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Short communication

Increased luminescence of the tetracycline–europium(III) system following oxidation by hydrogen peroxide

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1. Introduction

Tetracylines are well-known antibiotics, which have been and are still used for the treatment of a wide variety of bacterial infections. Ever more sensitive methods are required for the determination of these important antibiotics. Tetracycline-sensitized luminescence of europium(III) was first described by Hirschy et al. [1]. A subsequent paper from the same laboratory explained the binding characteristics of europium(III) to tetracycline [2]. Extra-weak chemiluminescence generated during oxidation of some tetracycline antibiotics (tetracycline, chlortetracycline, methacycline and oxytetracycline) has been observed in the presence of H_2O_2 in basic, air-saturated solutions [3,4].

In this communication, it is reported that the fluorescence intensity is increased 15 times when H_2O_2 is added to the fluorescent europium(III)–tetracycline system.

The method is based on the contact energy transfer of an oxidized form of the antibiotic to europium(III) followed by emission from a lanthanide localized $^5D_o \rightarrow {}^7F_2$ transition [5].

2. Experimental

Fluorescence measurements were made using a Perkin Elmer LS-5 spectrofluorimeter. A Shimadzu UV-160 U UV–visible recording spectrophotometer was used for absorbance measurements. The excitation and emission slitwidths were 10 nm. The fluorescence intensities of all solutions were measured in 10 mm quartz cells.

3. Materials

Tetracycline hydrochloride (Sigma), $Eu(NO_3)_3 \cdot 6H_2O$ (Alfa Products), H_2O_2 (Fisher Sci-

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entific), 1,4-diazabicyclo-(2,2,2) octane (DABCO) (Kodak), $HClO_4$ (Fisher Scientific) and ultrapure deionized water (Milli Q Millipore System) were used as supplied.

A 0.10 M stock solution of perchloric acid was slowly added to a 0.013 M 1,4-diazabicyclo-(2,2,2) octane solution in water to obtain a pH of 6.91.

4. Method

Known amounts of tetracycline were added to the solutions containing 3 μg ml^{-1} and 10 μg ml^{-1} europium(III). Ten microliters of 27.3 mg ml^{-1} H_2O_2 and 5 ml DABCO buffer solution were added to these solutions. Ten minutes later the fluorescence intensities were measured at excitation and emission wavelengths of 389 and 610 nm, respectively.

5. Results and discussion

Fig. 1 shows the fluorescence intensities when known amounts of tetracycline were added to the solutions of 3 and 10 μg ml^{-1} europium(III) in the presence and absence, respectively, of H_2O_2. As can be seen, in the presence of H_2O_2, the fluorescence intensities are much greater (15 times) than in the solutions without H_2O_2. No emission is observed when the sample is shielded from the exciting light, ruling out the possibility that the emission is related to chemiluminescence.

The effect of H_2O_2 under the same conditions as in Ref. [1], was studied and it was seen that when 8.02×10^{-4} M H_2O_2 was added to that system the fluorescence intensity was increased almost four times over that of the system without H_2O_2. Maximum enhancement of the fluorescence intensity is obtained at pH 6.91. The effect of pH

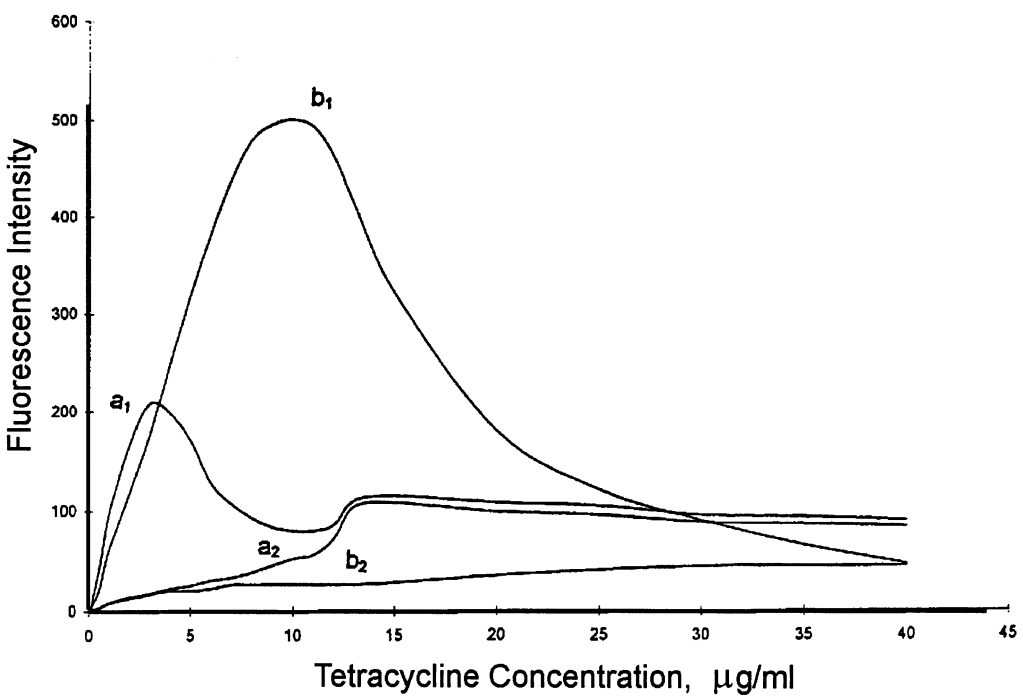

Fig. 1. Fluorescence intensities of complexes of 3 and 10 μg ml^{-1} europium(III) as a function of tetracycline concentration. a_1: 3 μg ml^{-1} Eu(III) with H_2O_2; a_2: 3 μg ml^{-1} Eu(III) without H_2O_2. b_1: 10 μg ml^{-1} Eu(III) with H_2O_2; b_2: 10 μg ml^{-1} Eu(III) without H_2O_2. The peroxide concentration was 8.02×10^{-4} M. The excitation wavelength was 389 nm with emission at 610 nm.

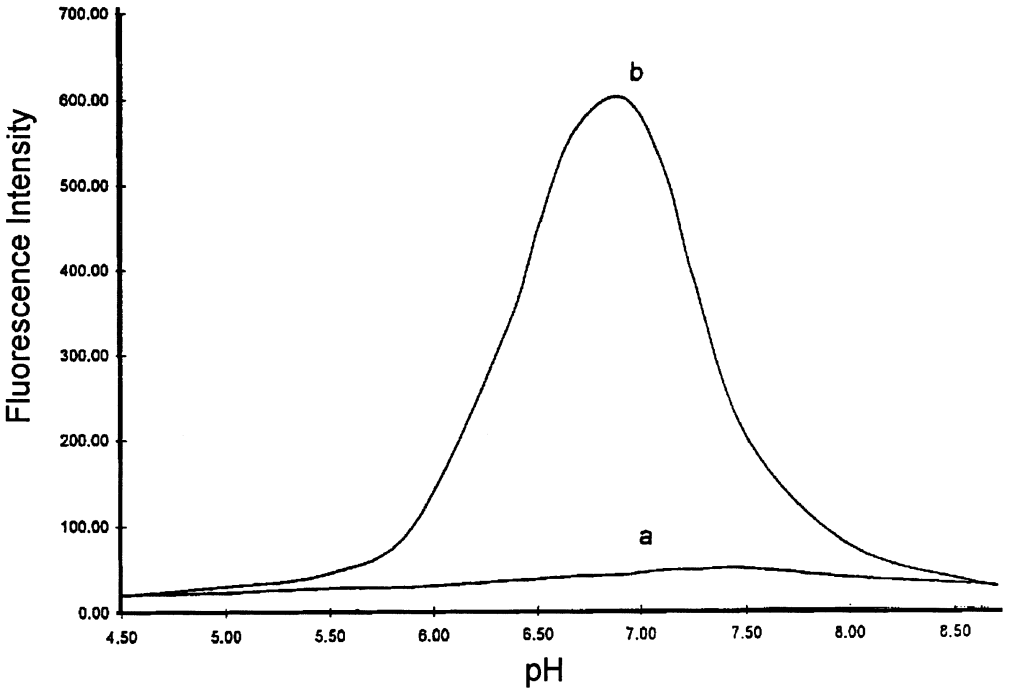

Fig. 2. Effect of pH on the fluorescence intensity of the europium (III)–tetracycline solutions; (a) without H_2O_2, (b) with 8.02×10^{-4} M H_2O_2. Europium(III) and tetracycline concentrations of 10 μg ml^{-1} were used with excitation at 389 nm, and emission at 610 nm.

on the florescence intensity of the new system is shown in Fig. 2.

The detection limit found by the present method is 10 ng ml^{-1}. This was calculated by multiplying the standard deviation of 16 blank measurements by three and dividing by the slope of the linear calibration curve. This can be compared with the method of [1], which when repeated in this laboratory gave a limit of detection of 143 ng ml^{-1} and so the proposed method is at least ten times more sensitive than that in the literature.

Acknowledgements

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