

Europium-sensitized Chemiluminescence of System Tetracycline–H₂O₂–Fe(II)/(III) and Its Application to the Determination of Tetracycline

M. Kaczmarek · A. Idzikowska · S. Lis

Received: 15 December 2007 / Accepted: 14 March 2008 / Published online: 4 May 2008
© Springer Science + Business Media, LLC 2008

Abstract Chemiluminescence (CL) of the reaction system tetracycline–H₂O₂–Fe(II)/(III)–Eu(III) was used for the determination of tetracycline hydrochloride in water, pharmaceutical preparations, and honey. The CL spectrum registered for this system shows emission bands typical of Eu(III) ions, with a maximum at $\lambda \sim 600$ nm, corresponding to the electronic transitions of ${}^5D_0 \rightarrow {}^7F_1$ and ${}^5D_0 \rightarrow {}^7F_2$. A strong chemiluminescence intensity characteristic of europium(III) ions in the system tetracycline–H₂O₂–Fe(II)/(III)–Eu(III), as contrasted to the emission of the system tetracycline–H₂O₂–Fe(II)/(III) without Eu(III), proves that the Eu(III) ion plays the role of a chemiluminescence sensitizer, accompanying tetracycline oxidation in the Fenton system (H₂O₂–Fe(II)/(III)). A linear dependence was observed for the integrated CL light intensity on the tetracycline concentration in the range of 2×10^{-7} to 3×10^{-5} mol l⁻¹ with the detection limit of 5×10^{-8} mol l⁻¹ in aqueous solution.

Keywords Chemiluminescence · Europium ions · Tetracycline · Energy transfer

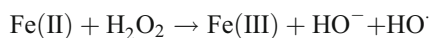
Introduction

Tetracyclines are well-known antibiotics, which have been and are still used for the treatment of a wide variety of bacterial infections. The majority of the methods described for the determination of tetracyclines (TC) are based on their ability to form colored metal complexes [1, 2]. Other methods use derivative fluorimetry [3], ion-selective electrodes [4], and ultraviolet [5] detection after high-performance liquid chromatographic separation.

Chemiluminescent (CL) methods can also be successfully used for the determination of tetracyclines. Extra-weak chemiluminescence generated during the oxidation of some tetracycline antibiotics has been observed in the presence of H₂O₂ in basic, air-saturated solutions [6]. Tetracyclines have been determined by a CL method based on the chemiluminescence produced by the action of *N*-bromosuccinimide on tetracyclines in alkaline solution [7].

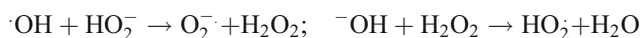
Upon complexation with trivalent europium ions, tetracyclines form stable chelates, which exhibit broad-banded absorption spectra and a narrow emission band centered at 615 nm, which is characteristic of the ${}^5D_0 \rightarrow {}^7F_2$ transition within the lanthanide ion [8]. Optimal conditions for enhanced fluorescence have been found to be at pH around 7.0 or pH=8 with Triton X-100, and pH=9 with cetyltrimethylammonium chloride [9, 10].

The Fenton reaction (H₂O₂–Fe(II)/(III)) is one of the main sources of reactive oxygen species in biological systems, with implication for oxidative stress and diseases. The hydroxyl radical, OH·, is formed by reduction of hydrogen peroxide with Fe(II) [11]

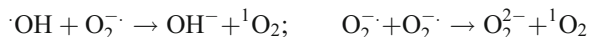


M. Kaczmarek · A. Idzikowska · S. Lis (✉)
Department of Rare Earths, Faculty of Chemistry,
Adam Mickiewicz University,
Grunwaldzka 6,
60-780 Poznań, Poland
e-mail: blis@amu.edu.pl

The overall reactions (recombination of radicals) can be described by the following



$\text{HO}_2 \rightarrow \text{O}_2^- + \text{H}^+$ with singlet oxygen ($^1\text{O}_2$) being formed [12–14]:



$\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH} + ^1\text{O}_2$ (Haber–Weiss reaction). Pairs $^1\text{O}_2$ molecules can combine their electronic excitations to give an emission from a dimol with four emission bands in the VIS region: at around 520, 580, 630 and 703 nm [13, 14].

This paper reports a CL study of the reaction system: TC– H_2O_2 –Fe(II)/(III)–Eu(III). The kinetic CL curves and CL spectral distribution were recorded. On the basis of the results obtained, it has been demonstrated that Eu(III) acts as a sensitizer. The CL observed in the reaction system: TC– H_2O_2 –Fe(II)/(III)–Eu(III) can be used for the determination of TC in pharmaceutical preparations and honey, without any pre-treatment of the samples.

Experimental

Ultra weak photon emissions (chemiluminescence) were measured with the use of the earlier described equipment [15]. The fluorescence spectra were recorded using a Perkin-Elmer MPF3 spectrofluorimeter.

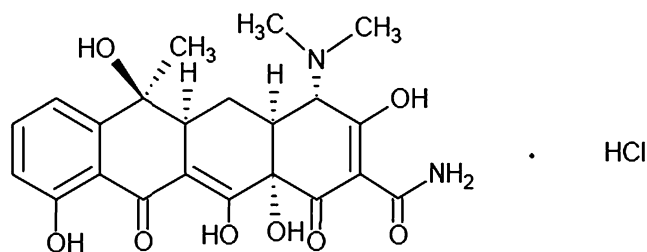
Chemicals used in this study were: tetracycline hydrochloride (Scheme 1, Sigma-Aldrich $\geq 95\%$), chloride of Eu(III) obtained by dissolving europium oxide (99.99%, Aldrich) in hydrochloric acid (spectroscopically pure, Fluka), hydrogen peroxide (30% solution, analytical grade, Merck), NaOH (analytical grade); FeCl_2 (analytical grade, Fluka).

Solutions

All solutions were prepared with the use of doubly distilled water. A stock solution of hydrogen peroxide (0.1 mol l^{-1}) was freshly prepared by cautious dilution of the 30% H_2O_2 solution with water. Stock solutions of other chemicals were: EuCl_3 (0.2 mol l^{-1}), FeCl_2 (0.02 mol l^{-1}), and tetracycline hydrochloride 4×10^{-5} and $4 \times 10^{-6} \text{ mol l}^{-1}$.

Procedure

All of the chemiluminescence studies were performed in the same manner. Hydrogen peroxide ($1 \times 10^{-2} \text{ mol l}^{-1}$) was



Scheme 1 Schematic presentation of tetracycline hydrochloride ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8 \cdot \text{HCl}$)

added to solutions containing tetracycline (1×10^{-8} to $1 \times 10^{-4} \text{ mol l}^{-1}$), Fe(II) ($5 \times 10^{-4} \text{ mol l}^{-1}$), and Eu(III) (4×10^{-4} to $1 \times 10^{-2} \text{ mol l}^{-1}$). The experiments were conducted in acidic solutions (pH ~ 3.5). This pH value was attained with additions of NaOH or HCl solutions. For all the solutions under study, curves of CL decay were obtained, and the integral of the CL light intensity were calculated as the area under these curves according to the following equation:

$$S = \int_{t_0}^t I \Delta t$$

where: I—CL intensity, t—measurement duration.

The determination of tetracycline in water, pharmaceutical preparations, and honey was carried out measuring the integrated CL light intensity of the reaction systems using cut-off filters. CL typical for Eu(III) ions was recorded with the use of a filter transmitting emission with $\lambda > 585 \text{ nm}$. This filter was placed between the photomultiplier and the measurement cell. The optimal conditions for tetracycline determination were found when analyte samples contained: TC of $2 \times 10^{-5} \text{ mol l}^{-1}$, the initial concentration of H_2O_2 — $1 \times 10^{-2} \text{ mol l}^{-1}$, Eu(III)— $2 \times 10^{-3} \text{ mol l}^{-1}$, and Fe(II)— $5 \times 10^{-4} \text{ mol l}^{-1}$, respectively.

Sample preparation for the determination of tetracycline in pharmaceutical preparations (powder) and honey:

Powder: a portion of the powdered sample was accurately weighed and diluted with doubly distilled water so that the final tetracycline concentration was within the working range.

Honey: an accurately weighed portion (of about 1 g) of a tetracycline-free honey sample was transferred into a 200 ml calibrated flask and diluted to volume with doubly distilled water.

Results and discussion

The chemiluminescence was measured of the systems containing tetracycline hydrochloride, Eu(III) ions, Fe(II) ions, and H_2O_2 , as an oxidizer, in aqueous solution at pH ~ 3.5. Figure 1 shows the CL kinetic curves of the system TC– H_2O_2 –Fe(II)/(III), recorded in the presence and in the

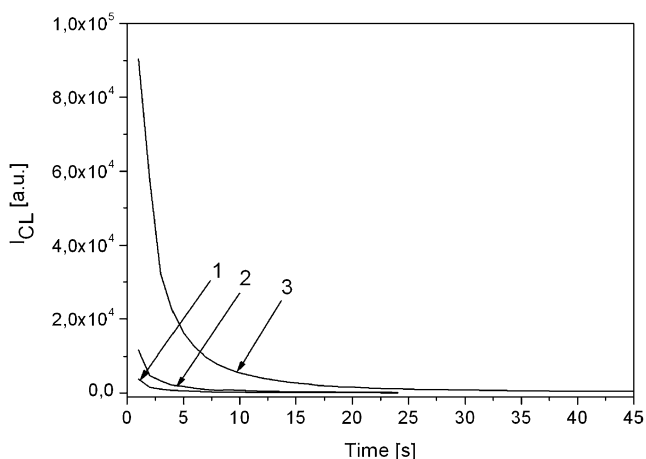


Fig. 1 The kinetic curves of CL decay in the systems: H_2O_2 -Fe(II)/(III)-Eu(III) (curve 1); tetracycline- H_2O_2 -Fe(II)/(III) (curve 2); and tetracycline- H_2O_2 -Fe(II)/(III)-Eu(III) (curve 3). The concentration of Eu(III) ions was $1 \times 10^{-3} \text{ mol l}^{-1}$; the initial concentration of $H_2O_2 = 1 \times 10^{-2} \text{ mol l}^{-1}$, tetracycline = $5 \times 10^{-6} \text{ mol l}^{-1}$, and Fe(II) = $2 \times 10^{-4} \text{ mol l}^{-1}$, pH ~ 3.5

absence of Eu(III) ions. The emission, generated as a result of the tetracycline oxidation in the Fenton system (Fe(II)/(III)- H_2O_2), was characterized by a very low intensity with a short duration.

This low intensity of CL emission appeared at the moment of H_2O_2 being introduced into the solution under investigation. This CL emission intensity decayed to the baseline in 5 min, when the initial concentration of TC was $5 \times 10^{-5} \text{ mol l}^{-1}$. When the concentration of TC was below $5 \times 10^{-6} \text{ mol l}^{-1}$, the presence of tetracycline had no influence on the chemiluminescence of the Fenton system. The presence of Eu(III) ions in the TC- H_2O_2 -Fe(II)/(III) resulted in an increase in the CL intensity and no change in the character of the kinetic curve (curve 3 in Fig. 1). In the reaction mixtures of TC- H_2O_2 -Fe(II)/(III)-Eu(III), an increase in the emission intensity with an increase in the Eu(III) concentration was observed. The maximal value of the integrated light-intensity of the system was observed for the molar ratio Eu:TC=100:1.

Spectral analysis of the chemiluminescence from the systems was performed using the method of cut-off filters as described earlier [16].

The CL spectral distribution of the system H_2O_2 -Fe(II)/(III) was typical for dimoles of singlet oxygen, with emission bands at $\lambda \sim 520, 580, \text{ and } 630 \text{ nm}$ (spectrum 1 in Fig. 2).

The spectral distribution of the system TC- H_2O_2 -Fe(II)/(III) displayed a broad emission band in the range of 450–

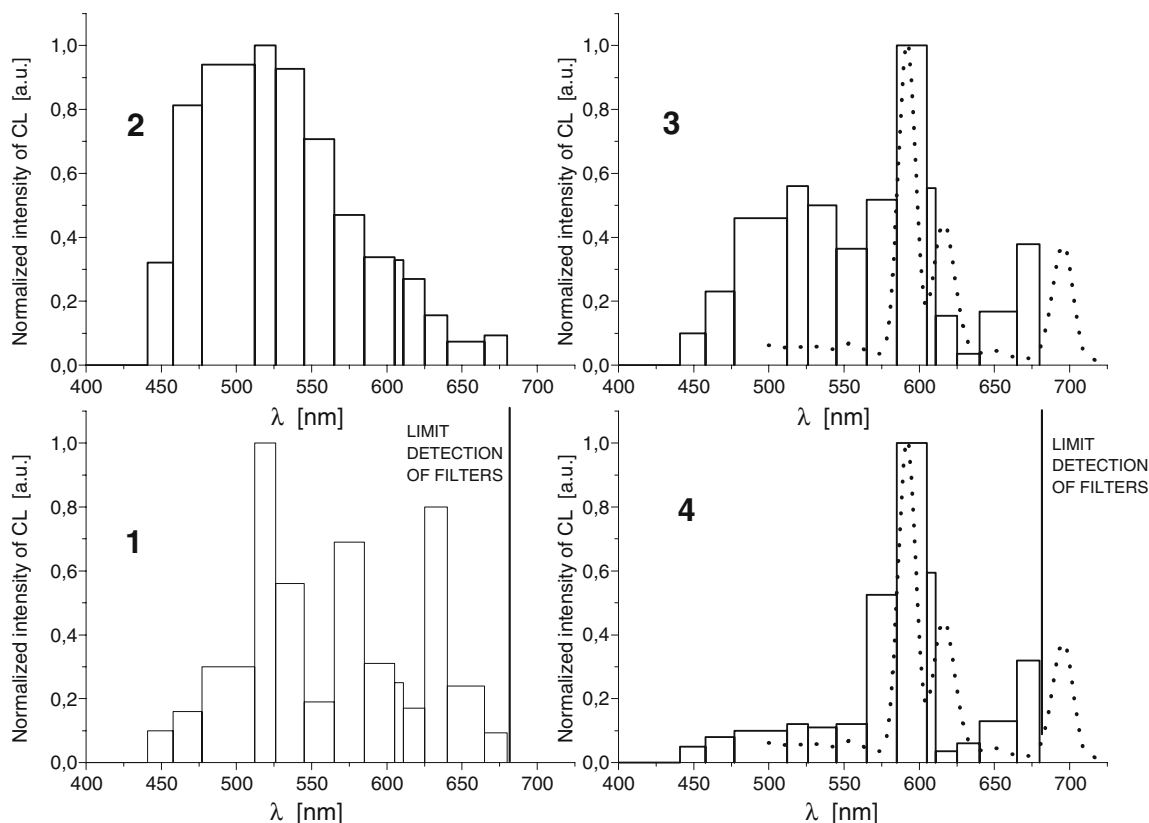


Fig. 2 The spectral distribution of chemiluminescence of the systems: H_2O_2 -Fe(II)/(III) (spectrum 1); tetracycline- H_2O_2 -Fe(II)/(III) (spectrum 2); tetracycline- H_2O_2 -Fe(II)/(III)-Eu(III) for molar

ratio Eu:TC=10:1 (spectrum 3) and 100:1 (spectrum 4). Spectra 3 and 4 contain additionally (dot lines) the luminescence spectra of uncomplexed Eu(III), ion $\lambda_{ex} = 394 \text{ nm}$, for comparison

650 nm with a maximum at ~ 520 nm (spectrum 2 in Fig. 2). This band is typical of excited organic molecules ($>C=O$)* [17, 18]. However, the CL spectrum of the reaction mixture containing both TC and Eu(III) is dependent on the concentration of europium(III) ions. The spectral distribution, observed when the molar ratio Eu:TC was 10:1, showed a broad band at 520 nm and a second band centered at $\lambda=600$ nm (spectrum 3 in Fig. 2), corresponding to the ${}^5D_0 \rightarrow {}^7F_1$ and ${}^5D_0 \rightarrow {}^7F_2$ transition of the Eu(III) ion. For higher molar ratio (Eu:TC above 80:1), the observed CL spectral distribution was typical for Eu(III) with a maximum at $\lambda \sim 600$ nm (spectrum 4 in Fig. 2). This means that the presence of the lanthanide ion is responsible for the increase in the CL intensity and for the lengthening of the duration of emission of the TC–H₂O₂–Fe(II)/(III)–Eu(III) system, compared to the emission time of the TC–H₂O₂–Fe(II)/(III) system. Replacement of the Eu(III) ions by Tb(III) generated CL of comparable intensity to that observed in the TC–H₂O₂–Fe(II)/(III) system itself. This lack of any Tb(III) excitation suggests that the product formed as a result of TC oxidation is of lower energy than the excited state (5D_4 –20,500 cm⁻¹) of the Tb(III) ion [8, 19].

In order to identify the role of the lanthanide ion in the reaction mixture under study, the process of Ln(III) complexation with TC was studied in the absence and in the presence of H₂O₂ in pH region from 3 to 4. Tetracycline formed complexes with the lanthanide ions in aqueous solutions at pH above 6. In these complexes, an efficient ligand-to-metal energy transfer takes place [9, 20]. At pH ~ 6.9 the Eu(III)-TC complex was shown to be a fluorescent probe for hydrogen peroxide [21]. For all molar ratios of Eu:TC (1:1 to 100:1) and concentrations of H₂O₂ (1×10^{-4} to 1×10^{-2} mol l⁻¹), the luminescence of Eu(III) was not observed in the investigated pH (3 to 4) region. The decay

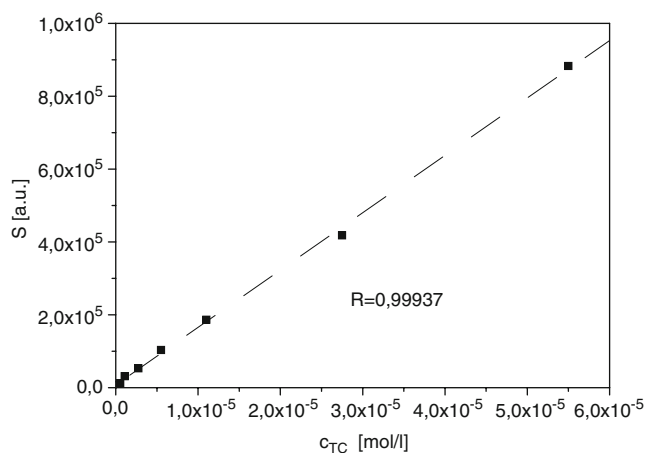


Fig. 3 The light sum of the system tetracycline–H₂O₂–Fe(II)/(III)–Eu(III) versus the concentration of TC. The initial concentration of H₂O₂ was 1×10^{-2} mol l⁻¹, Eu(III) ions: 2×10^{-3} mol l⁻¹, and Fe(II) ions 2×10^{-4} mol l⁻¹

Table 1 Determination of tetracycline in commercial pharmaceutical preparations using the proposed CL method

Pharmaceutical preparation	Amount	Found by using CL method
Vetacyclinum pulvis	5 g TC in 100 g of preparation	5.04±0.03 in 100 g preparation
Laudin	6.66 g TC in 100 g of preparation	6.60±0.05 in 100 g of preparation

time of the Eu(III) excited state was typical for uncomplexed europium with a value of ~ 110 μ s [8, 22]. This indicates that the excitation of Eu(III) in the chemiluminescent system TC–H₂O₂–Fe(II)/(III)–Eu(III) is a result of an energy transfer process from the excited product of the TC oxidation to the uncomplexed Eu(III).

The chemiluminescent system TC–H₂O₂–Fe(II)/(III)–Eu(III) was used for the determination of tetracycline. The optimum conditions were found using analyte samples of 2×10^{-5} mol l⁻¹ TC, and concentrations of H₂O₂— 1×10^{-2} mol l⁻¹, Eu(III)— 2×10^{-3} mol l⁻¹, and Fe(II)— 2×10^{-4} mol l⁻¹. Under these conditions, the decay curve of the TC–H₂O₂–Fe(II)/(III)–Eu(III) system could be described by first-order kinetics (curve 3 in Fig. 1). The CL intensity of this system depended only on the TC concentration. A linear dependence was found for the integrated CL light intensity (obtained by using an appropriate filter) on the tetracycline concentration in the TC concentration range of 2×10^{-7} to 3×10^{-5} mol l⁻¹ (Fig. 3) with a detection limit (S/N=3) of 5×10^{-8} mol l⁻¹.

The proposed CL method was also evaluated by analyzing commercial pharmaceutical preparations of tetracycline (Table 1). The results were compared with those obtained by the official method (provided by the manufacturer). The accuracy of the proposed CL method for the determination of TC in honey was examined by performing recovery experiments on solutions prepared from tetracycline (Table 2). Introduction of a tetracycline-free honey sample into the system H₂O₂–Fe(II)/(III)–Eu(III) by using filter 11 caused no changes—chemiluminescence did not

Table 2 Recovery experiments for TC added to aqueous solutions of commercial honey samples

Sample	TC		
	Added (μ g ml)	Found (μ g ml)	Recovery (%)
1	0.5	0.51	102.0
	0.7	0.71	101.4
2	0.5	0.492	98.4
	0.7	0.693	99
3	0.5	0.504	100.8
	0.7	0.707	101

occurred. However, introduction of TC into this reaction system resulted in a CL intensity increase, which was proportional to the amount of TC introduced. The results presented in Table 2 indicate a good agreement between the introduced amount of TC into honey and that determined using the CL method proposed.

Conclusion

The present study describes a method for determination of tetracycline which uses the chemiluminescence generated in the reaction system of TC–H₂O₂–Fe(II)/(III) which uses Eu (III) ions as the sensitizer. The proposed method is simple, accurate, and precise. The method enables the determination of tetracycline hydrochloride in pharmaceutical preparations and honey, without any sample pre-treatment. The measurement error is smaller than 3%.

References

- Sultan SM, Alzamil IZ, Alarfaj NA (1988) Complexometric-spectrophotometric assay of tetracyclines in drug formulations. *Talanta* 35:375–378
- Alwarthan AA, Al-Tamrah SA, Sultan SM (1991) Spectrophotometric determination of oxytetracycline by flow injection. *Analyst* 116:183–186
- Abdel-Hady Elsayed M, Barary MH, Mahgoub H (1985) Spectrofluorimetric assay of tetracycline and anhydrotetracycline in combination. *Talanta* 32:1153–1155
- Shoukry AF, Badawy SS (1987) Determination of tetracycline and related compounds using plastic membrane ion-selective electrodes. *Microchem J* 36:107–112
- Oka H, Ikai Y, Kawamura N, Uno K, Yamada M (1987) Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey. *J Chromatogr* 400:253–261
- Michalska T, Lichszfeld K, Nizinkiewicz K, Kruk I, Wronska J, Galembiowska D (1992) The extra-weak chemiluminescence generated during oxidation of some tetracycline antibiotics. II. Peroxidation. *J Photochem Photobiol B Biol* 16:305–318
- Halvatzis SA, Timotheou-Potamia MM, Calokerinos AC (1993) Continuous chemiluminometric determination of tetracyclines in pharmaceutical preparations and honey by oxidation with *N*-bromosuccinimide. *Analyst* 118:633–637
- Lis S (2002) Luminescence studies of lanthanide(III) ions in solution. *J Alloys Comp* 341:45–50
- Courrol LC, de Olivera Silva FR, Gomes L, Vieira Junior ND (2007) Energy transfer study of europium–tetracycline complexes. *J Luminescence* 122–123:288–290
- Amaud N, Georges J (2001) Sensitive detection of tetracyclines using europium-sensitized fluorescence with EDTA as co-ligand and cetyltrimethylammonium chloride as surfactant. *Analyst* 126:694–697
- Bautitz IR, Nogueira RFP (2007) Degradation of tetracycline by photo-fenton process-solar irradiation and matrix effects. *J Photochem Photobiol A Chem* 187:33–39
- Singh A (1982) Chemical and biochemical aspects of superoxide radicals and related species of activated oxygen. *Can J Physiol Pharmacol* 60:1330–1345
- Elbanowski M, Staninski K, Karczmarek M, Lis S (2003) A comparative study on chemiluminescence properties of some inorganic systems. *Int J Photoen* 5:239–242
- Khan AU, Kasha M (1970) Chemiluminescence arising from simultaneous transitions in pairs of singlet oxygen molecules. *J Am Chem Soc* 92:3293–3330
- Kaczmarek M, Lis S (2006) Luminescence characterisation of the reaction system histidine–KBrO₃–Tb(III)–H₂SO₄. *J Fluoresc* 16:825–830
- Tryka S (1998) Cut-off filter method for light-induced photon emission spectra estimation. *Computers Chem* 22:113–118
- Davies AK, Cundall RB, Dandiker Y, Slifkin MA (1988) Photooxidation of tetracycline adsorbed on hydroxyapatite in relation to the light-induced staining of teeth. *J Dent Res* 64:936–939
- Davies AK, McKellar JF, Phillips GO, Reid AG (1979) Photochemical oxidation of tetracycline in aqueous solution. *J Chem Soc Perkin Trans II*:369–375
- Arnaud N, Georges J (2003) Comprehensive study of the luminescent properties and lifetimes of Eu³⁺ and Tb³⁺ chelated with various ligands in aqueous solutions: influence of the synergic agent, the surfactant and the energy level of the ligand triplet. *Spectrochim Acta Part A* 59:1829–1840
- Lin Z, Wu M, Wolfbeis OS, Schäferling M (2006) A novel method for time-resolved fluorometric determination and imaging of the activity of peroxidase, and its application to an enzyme-linked immunosorbent assay. *Chem Eur J* 12:2730–2738
- Wolfbeis OS, Dürkop A, Wu M, Lin Z (2002) A Europium-ion-based luminescent sensing probe for hydrogen peroxide. *Angew Chem Int Ed* 41:4495–4498
- Lis S, Kimura T, Yoshida Z (2001) Luminescence lifetime of lanthanide(III) ions in aqueous solution containing azide ion. *J Alloys Comp* 323–324:125–127