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Enhancement of Europium Luminescence in Tetracycline–Europium Complexes in the Presence of Urea Hydrogen Peroxide

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An increase in the europium emission band was observed, for the first time, with addition of urea hydrogen peroxide to the tetracycline–europium (Tc–Eu)solution. We have observed that the wavelength, the band width and the area of $^5D_0 \rightarrow {}^7F_2$ europium transition change with the urea hydrogen peroxide concentration. We claim that the tetracycline–europium complexes can be used as probes of urea hydrogen peroxide concentration.

KEY WORDS: Lanthanides; optical sensor; glucose; urea hydrogen peroxide; tetracycline; energy transfer.

INTRODUCTION

Tetracyclines are drugs characterized by their exceptional chemotherapeutic efficacy against a wide range of Gram-positive and Gram-negative bacteria, and by their great tendency to form complexes with a number of chemical species [1]. Upon complexation with trivalent europium ions, tetracyclines form stable chelates which exhibit broadband absorption spectra and a narrow emission band centered around 617 nm and characteristic of the $^5D_0 \rightarrow {}^7F_2$ transition within the lanthanide ion [2]. Tetracyclines have several proton-donating groups which offer different possibilities of complexation with lanthanide ions depending on the pH. For pH around 7.0, lanthanides are probably bound to oxygen atoms (position 1 in Fig. 1). Chelation sites include the β-diketone system (positions 11 and 12, in Fig. 1) and the enol and carboxamide groups of the A ring [3,4].

A significant increase in the luminescence of lanthanides, particularly, in europium(III) tetracycline complexes was observed in the presence of hydrogen peroxide (HP) [5,6]. In fact, this effect has been used to improve the limits of detection in the determination of the drug tetracycline [7] and has a large potential in biomedicine, since almost all oxidades produce H_2O_2 during their activity [8,9]. It is also the first lanthanide based probe that can be directly excited by 405 nm diode laser.

H_2O_2 is unstable, easily subjected to degenerate into H_2O and O_2, and difficult to monitor in long-term incubation. In contrast, urea hydrogen peroxide (UHP), or carbamide peroxide in its obsolete name, is a stable form of H_2O_2 and a potential cytotoxic agent [10]. In this paper we report the observation that the europium fluorescence intensity is increased when urea hydrogen peroxide is added to the tetracycline-europium aqueous solution. This effect can be used to determine urea hydrogen peroxide levels [11].

MATERIALS AND METHODS

The absorption spectra of all samples were measured at room temperature in the range 200 nm–2500 nm

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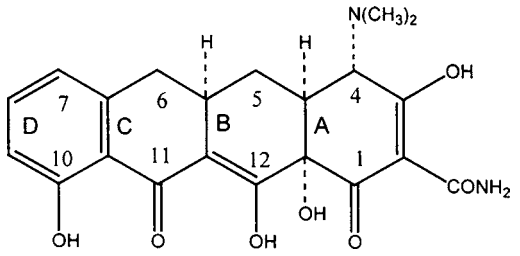

Fig. 1. Tetracycline-HCl structure.

using a Varian Spectrometer Cary 17 D. The emission spectra were obtained by exciting the samples (1 mm thickness) with a 150 W Xenon lamp. The emissions of the samples were analyzed with a 0.5 m monochromator (Spex) and a PMT detector. The signal was amplified with an EG&G 7220 lock-in and processed by a computer. A time-resolved luminescence spectroscopy technique was employed to measure the luminescence decays induced by resonant laser excitations. The excitation system consists of a tunable optical parametric oscillator (OPO from OPOTEK) pumped by the second harmonic of a Q-switched Nd-YAG laser from Quantel. This laser system delivers pulses of 10 mJ with time duration of 4 ns and repetition rate of 10 Hz. The time-dependence luminescence of the europium was detected by a S-20 Hammamatsu PMT and analyzed using a 200 MHz Tektronix TDS 410 digital oscilloscope. The relative errors in the emission measurements are estimated to be <5%, while errors in the lifetime measurements are <10%.

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} 3-(N-morpholino) propanesulfonic acid (Mops from Carl Roth, Germany) buffer (pH 6.9). Tetracycline-HCl used was a secondary pattern gently provided by Bunker Indústria Farmacêutica Ltda. Urea hydrogen peroxide 98% used in this work was obtained from Aldrich.

Solution I: Mops buffer, 544.1 mg of Mops salt in 200 mL of distillated water (pH = 6.9).

Solution II: 63 μmol^{-1} solution of Eu^{3+}, 2.3 mg of $EuCl_3$ $6H_2O$ in 10 mL solution I.

Solution III: 21 μmol^{-1} solution of tetracycline, 1.0 mg of tetracycline in 10 mL of solution I.

Solution IV: Tc–Eu solution, Mix 10 mL of solution II and 10 mL of solution III.

Solution V: Tc–Eu – hydrogen peroxide (HP) or urea hydrogen peroxide (UHP) solution, Mix 10 mL of solution II and 10 mL of solution III on 8 μmol/L HP or variable concentrations of UHP.

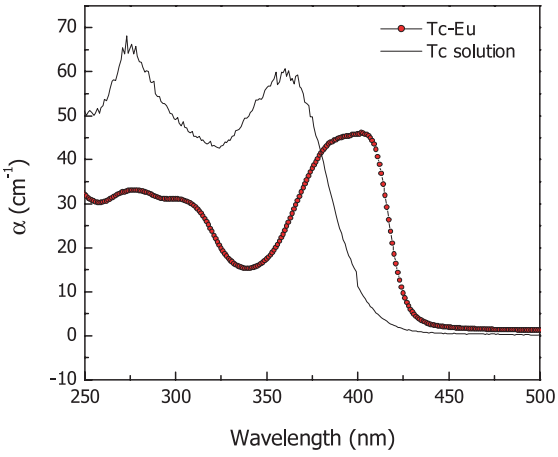

Fig. 2. Optical absorption of Tc–Eu complexes.

RESULTS

The optical absorption spectra of Tc and Tc–Eu solutions are shown in Fig. 2, where the *Y*-axis is the absorption coefficient $\alpha(\text{cm}^{-1})$ given by:

$$\alpha = \frac{2.303 \times \text{OD}}{d}$$

where OD is the optical density measured and d is the cuvette thickness. The absorption band (with peaks at 362 nm and 402 nm) is caused by the presence of the tetracycline ligand which, in its uncomplexed form, has a slightly blue shift absorption spectrum.

The effect of pH on the fluorescence intensity of Tc–Eu was measured (Fig. 3). The emission intensity of Tc–Eu is strongest at pH 6.6–7.2, and rapidly drops outside this range. The fluorescence and excitation spectra of Tc–Eu and Tc–Eu–HP and Tc–Eu–UHP are shown in the Fig. 4. The Tc–Eu complexes emission spectra were obtained exciting the samples with the Xenon lamp at 400 nm. As in other complexes of this type, the energy absorbed by the ligand is transferred from the triplet state of the ligand to the central Eu^{3+} ion with its typical emission spectra [12] of a main band which peaks at 617 nm ($^5D_0 \rightarrow {}^7F_2$) and several side bands centered at 579, 597, 654, and 688 nm, respectively. It is known that the quantum yield of Tc–Eu–Hp is 4.0%, while that of Tc–Eu is 0.3%, with tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate (TBDRH) used as reference [13]. An increase in the europium emission band was observed, for the first

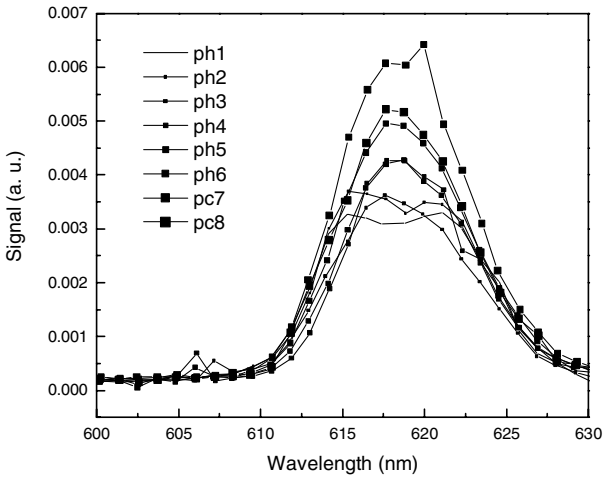

Fig. 3. Effect of pH on the emission intensity of Tc–Eu complexes. Each point represents an addition of 0.1 mL alkali. The pH = 6.9 results in the greatest increase in the emission.

time, with addition of urea hydrogen peroxide (UHP) to the solution, as can be seen in Fig. 4. The shape of the emission band and a shift to the blue region was observed compared with Tc–Eu solution. The fluorescence intensity of Tc–Eu–UHP is up to six times that of Tc–Eu for the best concentration condition. The appearance of two-peaks observed in Tc–Eu–HP (inside graphic in Fig. 4) indicates change of the crystal field at the europium ion site. In Tc–Eu–UHP complex the two peak structure appear only at higher concentrations. The HP and UHP both replace at least one water molecule binding to europium. Also a conversion of tetracycline into its fluorescent *iso* or anhydro compound was probably followed by a destruction of the tetracycline molecule. This hypothesis seems true since an uncolored solution is observed several hours after the complex Tc–Eu–UHP preparation.

Figure 5 shows the effect of UHP concentration in the Tc–Eu emission spectra. An increase in the UHP concentration results in an enhancement of the fluorescence. We notice that the wavelength, width and area of $^5D_0 \rightarrow {}^7F_2$ europium transition depend of UHP concentration variation, as can be seen in the Fig. 6.

The decay profiles of the Tc–Eu, Tc–Eu–HP and Tc–Eu–UHP complexes are shown in Fig. 7. Generally, complex systems may have multiple fluorescent species, and hence the fluorescence intensity decay cannot be fitted to a single exponential function. One needs to use a multi-exponential function and in that case, the fluorescence intensity decay equation becomes:

$$y = A_1 e^{(-t/\tau_1)} + A_2 e^{(-t/\tau_2)} \qquad (1)$$

where A_i and τ_i are the ith pre-exponential factor (amplitude) and the lifetime in the multiexponential decay, respectively. In the case of multiexponential decays the

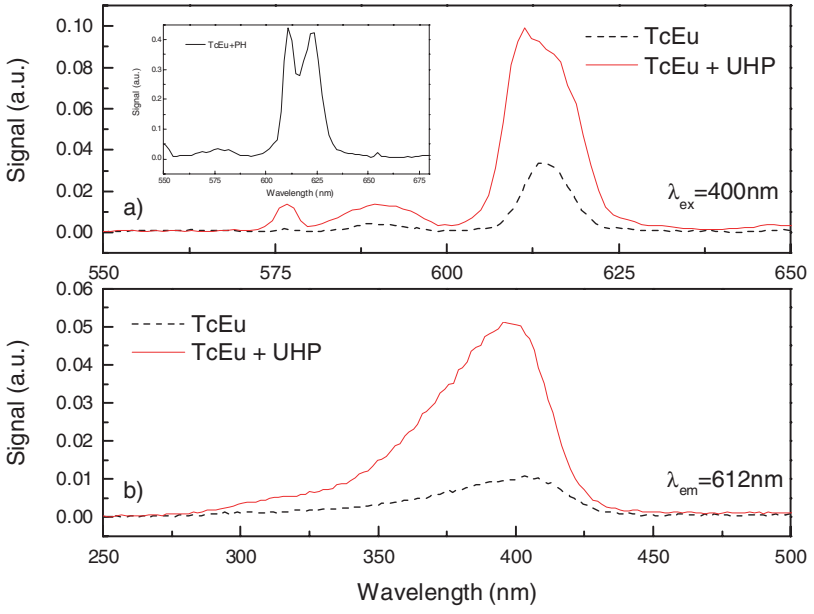

Fig. 4. (a) Emission spectra of Tc–Eu (solution IV) and Tc–Eu–UHP (solution V with 0.01 mL urea hydrogen peroxide) and in inside figure Tc–Eu–HP (solution V with 0.01 mL of hydrogen peroxide). Emission spectra were obtained exciting the samples at 400 nm. (b) Excitation spectra of Tc–Eu, and Tc–Eu–UHP solutions acquired fixing emission at 612 nm.

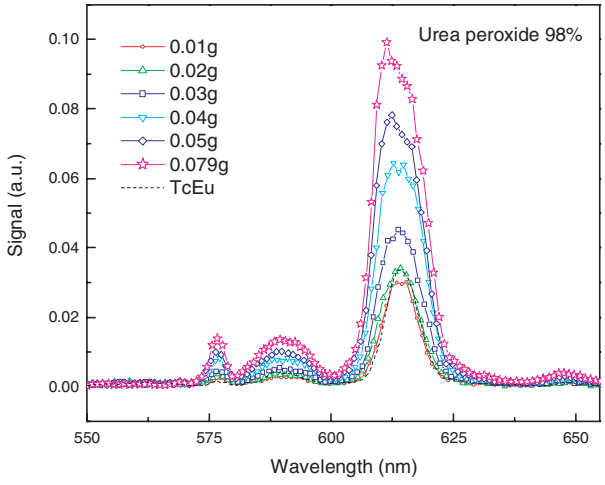

Fig. 5. Urea hydrogen peroxide concentration effect on Tc–Eu–UHP complex emission spectra.

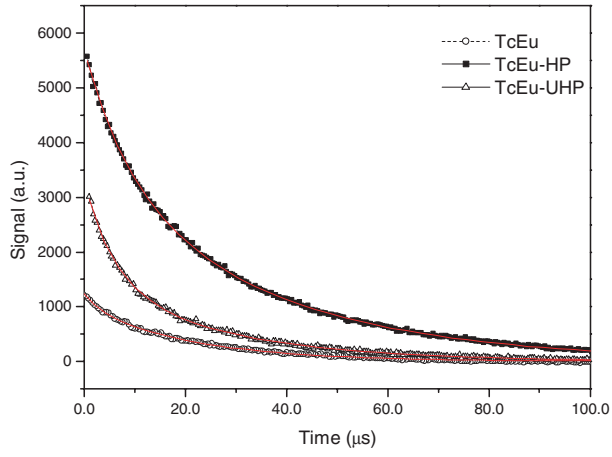

Fig. 7. Decay profile of the Tc–Eu probe and its complex with hydrogen peroxide (Tc–Eu–HP) and urea hydrogen peroxide (Tc–Eu–UHP).

average lifetime, which is proportional to the total area under the fluorescence decay curve is defined by:

$$\tau_{av} = \frac{\sum_i A_i \tau_i^2}{\sum_i A_i \tau_i} \tag{2}$$

The parameters obtained by fitting Eq. (2) to the decay curves are shown in Table I. It is possible to observe that the average lifetimes of the samples are 21 μs, 33 μs and 26 μs for TcEu, Tc–Eu–HP and Tc–Eu–UHP, respectively. The results show that the fluorescence of the Tc–Eu–HP and Tc–Eu–UHP systems are least quenched by molecular oxygen than Tc–Eu complex.

Urea hydrogen peroxide (UHP) is a stable form of HP and cytotoxic agent. The renal and cardiac levels of UHP are closely correlated with the levels of renal and cardiac pentosidine produced from Maillard Reaction [14]. Both UHP and HP have potential deleterious effects on various

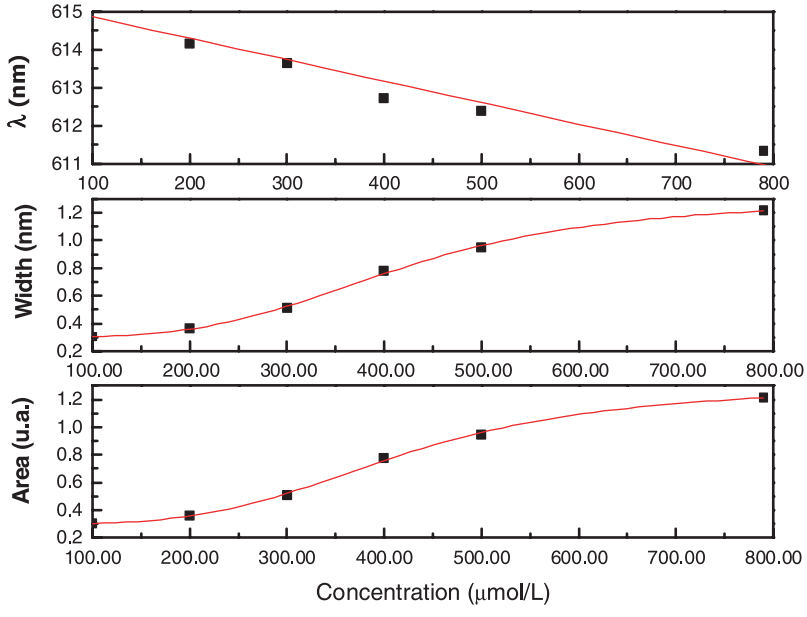

Fig. 6. Calibration of UHP concentration (μmol/L) in function of maximum wavelength peak, emission band width and emission area.

Table I. Lifetime Composition of Tc–Eu, Tc–Eu–HP, and Tc–Eu–UHP

Tc–Eu complexes	Parameters	Errors (±)	Amplitude (%)	τ_{av} (μs)
Tc–Eu				
A1	216.58	15.00	17.7	20.83
$\tau 1$ (μs)	3.49	0.40		
A2	1006.39	14.55	82.3	
$\tau 2$ (μs)	20.90	0.24		
Tc–Eu–HP				
A1	2149.87	34.91	37.7	33.48
$\tau 1$ (μs)	8.80	0.19		
A2	3548.18	38.65	62.3	
$\tau 2$ (μs)	34.00	0.22		
Tc–Eu–UHP				
A1	1405.15	23.32	53.7	25.58
$\tau 1$ (μs)	5.72	0.13		
A2	1527.16	26.16	46.3	
$\tau 2$ (μs)	26.08	0.29		

cells, including those of kidney and heart. The recognition that chronic renal failure is related to the increased UHP and glycoxidation in renal and cardiac matrix is expected to manage these toxins to delay functional damage of the heart and destroying kidney for predialysis patients or to accelerate the removal by developing more effective dialysis procedures for patients under dialysis [14]. We claim that the Tc–Eu complexes can be used as probes concentration for UHP for analysis of body fluids.

CONCLUSIONS

Tc–Eu complexes were studied and showed lanthanide emission. An enhancement and shift of Eu emission with introduction of urea hydrogen peroxide in Eu–Tc solution, suggests a new method for determination of urea in aqueous solutions using the luminescence enhancement of europium tetracycline complex upon biding of urea hydrogen peroxide. We claim that the Tc–Eu complexes can be used as probes of UHP concentration.

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