

# Chemiluminescence of the Reaction System Ce(IV) - Non-Steroidal Anti-Inflammatory Drugs Containing Europium(III) Ions and its Application to the Determination of Naproxen in Pharmaceutical Preparations and Urine

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**Abstract** The chemiluminescence (CL) of oxidation of non-steroidal anti-inflammatory drugs (NSAIDs) by Ce(IV) ions, was recorded in the presence and absence europium(III) ions, in solution of pH~4 of solution. Kinetic curves and CL emission spectra of the all studied systems were discussed. CL of measurable intensity was observed in the Ce(IV)–NP–Eu(III) reaction system only in acidic solutions. The CL spectrum registered for this system shows emission bands, typical of Eu(III) ions, with maximum at  $\lambda \sim 600$  nm. The chemiluminescent method, based on Eu(III) emission in reaction system of NP–Ce(IV)–Eu(III) in acid solution was therefore used for the determination of naproxen in mixture of non-steroidal anti-inflammatory drugs.

**Keywords** Chemiluminescence · Europium · Non-steroidal anti-inflammatory drugs

## Introduction

Naproxen(NP), ibuprofen(IB) and indomethacin (IM) are non-steroidal anti-inflammatory drugs (NSAID). Chemically, NP and IB belong to the 2-arylpropionic acid family, whereas IM belongs to the class of arylacetic acid. The NSAID as a group are diverse in chemical structure but are consistent in their ability to inhibit the enzyme cyclooxygenase, regarded as a marker for inflammation [1]. The complexation

processes of naproxen(NP) or ibuprofen(IB) or indomethacin (IM) with lanthanide ions depend of pH values. In acidic solution, the degree of complexation is small [2]. The trivalent lanthanide ions, especially Eu(III) and Tb(III), display spectroscopic properties of high photoluminescence efficiencies, long excited-state lifetimes band narrow emission spectra, all of which make them be widely used as analytical luminescent probes for a variety of chemical and biological applications [3–5].

Chemiluminescence can be achieved by direct reactions or by energy-transfer mechanisms. The CL phenomenon can be applied as detection technique for the monitoring of a wide variety of compounds in diverse fields, such as biomedical, environmental and can be considered as an alternative to other detection modes such as: UV/Vis spectroscopy, fluorescence [6]. In this work, the use of sensitized chemiluminescence of lanthanide ions for determination of naproxen in mixture of NSAIDs is studied. It was observed that only the CL signal of the Ce(IV)- Naproxen system was significantly increased in the presence of Eu(III) ions.

## Experimental

Ultra weak photon emission was recorded with the use of the earlier described equipment [7]. The fluorescence spectra were recorded using F-7000 HITACHI spectrofluorimeter.

## Chemicals

All chemicals used were of analytical reagent grade and doubly distilled water was used throughout the experi-

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ments. Naproxen sodium and indomethacin were from Sigma, ibuprofen sodium was from Fluka.

Their stock standard solutions ( $2 \times 10^{-3} \text{ molL}^{-1}$ ) were prepared by dissolving appropriate amount of naproxen sodium and ibuprofen sodium in water and diluting to 100 ml with water. A stock standard solution of indomethacin ( $6 \times 10^{-4} \text{ molL}^{-1}$ ) was prepared by dissolving an appropriate amount of IM with  $0.1 \text{ molL}^{-1}$  hydrochloric acid and then diluting to 100 mL with water. Working standard solutions of naproxen sodium were prepared of diluted the stock solution with water.

#### Optimization of Experimental Conditions

The optimum conditions for the naproxen determinations were carried out using the  $1 \times 10^{-6} \text{ molL}^{-1}$  concentration of NP. The effect of Ce(IV) concentration on the CL reaction was examined in the range of  $5.0 \times 10^{-4}$  to  $4.0 \times 10^{-3} \text{ molL}^{-1}$  in 0.015, 0.025, 0.05, 0.15 or  $0.3 \text{ molL}^{-1} \text{ H}_2\text{SO}_4$ . The most integrated CL light sum was obtained when the concentration of Ce(IV) was  $2.0 \times 10^{-3} \text{ mol L}^{-1}$  in  $0.015 \text{ molL}^{-1} \text{ H}_2\text{SO}_4$ . For the NP-Ce(IV)-Eu(III) system, the optimum concentration of europium(III) ions, using the following range of concentrations  $1.0 \times 10^{-4}$  -  $1.0 \times 10^{-2} \text{ molL}^{-1}$  was determined. In the case of concentration of Eu(III) ions above  $1.0 \times 10^{-3} \text{ molL}^{-1}$  the CL light intensity sums did not change, so as the optimal concentration of Eu(III) ions for the determination of NP  $2.0 \times 10^{-3} \text{ mol L}^{-1}$  was chosen.

#### Measurement Procedure

All of the chemiluminescence studies were performed in the same manner. Solution of cerium(IV) ions was added to solutions containing NSAID (1 mL solutions of appropriate concentrations) or NSAID and Eu(III) ions. The experiments were conducted in acidic solutions (pH~4). The total volume was 4 mL. The determination of naproxen in water, pharmaceutical preparations was carried out measuring the CL light intensity sums of the reaction systems applying the cut-off filters method, with the use of a filter transmitting emission of  $\lambda > 585 \text{ nm}$ , which was placed between the photomultiplier and the measurement cell. CL light intensity sums were calculated as the area under these curves [4].

#### Sample Preparation

*Tablets* Not less than 10 tablets were weighed and finely powdered. Specific amounts of analyte were weighed accurately, dissolved in water in a small beaker. The solution was filtered, and the residue was washed with water several times. Then it was transferred into a

250 mL calibrated flask and diluted to the specified volume with water. Working solutions were prepared by appropriate dilution of this sample solution so that the final analytic concentrations were within the working range.

*Ointment* Accurately weighed out amount of the ointment was dissolved in water ( $40^\circ\text{C}$ ) and 1 mL  $0.1 \text{ molL}^{-1} \text{ NaOH}$  while vigorously mixing. Next the solution was cooled in a refrigerator, in order to separate from vaseline, and transferred into a 100 ml calibrated flask and diluted to volume with water. The final concentration of NP was within the working range.

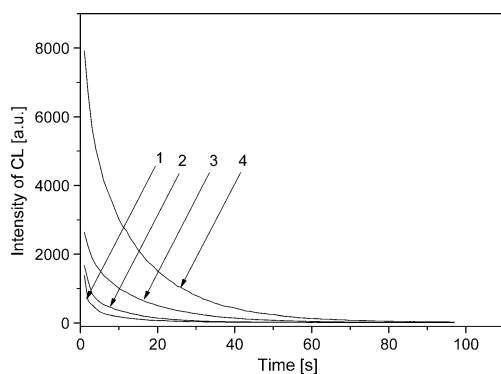
#### Urine

Urine samples were obtained from two healthy volunteers. Known amounts of standard solutions of naproxen were introduced into 1 mL of fresh urine samples. The typical naproxen urinary concentration was 0.2–20  $\mu\text{g/ml}$  [8]. This urine sample was mixed with a 10 mL of  $0.1 \text{ molL}^{-1} \text{ Ba}(\text{OH})_2$  solution and a 9 mL of  $0.1 \text{ molL}^{-1} \text{ ZnSO}_4$  solution in order to remove protein and reducing substances [9, 10]. The resulting solution was diluted to 50 mL with doubly distilled water and centrifuged at 3000 for 15 min. The supernatant solution was stored and maintained below  $5^\circ\text{C}$ . For analysis 1 ml of solution was used.

## Results and Discussion

#### Effect of Presence of the Europium(III) Ions on the CL Intensity of the Ce(IV)-NSAID Systems

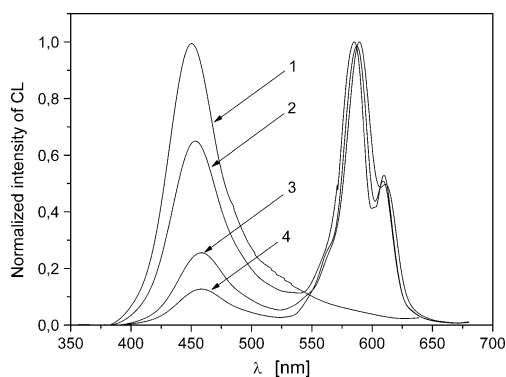
The chemiluminescence of systems containing NP or IB or IM and Ce(IV) ions as oxidizer in solution at pH~4 in the absence and in the presence europium(III) ions were studied. Chemiluminescence in the system NSAID-Ce(IV) was obtained, when the concentration of NSAID was higher than  $2 \times 10^{-7} \text{ molL}^{-1}$  for NP and  $4 \times 10^{-3} \text{ molL}^{-1}$  for IB and  $2 \times 10^{-4} \text{ molL}^{-1}$  for IM. For all systems the course of the kinetic curves of CL were similar, with maximum after initiation of the reaction by introducing Ce(IV) ions. The emission at the level of the background was obtained in about 30 s after the reaction initiation. Introduction of Eu(III) ions into the system NSAID-Ce(IV) resulted in a strong increase in the emission intensity only in the system containing naproxen without a change in the kinetic curve character (curves 3 and 4 in Fig. 1). The intensity of CL of the systems IB-Ce(IV) and IB-Ce(IV)-Eu(III) or IM-Ce(IV) and IM-Ce(IV)-Eu(III) were the same.



**Fig. 1** The kinetic curves of CL decay in the NP-Ce(IV) system versus the concentration of NP:  $6 \times 10^{-7} \text{ molL}^{-1}$  (curve 1);  $2 \times 10^{-6} \text{ molL}^{-1}$  (curve 2) and NP-Ce(IV)-Eu(III) system versus the concentration of NP:  $6 \times 10^{-7} \text{ molL}^{-1}$  (curve 3);  $2 \times 10^{-6} \text{ molL}^{-1}$  (curve 4). The concentration of Eu(III) ions was  $2 \times 10^{-3} \text{ molL}^{-1}$ ; the initial concentration of Ce(IV) was  $2 \times 10^{-3} \text{ molL}^{-1}$ , pH~4

The kinetics of the chemiluminescence decay of NP-Ce(IV) and NP-Ce(IV) – Eu(III) were monoexponential.

Spectral analysis of the chemiluminescence in the systems studied was performed using the method of cut-off filters as described earlier [11]. The spectra show a broad emission band in the range 400–600 nm, with a maximum at  $\lambda \sim 450 \text{ nm}$  for NP and 480 nm for IM or 420 nm for IB, which is typical of the CL systems with the compounds containing excited carbonyl groups as emitters [12]. The spectral distribution of CL of NP-Ce(IV)-Eu(III) system dependent on the concentration of europium(III) ions. The spectral distribution, observed for the molar ratio Eu: NP=10: 1, shows a broad band at 450 nm and a second band centered at  $\lambda = 600 \text{ nm}$  (spectrum 2 in Fig. 2) corresponding to the  $^5D_0 \rightarrow ^7F_1$  and  $^5D_0 \rightarrow ^7F_2$  transition of the Eu(III) ion [13]. For higher molar ratio (Eu: NP above 100: 1) the dominant band is band typical for Eu(III) with a maximum at  $\lambda \sim 600 \text{ nm}$  (spectrum 4 in Fig. 2). This means



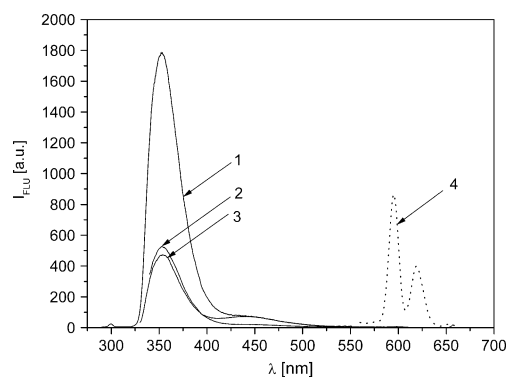
**Fig. 2** The normalized spectral distribution of CL of the systems: NP-Ce(IV) (spectrum 1) and NP-Ce(IV)-Eu(III) versus the molar ratio Eu (III): NP=10: 1 (spectrum 2); 40: 1 (spectrum 3) and 100: 1 (spectrum 4). The concentration of NP was  $1 \times 10^{-5} \text{ molL}^{-1}$ ; the initial concentration of Ce(IV) was  $2 \times 10^{-3} \text{ molL}^{-1}$ , pH~4

that the presence of the lanthanide ion is responsible for the increase in the CL intensity of NP-Ce(IV)-Eu(III) system.

In order to identify the role of the lanthanide ion in the reaction mixture NP-Ce(IV)-Eu(III), the process of Ln(III) complexation with NP in the pH range about 4–5 was studied. In the investigated pH region for all of the molar ratios used (Eu: NP=10:1; 40:1 and 100:1) the luminescence of Eu(III) was not observed, applying  $\lambda_{\text{exc}}$  typical for NP, i.e.: 270 nm, 317 nm and 327 nm. The recorded luminescence spectra show only one large unstructured band centered at around 353 nm (spectra 1, 2, 3 in Fig. 3), typical for this drug [14]. Using  $\lambda_{\text{exc}} = 394 \text{ nm}$  (typical for Eu (III) ions) the same luminescence spectrum (spectrum 4 in Fig. 3) as in the case of  $\text{EuCl}_3$  solution was obtained, which is typical for uncomplexed Eu(III) ions [11]. This indicates that the complex formation did not occur place in the chemiluminescent reaction system NP-Ce(IV)-Eu(III).

As follows from Fig. 1 the presence of Eu(III) ions have no effect on the rate of the naproxen oxidation with cerium (IV) ions. In the chemiluminescent experimental conditions europium(III) ions are uncomplexed and because of that a ligand to metal transfer process cannot appear (Fig. 3). The presence of europium(III) bands in the CL spectrum of the NP-Ce(IV)-Eu(III) system indicates that the excitation of Eu(III) ions in the reaction mixture is a result of the energy transfer process from the excited product of NP oxidation to the uncomplexed Eu(III) ions.

Oxidative decarboxylation of substituted phenylacetic acids can be mediated by metallic and nonmetallic oxidants or by electrolytic means [15]. When naproxen was treated with cerium (IV) or peroxydisulfate, the product pattern was similar to that from photolysis [16]. The major photoproducts obtained by irradiation of the drug under aerobic conditions are 6-methoxy-2-(1-hydroxyethyl)-naphthalene and 6-methoxy-2-acetyl-naphthalene, and a



**Fig. 3** The fluorescence spectrum of aqueous solution containing NP and Eu(III) ions at pH value 4–5. The concentrations of NP was  $4 \times 10^{-4} \text{ molL}^{-1}$  and Eu(III) was  $1 \times 10^{-2} \text{ molL}^{-1}$  and  $\lambda_{\text{exc}}$  were: 270 nm (spectrum 1); 318 nm (spectrum 2); 327 nm (spectrum 3) and 394 nm (spectrum 4); pH~4

**Table 1** Determination of naproxen in commercial formulations with the proposed CL method ( $n=5$ )

Drug sample	Label Amount (mg)	Found by proposed method (mg $\pm$ S.D.)	Recovery [%]
APO-NAPROXEN	250 mg	249 $\pm$ 3	99.6 $\pm$ 1.2
ALEVE	220 mg	223 $\pm$ 4	101.4 $\pm$ 1.8
NAPROXEN	500 mg	495.5 $\pm$ 7	99.1 $\pm$ 1.4
NAPROXEN	250 mg	255.3 $\pm$ 3	102.1 $\pm$ 1.2
NAPROXEN EMO gel	100 mg g <sup>-1</sup>	101.8 $\pm$ 2 mg g <sup>-1</sup>	101.8 $\pm$ 2

small amount of 6-methoxy-2-ethyl-naphthalene. The 6-methoxy-2-acetyl-naphthalene shows the emission band centered at around 440 nm [17], which correspond to the chemiluminescent spectrum.

### Analytical Application

The reaction mixture of NP-Ce(IV)-Eu(III) was used for the determination of the naproxen concentration, with applying the cut-off filters method. A linear dependence of the CL light intensity sums of the reaction system on the NP concentration, with the use of a filter transmitting emission of  $\lambda > 585$  nm, was obtained in the NP concentration range of  $4 \times 10^{-8}$  molL<sup>-1</sup> (9 ng/mL) to  $2 \times 10^{-6}$  molL<sup>-1</sup> (460 ng/mL) (correlation coefficient  $r=0.9998$ ) with the detection limit of  $1.1 \times 10^{-8}$  molL<sup>-1</sup> (2.5 ng/mL). It is six times lower than determination in reaction system without europium(III) ions [18].

### Interference

The effect of some common inorganic ion and excipient species on the determination of  $2.0 \times 10^{-7}$  molL<sup>-1</sup> naproxen solution was studied. The tolerance limit was taken as the recovery in the range 95%–105%. The tolerance ratios were as follows: 5000-fold for indomethacin, ibuprofen, K(I), Na (I), Mg(II), Ca(II); Zn(II); NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>; 500-fold for CO<sub>3</sub><sup>2-</sup>; 100-fold for Fe(III) starch, glycine, glucose; and 30-fold for

Fe(II). These tolerance ratios were higher than in system without europium(III) ions [18].

### Applications

The proposed method was applied to the determination of Naproxen in commercially available pharmaceuticals. The obtained results are shown in Table 1. As can be seen, there are no significant differences between the labeled values and those obtained by the suggested method. A mean recovery values were in the range 98–104%.

The accuracy studies for the determination of naproxen in human urine were conducted with three known concentrations of the compound. Results of recovery studies for urine are given in Table 2. Each value represents the mean of five measurements.

### Conclusion

The reaction system NSAID-Ce(IV) belongs to the group systems generating ultra-weak emission characteristic for excited carbonyl species formed as a result of NSAID oxidation. The presence of Eu(III) ions into the systems of IB-Ce(IV) and IM-Ce(IV) did not influence on CL intensity, however the Eu(III) ions are effective activators in the NP-Ce(IV) system. The CL reaction system of NP-Ce(IV)-Eu(III), as these studies have shown, can be analytically useful. This method gives a lower detection limit and a large tolerance of on the presence of inorganic ion and excipient species compared with the NP-Ce(IV) system. The proposed chemiluminescent method, based on emission of the Eu(III) ions in the NP-Ce(IV)-Eu(III) system, is simple, accurate and precise and allows the determination of naproxen in pharmaceutical preparations and in human urine.

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**Table 2** Determination of naproxen. in human urine with the proposed CL method

Sample	Added <sup>a</sup> (μg/ml)	Found $\pm$ SD ((μg/ml)	Recovery $\pm$ SD (%)
Urine-1	2	2.04 $\pm$ 0.04	102 $\pm$ 2
	4	4.02 $\pm$ 0.06	100.5 $\pm$ 1.5
	10	9.82 $\pm$ 0.16	98.2 $\pm$ 1.6
Urine-2	2	1.98 $\pm$ 0.04	99 $\pm$ 2
	6	6.02 $\pm$ 0.07	100.3 $\pm$ 1.2
	14	14.30 $\pm$ 0.15	102.1 $\pm$ 1.1

<sup>a</sup> in undiluted urine

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