Spectroscopic Study of Tetracycline - Lanthanides Complexes for biomedical applications

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ABSTRACT

Compared with the traditional organic fluorescent materials, the lanthanide coordination compounds have a large variety of applications, for example glucose sensing, based on the luminescence enhancement of a lanthanide-tetracycline complex due to enzymatically generated H_2O_2 at physiological glucose concentrations. It is known that the fluorescence is based on the energy-transfer from the ligand to the central lanthanide ion. The aim of this work was to study the optical properties of Europium, Erbium, Ytterbium, Holmium, Terbium and Neodymium tetracycline complexes. An increase in europium emission band was observed for the first time, with addition of urea peroxide in the solution. This method works at neutral pH and the luminescence was detected at visible lanthanides luminescence after a 10 min. incubation time of the samples.

Keywords: Lanthanides, optical sensor, glucose, urea, tetracycline, energy transfer.

INTRODUCTION

The luminescence properties from trivalent lanthanides ions (Ln^{3+}) in aqueous solution have been studied with purpose of developing new markers for chemical and biochemical applications[1]. Although they have weak absorption coefficients, it is possible to get highly luminescent lanthanides complexes chelating this ions in strongly absorbing ligand as the tetracycline structure. Tetracyclines have several proton-donating groups which offer different possibilities of complexation with lanthanide ions depending on the pH [2]. In these systems, intense ion luminescence originates from the intramolecular energy transfer through the excited state of the ligand to the emitting level of Ln^{3+} ion[3].

Sensitized 4f luminescence in lanthanides complexes is efficiently used also as alternative to organics fluorophores, particularly where there are problems of background auto-fluorescence due their spectral characteristics. These characteristics, include microsecond and millisecond lifetime, sharply spiked emission spectrum (<10 nm), large Stokes-shift, no self-quenching, high quantum yield for lanthanides luminescence (~1) and excellent solubility[4].

A significant increase in the luminescence of lanthanides particularly in Europium (III) tetracycline complexes, is observed in the presence of hydrogen peroxide (H_2O_2)[5]. In fact, this effect has been used to improve the limits of detection in the determination of the drug tetracycline, but appears to have an even larger potential in biomedicine since almost all oxidase produce H_2O_2 during their activity[6]. It is also the first lanthanide based probe that can be excited by 405nm diode laser.

In this work we report the optical properties of tetracycline – lanthanide ions (europium, terbium, ytterbium, neodymium, holmium and erbium) complexes. Since the absorption spectra of these lanthanides occurs in visible, IR and near infrared range, studying tetracycline-lanthanide-complexes (LnTc) is possible to map the best conditions to get an efficient complex formation. Optical parameters like, absorption and emission cross section, Ln lifetime, refractive index, density and energy transfer probability were obtained.

We report also the optical properties of tetracycline–europium (EuTc) which increased when urea peroxide is added to the tetracycline-europium aqueous solution. It was conceived that this enhancement can be used to determine urea. Urea is primarily produced in the liver and secreted by the kidneys and is the major end product of protein

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catabolism in animals. It is the primary vehicle for removal of toxic ammonia from the body. Urea determination is very useful for the medical clinician to assess kidney function of patients. In general, increased urea levels are associated with nephritis, renal ischemia, urinary tract obstruction, and certain extra renal diseases, congestive heart failure, liver diseases and diabetes. Decreased levels indicate acute hepatic insufficiency or may results from over-vigorous parenteral fluid therapy[7].

MATERIALS AND METHODS

The absorption spectra of all samples were measured at room temperature in the range 200nm-2500nm using a Varian Spectrometer Cary 17 D. The emission spectra were obtained by exciting the samples with 300 Watts 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.

To determine the luminescence decays induced by resonant laser excitations was used an excitation system that consists of a tunable optical parametric oscillator (OPO from OPOTEK) pumped by the second and third harmonics of a Q-switched Nd-YAG laser from Quantel. This laser system delivers pulses of 20mJ with time duration of 4ns and repetition rate of 10Hz, and can be tuned from $0.41\mu m$ to $2.0\mu m$. The time-dependence luminescence of the acceptor was detected by S-20 PMT and analyzed using a signal-processing box-car averager (PAR 4402), or a 200MHz 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%.

Refractive indexes were obtained with a Zeiss Abbe refratometer and densities were obtained by Archimeds method.

Materials.

All inorganic salts were of 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. Tetracycline-HCl used was a secondary pattern gently obtained by Bunker Indústria Farmacêutica Ltda. Urea peroxide 16% used in this work is a composition of 16% Urea Peroxide, 3% Potassium Nitrate and 0,11% Fluoride from F & A Laboratório Farmacêutico Ltda.

Solution I: a 10 mmol l^{-1} 3-(N-Morpholino) propanesulfonic acid (Mops from Carl Roth, Germany) buffer. 23 g of Mops salt in 100 ml of distilled water with NaOH to adjust the PH to 6.9.

Solution II: 2.1 mmol^{-1} solution of Ln^{3+} (Eu, Tb, Er, Ho, Nd, Yb). Correspond mass in grams of $LnCl_3.6H_2O$) in 10 ml of Mops buffer solution.

Solution III: 2.1 mmol^{-1} solution of tetracycline. 50.5mg of tetracycline in 50mg of tetracycline hydrochloride in 10 ml of Mops buffer solution.

Solution IV: LnTc solution. Mix 10 ml of solution II and 10 ml of solution III.

RESULTS

Optical absorptions of all LnTc solutions were measured and are shown in figure 1. In this figure we observe that the studied lanthanide, Europium (Eu), Erbium (Er), Neodymium (Nd), Terbium (Tb) and Ytterbium (Yb), can form complexes with tetracycline molecule. In all LnTc complexes, tetracycline absorption band shifts to red when perturbed by lanthanides ions. YbTc complex has the lower shift, ~5 nm, and EuTc complex has the higher shift ~40 nm. The concentrations of lanthanides ions are extremely small and only the tetracycline band is observed in the absorption spectra. The emission of tetracycline occur around 520 nm (figure 2), and this excitation must be transferred to lanthanides ions that emit light with their main properties. The studied lanthanides, Eu, Er, Ho and Nd have absorption bands in the range of tetracycline emission, and Tb and Yb absorption bands have small resonance with this emission band.
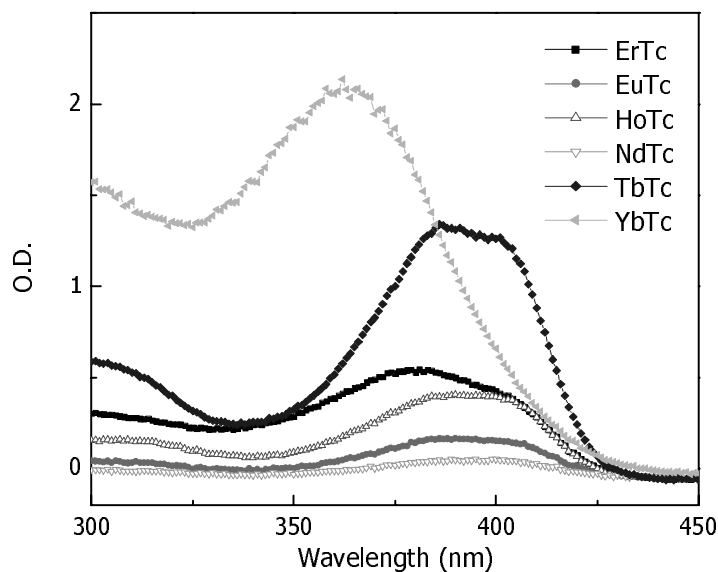
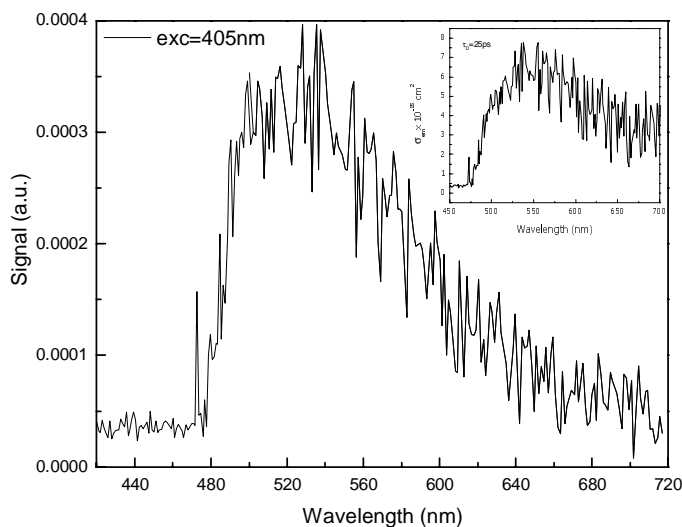Figure 1. Optical absorption of LnTc complexes.

Figure 2. . Emission of Tetracycline excited at 405 nm. Inside figure represents de emission cross section obtained for this emission and a emission lifetime of 25 ps.

The refractive index of each sample was also measured and the results are shown in table 1. Studying the refractive index dependence on the lanthanide concentration, we took a higher concentrated NdTc complex, prepared containing always the same volume, controlled adding solution II in complementary parts in the solution IV, keeping a volume of 1ml for each sample. NdTc complex refractive index values were plotted in a graph as a function of

absorption coefficients (α) of each studied sample and shown in figure 3. We can observe for the refractive index a linear dependence on the Nd concentration (α is proportional on concentration). This linear behavior can be explained considering that the enlargement of Nd concentration, promote an enlargement on solution density and consequently a loss of light velocity, increasing the refractive index.

Complex	Composition	Refractive index
Europium		
EuTc (yellow)	$EuCl_3.H_2O$ + Tetracycline +MOPS	1,3385
EuTc (red)	$EuCl_3.H_2O$ + Tetracycline +MOPS +uv ligth	1,3465
EuTcPH (orange)	$EuCl_3.H_2O$ + Tetracycline +MOPS + H_2O_2	1,3387
EuTcPC (yellow)	$EuCl_3.H_2O$ + Tetracycline +MOPS + urea peroxide	1,3565
EuTcEDTA (yellow)	$EuCl_3.H_2O$ + Tetracycline +MOPS +EDTA	1,3370
Holmium		
HoTc (dark yellow)	$HoCl_3.H_2O$ + Tetracycline +MOPS	1,3380
Terbium		
TbTc (yellow)	$TbCl_3.H_2O$ + Tetracycline +MOPS	1,3376
Ytterbium		
YbTc (brown)	$YbCl_3.H_2O$ + Tetracycline +MOPS	1,3370
Neodymium		
NdTc (yellow)	$NdCl_3.H_2O$ + Tetracycline +MOPS	1,3396

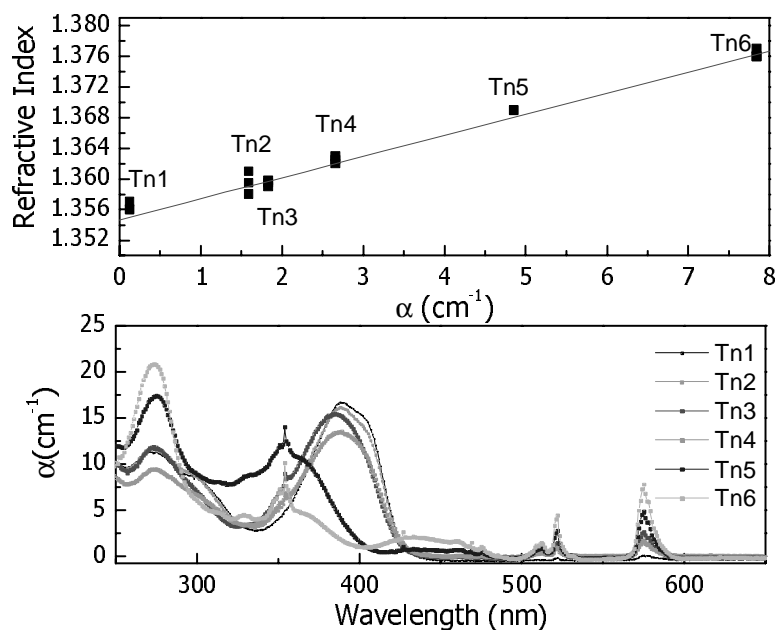Table 1. Refractive indexes of studied Ln-Tc complexes.

Figure 3. Dependence of refractive index of NdTc complexes with Nd absorption coefficients.

The LnTc complexes emission spectra were obtained exciting the samples with a Xenon lamp and a 405 nm line filter. Only the EuTc complex showed a strong red emission at 619 nm which intensity is a function of pH, how we can see in figure 4. As is known, the addition of hydrogen peroxide in the pH controlled solution results in a grown in intensity of emission band. A corrected pH concentration (7,0) was mapped by fluorescence lifetime measurement (figure 5), adding a controlled volume of NaOH in the solution IV. It was observed a little shortening in fluorescence lifetime (40μs to 20μs) with growing in pH.
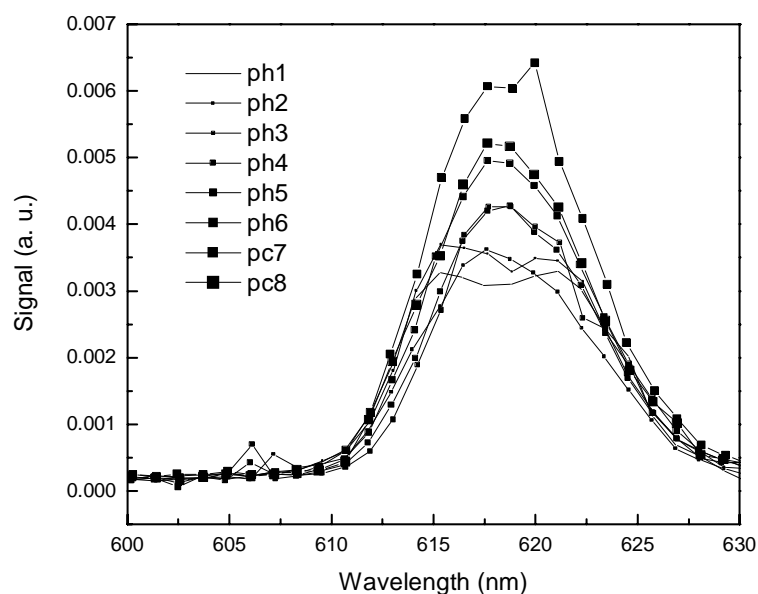

Figure 4. Effect of pH on the emission intensity of EuTc complexes. Each point represents an addition of 0.1ml alkali with regard to before one.
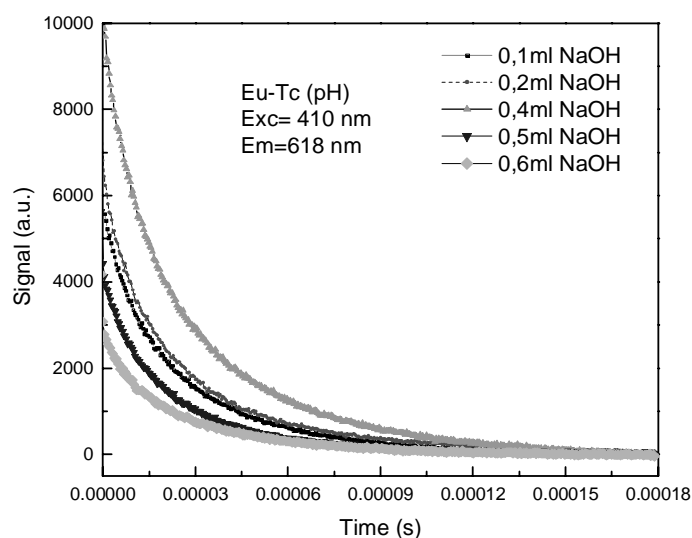

Figure 5. Mapping of best pH intensity condition for EuTc complex by fluorescence lifetime.

With the ideal pH condition was observed, for the first time, an increase in europium luminescence with addition of urea peroxide in the solution, as we can see in figure 6. The shape of the emission band and a shift for the blue region with the composition of the solutions, revealing that the Eu ion can occupies different sites in the molecule structure.
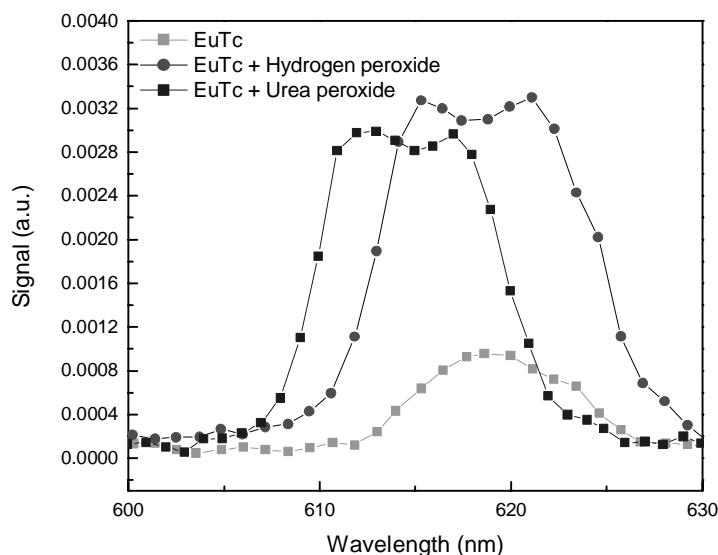

Figure 6. Effects of Europium emission band on composition of the complexes.

The intense europium luminescence originates from the intramolecular energy transfer through the excited state of the tetracycline to the emitting level of Eu^{3+}. The process of resonant energy transfer takes place when a donor in an electronically excited state transfers its excitation energy to a nearby, the acceptor. If the fluorescence emission spectrum of the donor molecule overlaps the absorption spectrum of the acceptor ion, and the two are within a minimal spatial radius, the donor has a high probability of transferring its excitation energy to the acceptor through long-range dipole-dipole intermolecular coupling. A theory proposed by Theodor Förster[8] in the late 1940s initially described the molecular interactions involved in resonance energy transfer, Förster also developed a formal equation defining the relationship between the transfer rate, inter-chromophores distance, and spectral properties of the involved chromophores.

Using the resonant energy transfer theory, a non-radiative quantum mechanical process that does not require collisions and as consequence production of heat, for tetracycline-europium (EuTc) complex we obtained several important parameters. The microparameter (C_{DA}) involved in the energy transfer from the excited states of donor (Tc) to the acceptor (Eu^{3+}) can be calculated by:
$$C_{DA} = \frac{R_{c(D-A)}^6}{\tau_D}$$

where τ_D is the total lifetime of the donor state without the presence of the acceptor. The critical radius R_C is calculated using the overlap integral method based on the calculation of the emission (donor) and the absorption (acceptor) cross-section overlap using the following expression valid for a resonant energy transfer:

$$R_c^6 = \frac{6c\tau_D}{(2\pi)^4 n^2} S(\lambda)$$

where c is the speed of light, n the refractive index of the medium, and $S(\lambda)$ is the overlap integral between the emission and absorption cross sections of donor and acceptor (4.88×10^{-32} cm^2), respectively, calculated for *D-A* transfer. The emission cross-section of Tc was obtained from the emission shown in figure 2.:

$$\sigma_e(\lambda) = \frac{\lambda^4}{n^2 8\pi c \tau_R} \frac{SE(\lambda)}{\int SE(\lambda)d\lambda}$$

where n is refracticve index of the EuTc complex, $SE(\lambda)$ is the emission spectra, τ_R is radiative lifetime (considered ~10ns dipole-dipole permitted transition) and the τ_D donor emission lifetime of 25 ps[9].

The absorption spectra of a EuCl$_3$.H$_2$0 solution (solution II) was measured and absorption cross section of Eu^{3+} (~5 x 10^{-18} cm^2 , $\sigma_{abs} = \alpha/[Ln^{3+}]$) was estimated. The observed overlap predicts an efficient resonant energy transfer from Tc to Eu^{3+}. The microscopic parameter of energy transfer obtained using the overlap integral calculation was C_{DA}~3.15x 10^{-31} cm^6/s an R$_c$~14.1 $\overset{\circ}{A}$.

For these values it was possible to calculate the energy transfer probability (3.9 x10^{10} s^{-1}) given by:

$$W_{DA} = \frac{C_{DA}}{R_c^6}$$

With these parameters we obtained a dipole-dipole energy transfer efficiency of only 0.015%. We consider that the electron exchange mechanism of energy transfer must be more effective that dipole-dipole mechanism in EuTc complex. For exchange mechanism, with 50% luminescence quantum yields we obtained $R_C = 2.6 \overset{\circ}{A}$.

CONCLUSIONS

The complexes LnTc were studied and only EuTc complex showed a high lanthanide emission. This effect can be attributed to the branching ratios of the studied lanthanides, Er^{3+}, Ho^{3+}, Nd^{3+}, leading a non-radiative emissions or the non-resonance with tetracycline emission band (Tb^{3+} and Yb^{3+}).

A study of refractive index of solutions was made showing that this parameter is very important in the estimative of lanthanides concentration.

The energy transfer between Tc and Eu was studied and an interaction critical radius of 14.1 $\overset{\circ}{A}$ for dipole-dipole transfer was obtained. For exchange mechanism the calculated critical radius was 2.6 $\overset{\circ}{A}$

Finally the enhancement of Eu emission with introduction of urea peroxide in EuTc solution suggest a new method for determination of urea in aqueous solutions using the luminescence enhancement of europium tetracycline complex upon biding of urea peroxide.

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