(Tetracycline)europium(III) Complex as Luminescent Probe for Hydrogen Peroxide Detection

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Dedicated to Professor *Jean-Claude Bünzli* on the occasion of his 65th birthday

Luminescence spectra of aqueous solutions containing a fixed concentration of tetracycline (TC) and increasing concentrations of Eu^{3+} were recorded both in the absence and presence of hydrogen peroxide (H_2O_2). In H_2O_2-free solutions in which the Eu/TC molar ratio was varied from 1:1 to 8:1, the $^5D_0 \rightarrow {}^7F_0$ transition consisted of only one peak at 580 nm. In the presence of H_2O_2, an extra peak appeared in the spectrum at 578 nm when the Eu/TC molar ratios were above 2.5. A detailed analysis of this spectral region revealed that at lower Eu/TC molar ratios (up to 2:1), the $^5D_0 \rightarrow {}^7F_0$ transition experienced a slight blue shift. This indicates that at low Eu/TC molar ratios, the presence of H_2O_2 leads to two different environments of the trivalent europium ions, which most likely form bridged peroxide complexes with hydrogen peroxide (μ-H_2O_2 ligand). Luminescence spectra measured in the presence of molybdate ions, which catalytically decompose H_2O_2, led to the disappearance of the extra europium(III) site that was formed in the presence of H_2O_2. The intensity of the hypersensitive $^5D_0 \rightarrow {}^7F_2$ transition did not linearly depend on the H_2O_2/TC molar ratio. For H_2O_2/TC ratios up to 10, a sharp linear increase of the peak intensity was observed, but with further increase of the H_2O_2 concentration, the intensity remained nearly constant. For H_2O_2/TC ratios above 100, the intensity of this transition even started to decrease, which limits the use of the (tetracycline)europium(III) system to quantify hydrogen peroxide in solution.

Introduction. – The development and characterization of molecular optical probes for the detection and quantification of different kinds of molecules is nowadays an ever-increasing field of interest. Especially molecular sensors based on luminescent lanthanide ions are attracting considerable attention [1–9]. They represent a versatile class of materials which can be used for the detection of cations, anions, and neutral species, including hydrogen peroxide (H_2O_2) [10]. Hydrogen peroxide is well known for its strong oxidizing properties, and in industry it is mainly used for paper-pulp bleaching and for water treatment. In addition, many applications result from its excellent disinfection and cleaning properties [11]. Although in nature most oxidases convert dioxygen to hydrogen peroxide, the effects of hydrogen peroxide on biological molecules such as DNA cannot be neglected. H_2O_2 can induce damage in several types of DNA in animal and plant cells [12]. Considering the practical and biological importance of H_2O_2, the development of molecular sensors for H_2O_2 detection and quantification is of great importance.

Optical detection of H_2O_2 can be achieved in alkaline solutions (pH > 8) by the use of chemiluminescence [13] or electroluminescence [14]. Visualization and detection of

H_2O_2 is also possible *via* reaction of H_2O_2 with phenols in the presence of peroxidase, resulting in the production of fluorescent dimers [15]. Although this analytical technique is very sensitive, it requires the use of several reagents such as a luminophor precursor, a europium(III) source, and a peroxidase enzyme. A novel probe, based on a (tetracycline)europium(III) complex ([$Eu^{III}(TC)$]), which does not require the presence of an enzyme or an enzyme mimic to form a luminescent molecule has been developed by *Wolfbeis et al.* [10]. The probe was based on an earlier study by *Schulman* and co-workers who reported an increase in luminescence intensity up to a factor of 15 upon addition of H_2O_2 to the (tetracycline)europium(III) system [16]. These observations served as the starting point for the development of a new type of fluorescence assay for the determination of peroxidase activity [17].

Despite the potential of the (tetracycline)europium(III) probe for the use in high-throughput-type assays for the screening of enzyme inhibitors or activators, the molecular basis for the unprecedented increase of europium(III) luminescence in the [$Eu^{III}(TC)$] system upon addition of H_2O_2 has not been fully understood yet. As full understanding of the [$Eu^{III}(TC)$] system can aid the further development of more efficient probes for H_2O_2, we attempt in this study to characterize the nature of the (tetracycline)europium(III) complex in the presence of H_2O_2 under various conditions and to investigate the nature of the luminescence enhancement caused by the addition of H_2O_2.

Results and Discussion. – *Complexation of Eu^{III} with Tetracycline.* Tetracycline (TC) is a broad-spectrum antibiotic [18] which forms stable complexes with lanthanide(III) ions. As can be seen from *Fig. 1*, TC has several proton-donating groups resulting in different complex formation possibilities depending on the pH conditions [19][20].
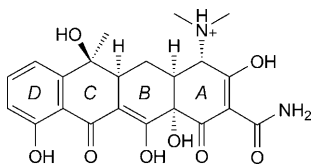

Fig. 1. *Structure of tetracycline* (TC)

Several studies have examined the binding of lanthanide(III) ions to tetracycline, and it has been found that the coordination site is largely influenced by the nature of the solvent and the pH of the solution [19][20]. In general, in aqueous solution, two coordination sites for lanthanide(III) ions have been identified. At low pH values (pH 2), the lanthanide(III) ions coordinate in a bidentate manner to two carbonyl groups of the *A* ring of tetracycline [19]. It was suggested that upon increase of pH, coordination of tetracycline to lanthanide(III) ions changes from the *A* ring to the *B*, *C*, and *D* rings and most likely occurs *via* coordination to the carbonyl group of the *C* ring and to the OH group of the *B* ring and/or the *D* ring [20].

The [$Eu^{III}(TC)$] luminescent probe used for the detection of H_2O_2 contains europium(III) and tetracycline in a molar ratio 3:1 ([$Eu_3(TC)$]) at pH 6.9 [10]. The structure of the complex has not been determined yet, and it remains unclear why the

threefold molar excess of Eu^{III} is necessary for the luminescent probe to be effective. Although TC offers multiple coordination sites for the binding of Eu^{III}, the limited amount of TC ligand used in the probe suggest that most of the coordination sites of Eu^{III} are being occupied by H_2O molecules. In addition, it remains unclear how the structure of the $[Eu^{III}(TC)]$ complex changes upon addition of H_2O_2, and whether H_2O_2 directly coordinates to Eu^{3+} by replacing H_2O molecules or whether it acts by modifying the $[Eu^{III}(TC)]$ complex by partially oxidizing the TC ligand.

Luminescence Spectroscopy of the [Eu^{III}(TC)] System at Different Eu/TC Ratios. To get more information on the complex formation between trivalent europium ions and tetracycline, luminescence spectra of aqueous solutions containing a fixed concentration of TC ($2.08 \cdot 10^{-5}$ M) and increasing concentrations of Eu^{3+} both in the absence and presence of H_2O_2 were recorded between 570 and 640 nm. This spectral region covers the transitions $^5D_0 \rightarrow {}^7F_0$, $^5D_0 \rightarrow {}^7F_1$, and $^5D_0 \rightarrow {}^7F_2$. The hypersensitive transition $^5D_0 \rightarrow {}^7F_2$ is by far the most intense transition in the luminescence spectrum. The luminescence spectra are shown in *Figs. 2* and *3*.
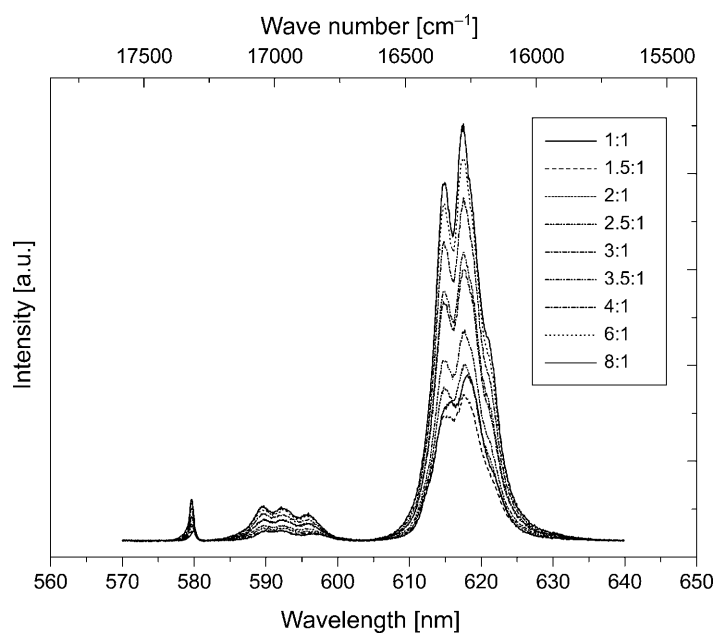

Fig. 2. *Influence of the Eu^{3+}/TC ratio on the luminescence spectrum in aqueous solutions at pH 6.9* (MOPS buffer). $[TC] = 2.08 \cdot 10^{-5}$ M, $[Eu^{3+}]$ from $2.08 \cdot 10^{-5}$ M to $1.66 \cdot 10^{-4}$ M (variation of Eu^{3+}/TC ratio between 1:1 and 8:1).

In solutions in which the Eu^{3+}/TC molar ratio was varied from 1:1 to 8:1 in the absence of H_2O_2, the $^5D_0 \rightarrow {}^7F_0$ transition consists of only one peak at 580 nm. The presence of a single peak suggests the presence of only one type of coordination environment for the Eu^{3+} ion. However, in the presence of H_2O_2, an extra peak at 578 nm appeared in the spectrum when the Eu^{3+}/TC molar ratio was higher than 2.5 (*Fig. 4*). This indicates that at high Eu^{3+}/TC molar ratios, the presence of H_2O_2 leads to
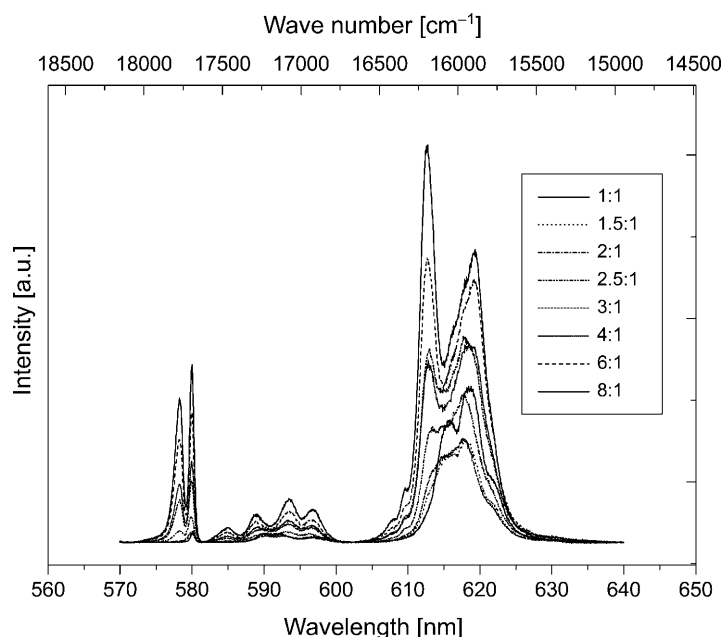
Fig. 3. *Influence of the Eu^{3+}/TC ratio on the luminescence spectrum in aqueous solutions at pH 6.9* (MOPS buffer) *in the presence of H_2O_2.* $[TC] = 2.08 \cdot 10^{-5}$ M, $[H_2O_2] = 4.00 \cdot 10^{-4}$ M, $[Eu^{3+}]$ from $2.08 \cdot 10^{-5}$ M to $1.66 \cdot 10^{-4}$ M (variation of Eu^{3+}/TC ratio between 1:1 and 8:1).

the existence of two Eu^{3+} environments, since the $^7F_0$ energy state is nondegenerate and only one transition can occur. The existence of two different Eu^{3+} sites in the presence of H_2O_2 is also evident from the observation of four crystal-field lines in the spectral region of the $^5D_0 \rightarrow {}^7F_1$ transition. A detailed analysis of the $^5D_0 \rightarrow {}^7F_0$ spectral region revealed that at lower Eu^{3+}/TC molar ratios (up to 2:1), the peak that was originally located at 580 nm experienced a slight blue shift to 579.5 nm. This shows slight changes in the europium(III) environment at low Eu^{3+}/TC molar ratios.

The *Table* gives the intensity ratio of the two peaks at 580 nm and 578 nm that can be observed for the $^5D_0 \rightarrow {}^7F_0$ transition at high Eu^{3+}/TC molar ratios (above 2.5). It is interesting to note that after the large initial decrease that was observed when the Eu^{3+}/TC molar ratio was increased from 2.5 to 3.0, the intensity ratio stayed constant upon further increase of the Eu^{3+} concentration. The intensity of the two peaks increased in a similar manner when the Eu^{3+}/TC ratio reached the value of 8 indicating that the TC ligand also changed in such a way as to induce better energy transfer to the Eu^{3+} ion.

Figs. 5 and *6* graphically show the ratio between the peak intensities of the $^5D_0 \rightarrow {}^7F_2$ and the $^5D_0 \rightarrow {}^7F_1$ transition for the Eu^{3+}/TC system in the presence and in the absence of H_2O_2. In both cases, it is apparent that at molar ratios of Eu^{3+}/TC below 3, a significant increase in the $I(^5D_0 \rightarrow {}^7F_2)/I(^5D_0 \rightarrow {}^7F_1)$ intensity ratio occurs, but that this ratio remains constant upon further increase of the Eu^{3+} concentration.

The observation of different europium(III) sites in the presence of hydrogen peroxide is not surprising since it has been established that lanthanide(III) ions are able
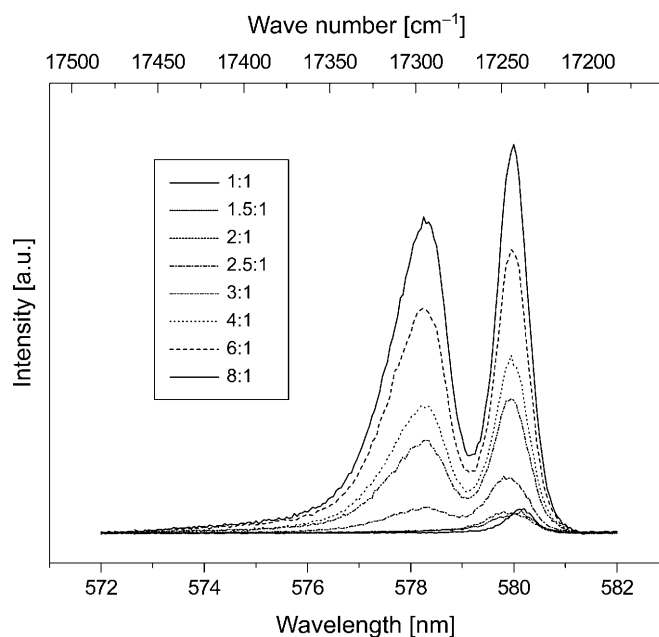
Fig. 4. *Influence of the Eu^{3+}/TC ratio on the $^5D_0 \rightarrow {}^7F_0$ transition in aqueous solution at pH 6.9* (MOPS buffer) *in the presence of H_2O_2.* [TC] $= 2.08 \cdot 10^{-5}$ M, [H_2O_2] $= 4.00 \cdot 10^{-4}$ M, [Eu^{3+}] from $2.08 \cdot 10^{-5}$ M to $1.66 \cdot 10^{-4}$ M (variation of Eu^{3+}/TC ratio between 1:1 and 8:1).

Table. *Ratio of the Peak Intensities for the $^5D_0 \rightarrow {}^7F_0$ Transition as a Function of the Eu^{3+}/TC Ratio in Solutions Containing Hydrogen Peroxide.*

Eu^{3+}/TC ratio	I(580 nm)/I(578 nm)
2.5	2.21
3.0	1.45
4.0	1.39
6.0	1.26
8.0	1.24

to form bridged peroxolanthanide(III) complexes with hydrogen peroxide [21]. It is possible that these structures are responsible for the formation of an extra europium(III) site since only in the presence of H_2O_2, more than one $^5D_0 \rightarrow {}^7F_0$ transition was observed. To support this hypothesis, we examined the effect of the sterically more hindered *tert*-butyl hydroperoxide on the luminescence spectrum of the (tetracycline)europium(III) system. Interestingly, the intensity increase that was observed when H_2O_2 was added to the Eu^{3+}/TC system was not observed when *tert*-butyl hydroperoxide was added. This is most likely because the less coordinating and bulky *tert*-butyl group prevents the formation of bridged complexes.

Luminescent spectra of a solution containing an excess of TC with respect to Eu^{3+} were also recorded. The data both in the absence and presence of H_2O_2 are shown in
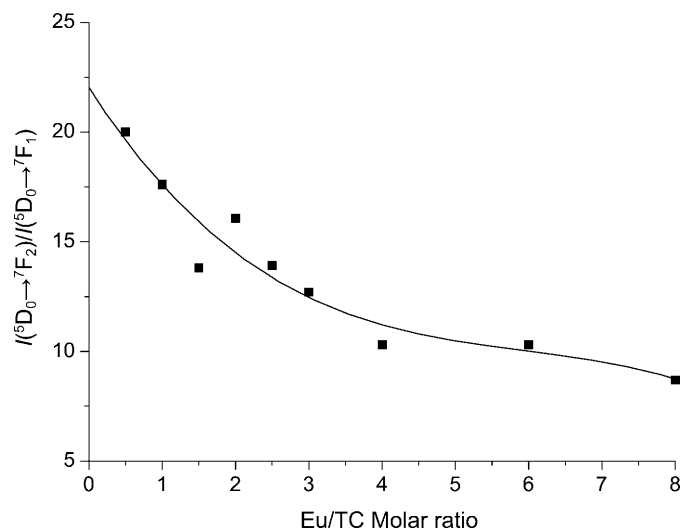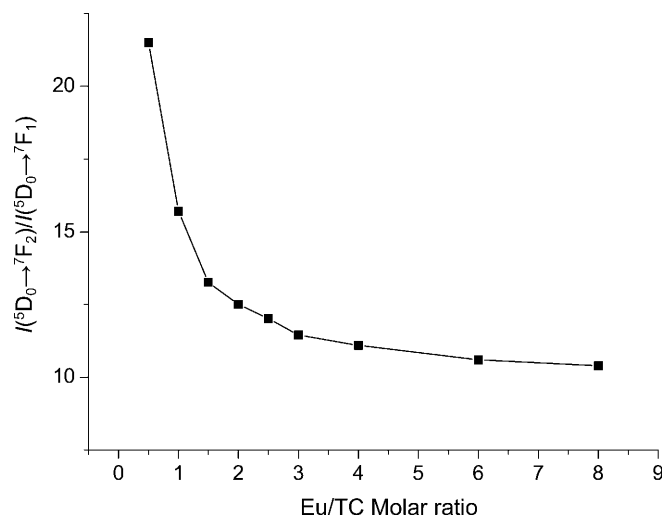
Fig. 5. *Ratio of peak intensities of the $^5D_0 \rightarrow ^7F_2$ and the $^5D_0 \rightarrow ^7F_1$ transition in aqueous solutions containing $2.08 \cdot 10^{-5}$ M of TC at pH 6.9* (MOPS buffer). [Eu^{3+}] from $2.08 \cdot 10^{-5}$ M to $1.66 \cdot 10^{-4}$ M (variation of Eu^{3+}/TC ratio between 1:1 and 8:1).

Fig. 6. *Ratio of peak intensities of the $^5D_0 \rightarrow ^7F_2$ and the $^5D_0 \rightarrow ^7F_1$ transition in aqueous solutions containing $2.08 \cdot 10^{-5}$ M of TC and $4.00 \cdot 10^{-4}$ M of H_2O_2 at pH 6.9* (MOPS buffer). [Eu^{3+}] from $2.08 \cdot 10^{-5}$ M to $1.66 \cdot 10^{-4}$ M (variation of Eu^{3+}/TC ratio between 1:1 and 8:1).

Figs. 7 and *8*. In the absence of H_2O_2, an increase of the $^5D_0 \rightarrow ^7F_2$ signal intensity was observed at Eu^{3+}/TC molar ratios 1:1 and 1:2. This is most likely because an excess of the TC ligand results in a more favorable complexation to the Eu^{3+} ion, leading to an increase of the energy transfer to Eu^{3+}. Interestingly, at higher Eu^{3+}/TC molar ratios
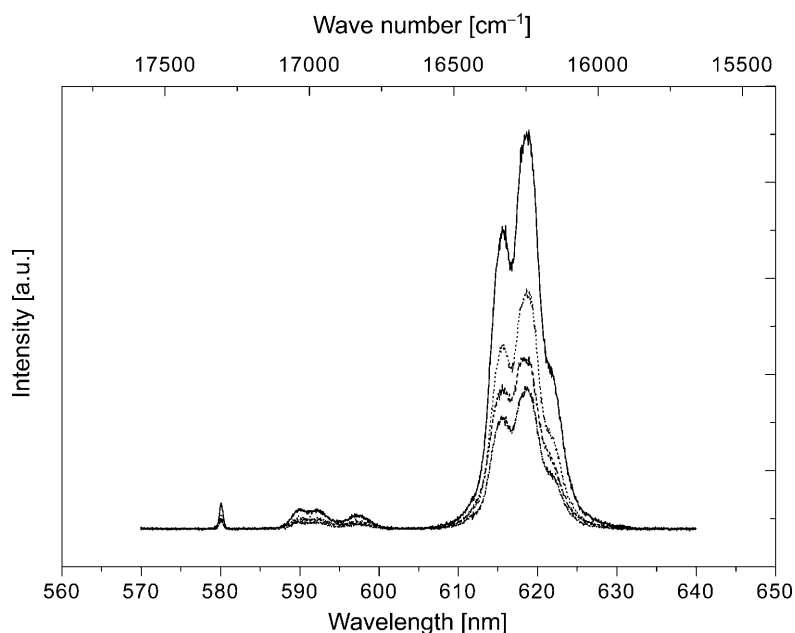
Fig. 7. *Influence of the Eu³⁺/TC ratio on the luminescence spectrum in aqueous solutions at pH 6.9* (MOPS buffer). [Eu³⁺] = 2.08 · 10⁻⁵ M; variation of Eu³⁺/TC ratio from 1:1 (– – –), to 1:2 (——), 1:3 (···), and 1:4 (– · – ·).

(1:3 and 1:4), a decrease of the luminescence intensity was observed. It is likely that due to the high TC concentration, not all TC molecules take part in the complex formation resulting in absorption of energy without energy transfer to the Eu^{3+} center. When H_2O_2 was present, an intensity drop was observed at higher TC concentrations, suggesting that under these conditions, the concentration of the strongly luminescent (tetracycline)europium(III) complex decreases in solution.

Possible Oxidation of the [Eu^{III}(TC)] Probe. So far, it has been postulated that the direct coordination of H_2O_2 to the Eu^{3+} ion was responsible for the large increase in luminescence intensity upon its addition to the Eu^{3+}/TC solution. The possibility that H_2O_2 could have an oxidizing effect on the TC ligand or the [Eu^{III}(TC)] complex has not been considered so far. It is known that in the presence of air, the TC ligand undergoes autooxidation which is accompanied by a very weak chemiluminescence [22]. In alkaline solutions, the weak chemiluminescence during the autooxidation of TC is significantly increased in the presence of H_2O_2 as additional oxidant [22]. It has been suggested that under these conditions, $^1O_2$ species are generated which play an important role in the oxidation of TC. To verify this, we recorded luminescence spectra of Eu^{3+}/TC solutions containing H_2O_2 to which molybdate ions had been added. Molybdate is known to act as a catalyst for the formation of $^1O_2$ from H_2O_2. As it can be seen in *Fig. 9*, addition of sodium molybdate led to an immediate decrease in luminescence intensity. The spectra measured at different time increments indicated that the intensity was continuously dropping and that no recovery of the luminescence
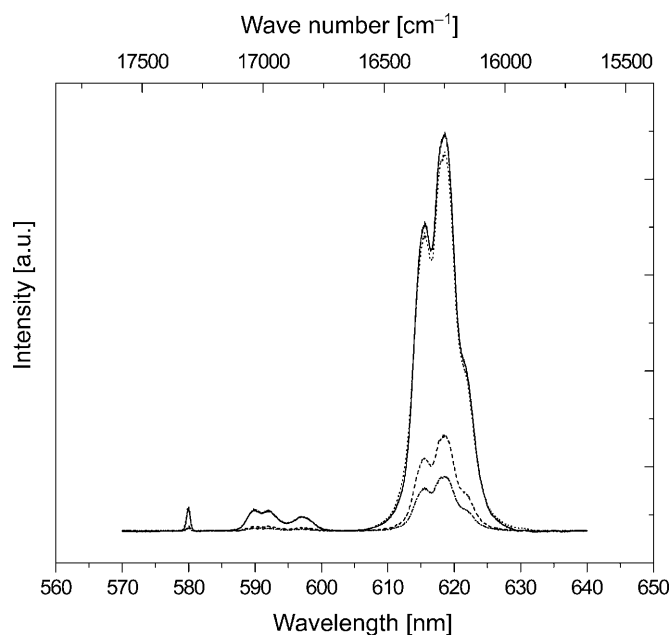
Fig. 8. *Influence of the Eu³⁺/TC ratio on the luminescence spectrum in aqueous solutions containing H₂O₂ at pH 6.9* (MOPS buffer). [Eu³⁺] = 2.08 · 10⁻⁵ M, [H₂O₂] = 4.00 · 10⁻⁴ M); variation of Eu³⁺/TC ratio from 1:1 (···), to 1:2 (– · – ·), 1:3 (---), and 1:4 (——).

intensity could be observed when H_2O_2 was added. The results indicate that under the examined conditions, TC most likely does not undergo an oxidation reaction, since the oxidation should result in the formation of species which show better chemiluminescent properties. It is more likely that addition of molybdate results in the conversion of H_2O_2 in $^1O_2$ leading to the decomposition of the bridging peroxo structures. The disappearance of the extra europium(III) site that is being formed in the presence of H_2O_2 when molybdate is added, supports this hypothesis (*Fig. 9*).

Effect of H_2O_2 Concentration. The effect of the H_2O_2 concentration on the luminescence properties of the (tetracycline)europium(III) system has been investigated earlier [19]. Commonly, to increase the stability of H_2O_2, urea is added to aqueous solutions. The results from the previous study indicated that the intensity of the peaks in the emission spectra increases as the concentrations of urea and hydrogen peroxide increase. In this study, we examined the influence of pure H_2O_2 solutions on the emission spectra of [Eu^{III}(TC)] as this seems to be more relevant for the performance of the optical sensor.

The influence of the H_2O_2/TC ratio on the intensity of the $^5D_0 \rightarrow {}^7F_2$ transition is shown in *Fig. 10*. The concentration of H_2O_2 was varied with respect to the TC concentration up to the H_2O_2/TC ratio of 200. As it can be seen from *Fig. 10*, the intensity of the $^5D_0 \rightarrow {}^7F_2$ transition does not change linearly within this concentration range. For a H_2O_2/TC ratio up to 10 a sharp linear increase of the peak intensity was observed. However, with further increase of the H_2O_2 concentration, the intensity
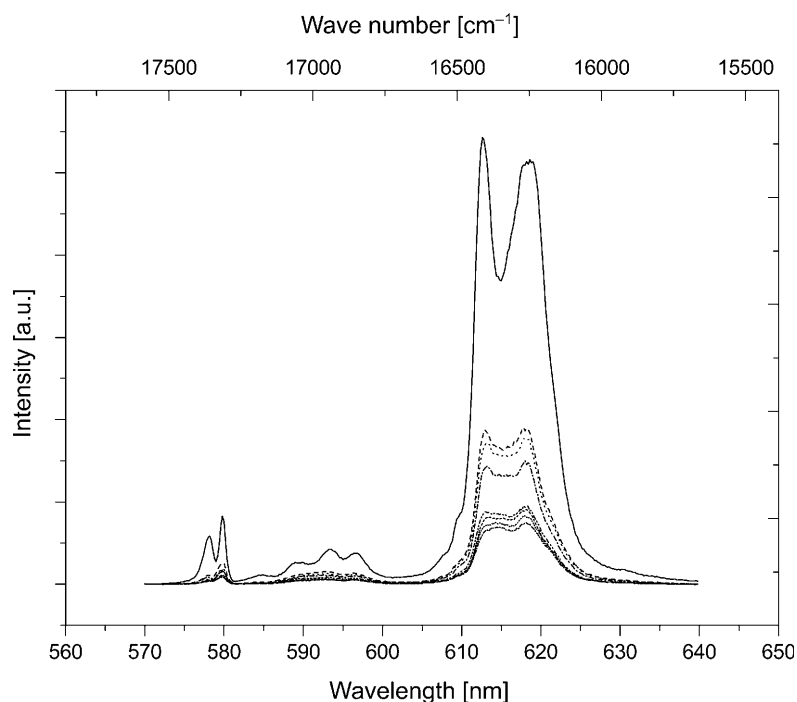
Fig. 9. *Luminescence spectra of the Eu^{3+}/TC 3:1 solution in the absence of molybdate* (—), *when molybdate is added* (– – –), *10 min after adding molybdate* (···), *after 20 min* (– · – ·), *and after 100 min* (- - - -). *Spectrum recorded immediately after addition of H_2O_2* (– · –– · –), *10 min after adding H_2O_2* (·····), *and 20 min after adding H_2O_2* (– · – · – ·).

remained nearly constant. For the H_2O_2/TC ratio higher than 100, the intensity of this transition even started to decrease. This severely limits the use of the (tetracycline)europium(III) systems for sensing H_2O_2 in solutions.

Conclusions. – The luminescence of (tetracycline)europium(III) complexes were studied in the absence and presence of hydrogen peroxide to establish the usefulness of this systems for sensing H_2O_2 in aqueous solutions. The response of the system to the H_2O_2 concentration was found to depend on the Eu^{3+}/TC molar ratio, and the optimal ratio was determined to be 2.5. At higher Eu^{3+}/TC ratios a decrease of the luminescence intensity was observed most likely because not all TC molecules take part in the complex formation resulting in absorption of energy without energy transfer to the Eu^{3+} center. The luminescence spectroscopy of the Eu^{3+}/TC system indicated that upon addition of H_2O_2, the coordination around Eu^{3+} ion changes to yield two different Eu^{3+} environments that most likely contain bridged peroxo-europium(III) complexes. A study of the influence of the H_2O_2 concentration on the intensity of the $^5D_0 \rightarrow {}^7F_2$ transition showed that there is no linear relationship between the intensity of this transition and the H_2O_2 concentration, so that the europium(III) system is of
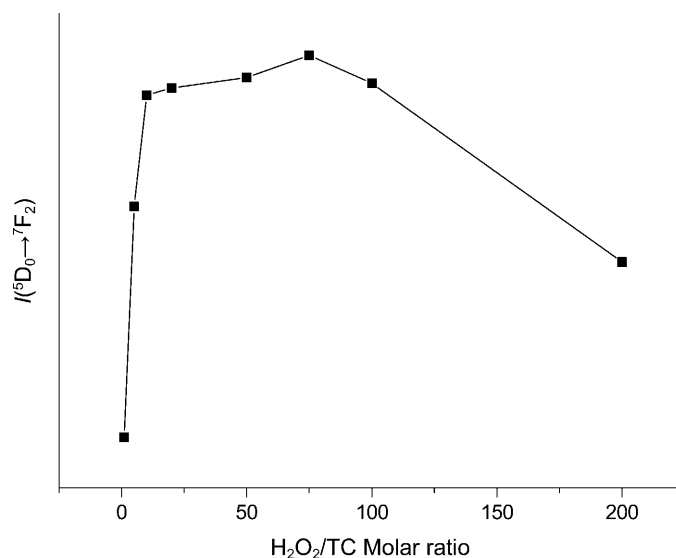
Fig. 10. *Influence of the H_2O_2 concentration on the peak area of the $^5D_0 \rightarrow {}^7F_2$ transition of the $[Eu^{III}(TC)]$ system*

limited value for its further development as a luminescence sensor for hydrogen peroxide.

Experimental Part

Tetracycline (TC) was obtained from *AppliChem*. The 3-morpholinopropanesulfonic acid (MOPS) was purchased from *Sigma Aldrich*. $Eu(NO_3)_3 \cdot 6 H_2O$ was synthesized from Eu_2O_3. Eu_2O_3 (2.60 g, 7.40 mmol) was added to a 65% HNO_3 soln. (3.07 ml, 45 mmol) and dist. H_2O (50 ml). After stirring overnight, the unreacted Eu_2O_3 was removed by centrifugation. After evaporation of the solvent, $Eu(NO_3)_3 \cdot 6 H_2O$ was obtained. The experiments were performed at pH 6.9 by using a MOPS buffer unless mentioned otherwise.

Luminescence spectra were recorded with an *Edingburgh Instruments FS 900* spectrofluorimeter equipped with a 450 W Xe lamp, and a red-sensitive photomultiplier (*Hamamatsu R-928*) was used.

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