtained for the EuTcs complexes in the presence of hydrogen peroxide

275 nm is not affected. The same occurs for the other tetracyclines studied, as can be observed in Fig. 2b and Table 1. The lower energy band experiences the largest red-shift, of approximately 50 nm, in the EuCTc, being displaced to above 400 nm.

When the EuTcs solutions are excited around 390 nm, the fluorescence emission occurs accordingly to the schema shown in Fig. 3: a) the tetracyclines absorb energy ($S_0 \rightarrow S_1$), then proceed to internal conversion; b) intersystem crossing process from the lowest vibrational level of S_1 to the T_1 level take place; c) the Tetracyclines molecules act as antennas, absorbing the excitation light and transferring the energy to the lanthanide ion; d) energy is transferred from the T_1 tetracyclines level to the $^5D_1$ europium ion level, and $S_1 \rightarrow S_0$ and $T_1 \rightarrow S_0$ transitions occur; e) multiple emissions are observed corresponding to the desexcitations $^5D_0 \rightarrow {}^7F_J$ (J=0, 1, 2, 3, 4) and $^5D_1 \rightarrow {}^7F_J$ (J=1, 2, 3, 5, 6); the most intense transitions are the $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_1$ peaking emissions around 610–660 nm and 585–600 nm as can be seen in Fig. 4a. Since the $^5D_0$ level is nondegenerate, no crystal field transitions contribute to the $^5D_0 \rightarrow {}^7F_J$ emission spectra. It is know that, for systems in which there is only one type of Eu^{3+} binding site or only one type of Eu^{3+} complex, the $^5D_0 \rightarrow {}^7F_1$ emission band can split at most into just three components, and the $^5D_0 \rightarrow {}^7F_2$ emission band can split at most into five components. The $^5D_0 \rightarrow {}^7F_2$ transition is an electric dipole allowed one, and its intensity is hypersensitive to variations of the Eu^{3+} ions bonding environment, while the $^5D_0 \rightarrow {}^7F_1$ is a magnetic dipole allowed transition and its intensity hardly varies with the Eu^{3+} ions bonding environment. Figure 4b shows the normalized emission spectra for EuCTc, EuMTc, and EuOTc. We observe that each complex presents a different emission profile. For EuCTc complexes the emission spectra present only one peak around 615 nm, while EuOTc presents two peaks around 613 and 617 nm, and EuMTc presents three peaks at 610, 613 and 617 nm. The intensity ratio (R) between the $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_1$

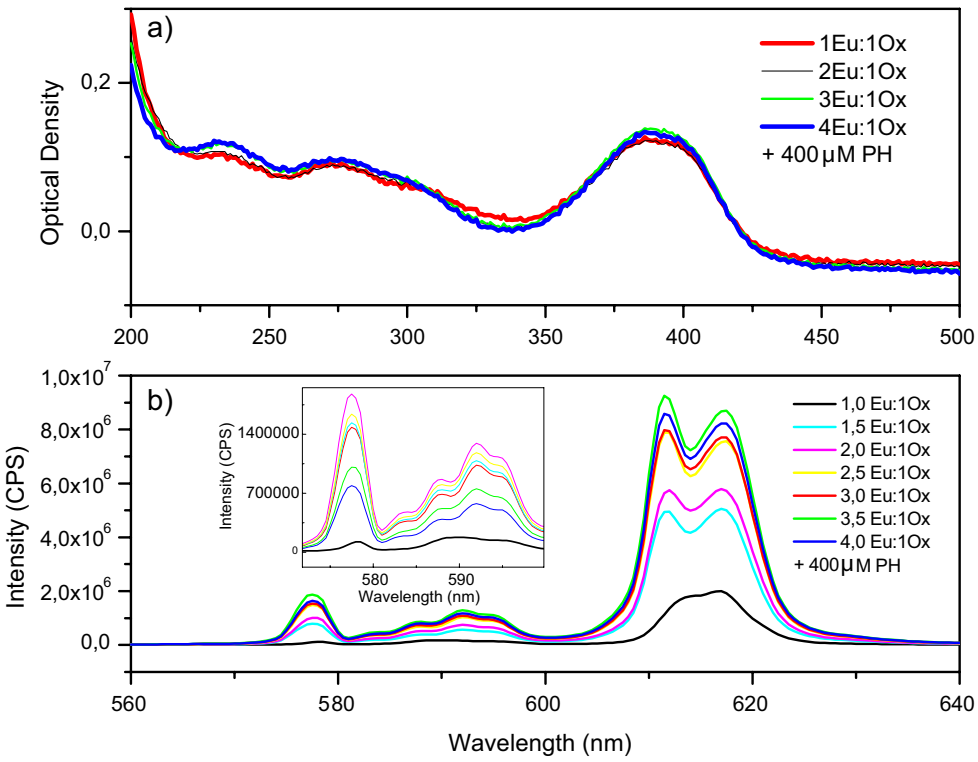

Fig. 6 EuOTc complexe in different molar ratios: **a** Absorption spectra; **b** emission spectra obtained with excitation around 390 nm

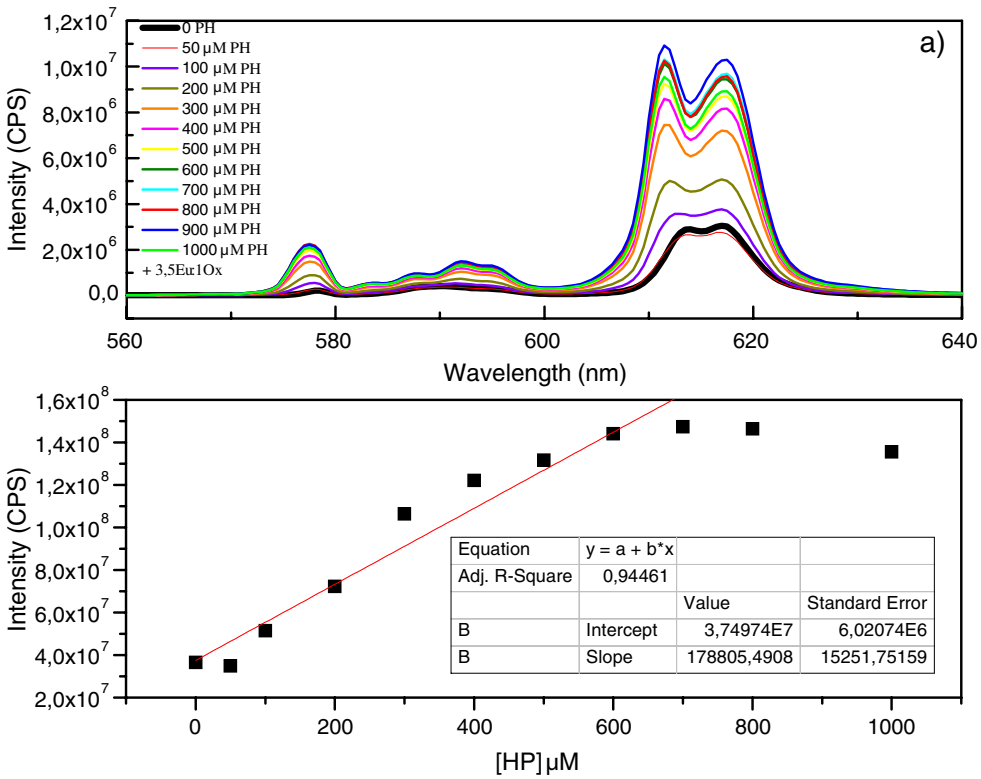

Fig. 7 **a** EuOTc 3.5:1 emission spectra with different HP concentrations and **b** HP calibration curve

transitions increases as the degree of Eu–O covalence increases. Due to these phenomena, the intensity ratio is widely used to investigate the bonding environment of the Eu^{3+} ions. It can be seen in Fig. 4b that *R* remains constant for the four tetracycline complexes studied, evidencing that the degree of Eu–O covalence is similar for all complexes.

Optical characterization of EuTcs complexes in the presence of hydrogen peroxide (HP)

Aiming to compare the changes in the EuTcs complexes fluorescence in the presence of hydrogen peroxide, we performed a study keeping the HP concentration (200 µM) constant. Figure 5 shows comparisons between the europium emission spectra obtained in different EuTcs complexes, in the presence of hydrogen peroxide, and it can be verified that the EuOTc exhibits the highest signal intensity in the presence of hydrogen peroxide.

In order to verify the performance of EuOTc in the presence of hydrogen peroxide, a study was performed starting with the determination of the optimal molar ratio between Eu and OTc in the formation of EuOTc complex in the presence of 400 µM PH. While the absorption spectra does not change significantly with the Eu increase (Fig. 6a), the emission intensity reaches a maximum when the molar ratio is 3.5 Eu:1 OTc, as can be seen in Fig. 6b. In this figure can be seen that the 615 nm emission, corresponding to the $^5D_0 \rightarrow {}^7F_2$ transition, is splitted in two, and the emission in the range between 580–600 nm, corresponding to the $^5D_0 \rightarrow {}^7F_1$ transition, is splitted in 4 bands in molar ratios ranging from 1.5:1 to 4:1, as can be seen in the inset graph. From the $^5D_0 \rightarrow {}^7F_0$ emission spectra (Fig. 6b), it can be seen that the $^5D_0 \rightarrow {}^7F_0$ transition band is blue-shifted as the Eu^{3+} concentration increases. The $^5D_0 \rightarrow {}^7F_0$ transition is an effective probe for the Eu^{3+} bonding environment because the levels $^5D_0$ and $^7F_0$ are not splitted by the crystal field. Due to this, the $^5D_0 \rightarrow {}^7F_0$ excitation and emission spectra also provide information on the covalence of the Eu^{3+} bonding. The results presented here further indicates that the covalence degree of the Eu^{3+} ions increases, revealing the formation of Eu^{3+}–O^{2-} bondings. Similarly to EuTc-HP[31], EuOTc-HP probe is a ternary complex system, in which the H_2O_2 added to the EuOTc

Table 2 EuOTc 3.5:1 complex with different HP concentrations

0 PH	1 mL MOPS + 1 mL 3,5Eu:1Ox
10 HP	10 µL PH + 990 µL MOPS + 1 mL 3.5Eu:1Ox
50 HP	50 µL PH+ 950 µL MOPS + 1 mL 3.5Eu:1Ox
100 HP	100 µL PH+ 900 µL MOPS + 1 mL 3.5Eu:1Ox
200 HP	200 µL PH + 800 µL MOPS + 1 mL 3.5Eu:1Ox
300 HP	300 µL PH + 700 µL MOPS + 1 mL 3.5Eu:1Ox
400 HP	400 µL PH + 600 µL MOPS + 1 mL 3.5Eu:1Ox
500 HP	500 µL PH + 500 µL MOPS + 1 mL 3.5Eu:1Ox
600 HP	600 µL PH + 400 µL MOPS + 1 mL 3.5Eu:1Ox
700 HP	700 µL PH + 300 µL MOPS + 1 mL 3.5Eu:1Ox

replaces water molecules bonded to Eu^{3+} to form a new ligand, without, however, undergoing any significant redox reactions.

Figure 7a shows that the europium emission band in the 3.5Eu:1OTc complex is enhanced with the increase of the hydrogen peroxide concentration (Table 2).The calibration curve for EuOTc:HP, shown in Fig. 7b, is linear for Hp concentrations ranging from 0 to 700 μM, and the detection limit was determined to be 0.25 μM. The obtained results suggest a method for determination of hydrogen peroxide in aqueous solutions using the luminescence enhancement of europium oxytetracycline complex upon hydrogen peroxide biding. Durkop et al [22] presented a detailed study on the use of the europium–tetracycline probe for determination of hydrogen peroxide (HP). HP could be quantified in aqueous solution of pH 6.9 over a 2–400 μM concentration range with a limit of detection of 0.96 μM. They considered the method critically assessed with respect to other common optical methods for determination of HP. We observed similar properties for EuOTc complex, and the concentration range of the determination could be increased.

Conclusions

We can see that, despite all the tetracyclines studied suffer the same process of complexion with europium ions and similar effects in optical absorption, the emission spectra undergoes some changes. The shape of the europium emission band changes according to the tetracycline complex, being splitted into two peaks for the Oxytetracycline, three peaks for the Methatetracycline and remaining at one peak for the Chlortetracycline.

A detailed study on the use of the 3.5Eu:1 OTc ccomplex for determination of hydrogen peroxide (HP) was performed. Data were obtained for the emission spectra of both the EuOTc and its EuOTc- HP complex, and on the effect of stoichiometry between Eu^{3+} and Tc. Also, we were able to determine that HP can be quantified in aqueous solution of pH 6.9 over a 2–700 μM concentration range.

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