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Chemiluminescence analysis of captopril: comparison between luminol and rhodamine B-sensitized cerium(IV) methods*

Z. XINRONG,‡§ W.R.G. BAEYENS,†‡ G. VAN DER WEKEN,‡ A.C. CALOKERINOS‖ and K. NAKASHIMA¶

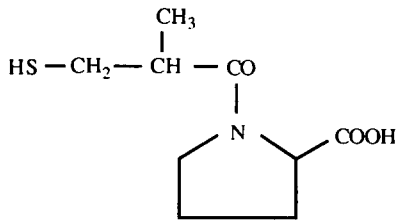
‡ *University of Ghent, Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Analysis, Laboratory of Drug Quality Control, Harelbekestraat 72, B-9000 Ghent, Belgium*
‖ *University of Athens, Chemistry Department, Laboratory of Analytical Chemistry, Panepistimiopolis 157 71, Athens, Greece*
¶ *Nagasaki University, School of Pharmaceutical Sciences, 1–14 Bunkyo-machi, Nagasaki 852, Japan*

Abstract: Two chemiluminescence methods for the determination of captopril are compared in the present paper. The first method is based on the reaction of captopril with the luminol–hydrogen peroxide–copper(II) system, the copper(II) ion acting as a key species in the light-emitting process. The catalytic activity of copper(II) decreases due to complex formation with the sulphhydrylic captopril analyte. Application of this indirect method allows captopril determinations in the 8.0–1.0 μM range as starting concentrations. Similarly, captopril was capable of generating chemiluminescence from acid cerium(IV) solutions, a reaction that could be analytically exploited by the inclusion of the rhodamine B fluorophore yielding a sensitized-type of chemiluminescence emission that allowed quantitation of captopril concentrations in the 0.1–6.0 μM range with a detection limit of 0.037 μM (original concentration). For both types of reaction the experimental conditions were optimized and a direct application was carried out on a commercial drug formulation.

Keywords: *Chemiluminescence; captopril; luminol; cerium(IV); pharmaceutical analysis; rhodamine B.*

Introduction

Captopril (1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline) (**I**) is an orally active inhibitor of the angiotensin-converting enzyme. Combination with the diuretic hydrochlorothiazide increases its strong antihypertensive effects for which it is frequently used. A variety of methods have been reported for the quantitative determination of captopril, including titrimetry [1], electroanalytical methods [2], HPLC [3, 4], gas–liquid chromatography [5], spectrophotometry [6] and fluorimetry [7].



(**I**)

Analytical procedures applying chemiluminescence (CL) methods combine the advantages of simplicity and sensitivity of detection and have been frequently used for the analysis of pharmaceutical compounds [8–15]. Some thiol-containing drugs such as cysteine, *N*-acetylcysteine, penicillamine, 2-mercaptopropionylglycine and thiouracil had been determined by the inhibition effects on the copper(II)-catalysed luminol–hydrogen peroxide CL reaction [16].

The aim of this work was to develop a CL method for the determination of the captopril thiol in pharmaceuticals, with the aim of extrapolating the results towards liquid chromatographic systems. Two CL reactions were investigated in the present paper. One involves

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†Author to whom correspondence should be addressed.
§On leave from the Department of Chemistry, Shaanxi Normal University, Xian — Shaanxi 710062, P.R. China.

the CL reaction of the copper(II)-catalysed luminol–hydrogen peroxide system in alkaline medium, copper(II) acting as a key species in the light-emitting process. The catalytic activity of copper(II) decreases due to its complex oxidation reaction with the thiolic captopril. The other reaction utilizes the CL emitting reaction between cerium(IV) and captopril in acidic medium [17]. This reaction could be sensitized by the use of the strong rhodamine B fluorophore yielding a sensitized-type of CL emission providing a 9-fold improved sensitivity when compared to the reaction without sensitizer. Both procedures for the determination of captopril samples were optimized and the proposed methods were compared and applied to the assay of captopril in a commercial formulation.

Experimental

Apparatus

CL emission was measured using a Bio-Orbit (LKB Wallac) 1250-Luminometer equipped with two Bio-Orbit automatic injectors (Bio-Orbit Dispenser SVD and Bio-Orbit Dispenser Controller DC). No wavelength discrimination is involved. CL data were handled by an IBM-compatible computer (PC Systems) employing the luminometer software for graphical viewing of the measured values and for calculation. Printing was done on a Citizen 120D matrix printer.

Reagents

All chemicals were of analytical or reagent grade and the solutions were prepared with distilled deionized water. Luminol 5.0 mM solution was prepared by dissolving luminol (Fluka, Switzerland) in NaOH medium at pH 13; hydrogen peroxide 1.0 mM solution was freshly prepared by dilution of the 30% H_2O_2 (Merck, Darmstadt, Germany) solution with water; the Cu(II) ion solution of 20 μM was prepared by dissolving $CuSO_4 \cdot 5H_2O$ (Merck, Darmstadt, Germany) in water; Ce(IV) ion solution of 1.0 mM was prepared by dissolving $Ce(SO_4)_2$ (UCB, Leuven, Belgium) in 0.1 M H_2SO_4 medium and an aqueous rhodamine B (UCB, Leuven, Belgium) solution of 20 μM was used.

Stock captopril solutions of 1.0 mM were freshly prepared by dissolving captopril (the Sigma Chemical Company, St Louis, USA) in water. When not in use they were stored at about 4°C in a dark bottle. The analytical standard solutions were prepared by diluting an accurately measured volume of the stock solution in the proposed medium.

Procedures

Luminol–H_2O_2–Cu(II)–captopril system. Luminol is oxidized in alkaline solution to produce the excited-state aminophthalate dianion which is the light emittor, this reaction is catalysed by Cu(II)-ions.

An accurately measured volume of standard captopril or sample solution is mixed with 5.0 ml 20 μM Cu(II) ion solution and diluted to 50 ml with aqueous 0.1 M H_2SO_4 solution providing a solution with a final pH of 2.0. A 0.5-ml portion of this solution is pipetted into the CL cell which is positioned into the cell holder of the luminometer. After rotating the cell holder to the detection position, 0.25 ml of the 1.0 mM H_2O_2 solution is injected into the CL cell by means of the automatic injector A and 0.5 ml luminol of 5.0 mM solution is subsequently injected into the CL cell by the automatic injector B with a 2 s delay time. The produced CL signal is sent to the computer for graph viewing of the measured values, calculations, printing and saving of data.

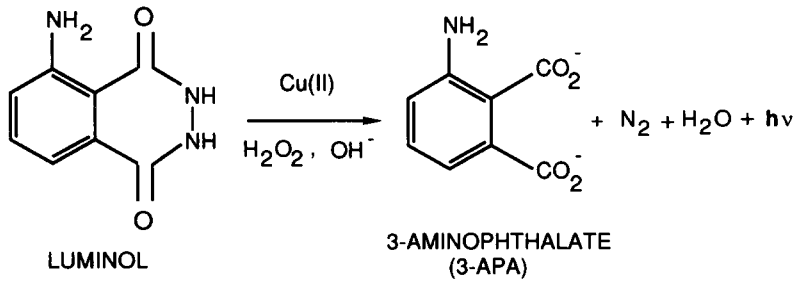
Cerium(IV)–captopril system. An accurately measured volume of standard captopril solution or sample solution is mixed with 5.0 ml 20 μM rhodamine B solution and diluted to 50 ml with aqueous 1.0 M H_2SO_4 solution providing a solution with a final acid concentration of 0.1 M H_2SO_4. A 0.5-ml portion of this solution is pipetted into the CL cell which is positioned into the cell holder of the luminometer. After rotating the cell holder to the detecting position, 0.5 ml of the 1.0 mM Ce(IV) ion solution in 0.1 M H_2SO_4 is injected into the CL cell by the automatic injector. The produced CL signal is sent to the computer for graph viewing of the measured values, calculations, printing and saving of data.

All measurements are performed at ambient temperatures.

Results and Discussion

CL reaction of captopril with luminol–H_2O_2–Cu(II)

Luminol is oxidized in alkaline solution to produce the excited-state aminophthalate dianion which is the light emitter, the chemi-



luminescence reaction being catalysed by Cu(II)-ions as shown in Scheme 2 above.

Several experiments are cited in literature to establish optimal CL intensities from the luminol–H_2O_2–Cu(II) reaction [18–20]. As the present method is based on the CL *quenching* effect by captopril of the luminol–H_2O_2–Cu(II) reaction, reagent concentrations were optimized so as to obtain optimum CL quenching conditions.

Negative peaks appeared because the catalytic activity of Cu(II)-ions decreases on complexation with captopril

$$Cu^{2+} + RSH \rightarrow Cu\,(RS^{-})^{+} + H^{+}$$

or

$$Cu^{2+} + 2RSH \rightarrow Cu\,(RS^{-})_2 + 2H^{+}$$

(RSH: captopril)

No catalytic activity is observed when all four coordination sites are occupied by the ligands. Comments on the reaction mechanism can be found in reference (17).

Variation of pH and concentration of luminol solutions were studied to achieve a maximum rate of non-quenched CL intensity ($I_{CL(0)}$) to quenched CL intensity ($I_{CL(Q)}$), ($I_{CL(0)}/I_{CL(Q)}$). The results showed that the optimum was situated at a 5.0 mM luminol concentration and pH 13. The effect of the H_2O_2-concentration was studied in the range 0.5–10 mM; only slight changes of the $I_{CL(0)}/I_{CL(Q)}$ value was observed within this range (Fig. 1); therefore, a 1.0 mM hydrogen peroxide solution was used for further work.

Under the selected experimental conditions described above, the calibration graph was linear in the captopril concentration range of 1.0–8.0 μM, the regression equation being $I_{CL} = -0.104C + 0.960$, $r = 0.997$. RSD for 1.0 μM captopril: 4.16% ($n = 10$).

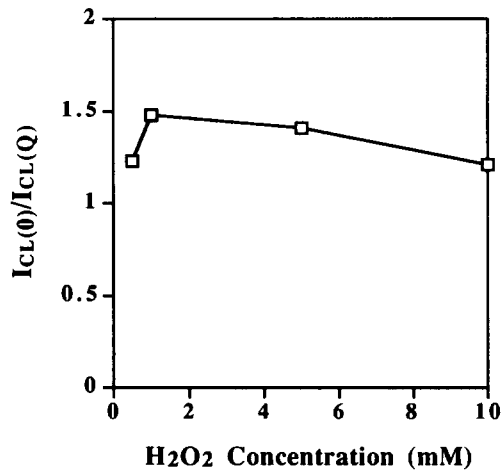


Figure 1
Effect of H_2O_2 concentration on the inhibition of the luminol–H_2O_2–Cu(II) CL reaction with captopril. Luminol concentration: 5.0 mM at pH 13, Cu(II) ion: 2.0 μM; captopril concentration: 1.0 μM.

CL reaction of captopril with rhodamine B-sensitized Ce(IV)

The CL properties of fluorophore-sensitized Ce(IV) reactions with sulphite in sulphuric acid medium have been widely investigated by Calokerinos' group from Athens [21–23]. It was shown that Ce(IV) may not only react with sulphite but also with some thiols in sulphuric acid medium. Captopril is an excellent example-molecule to be determined by the actual CL reaction, and a new method for captopril determination was suggested by this group elsewhere [17]. The conditions for the production of analytically useful CL emission from the cited reaction were optimized so as to achieve maximum light emission. The influence of Ce(IV) and H_2SO_4 concentrations were studied in the 0.1–50 mM and 0.01–0.5 M ranges, respectively, employing a fixed captopril concentration of 5.0 μM. Maximum CL intensities are obtained for 1.0 mM Ce(IV) and 0.1 M H_2SO_4 concentrations, concentrations higher than 1.0 mM Ce(IV) caused a

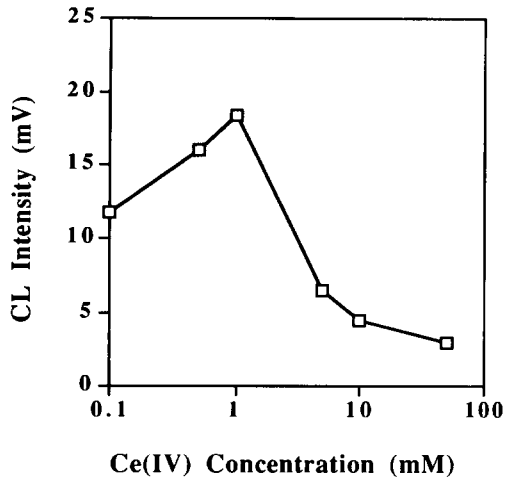


Figure 2
Effect of Ce(IV) concentration on rhodamine B-sensitized cerium-induced captopril CL emission. Ce(IV) is prepared in 0.05 M H_2SO_4 medium; captopril concentration: 5.0 μM.

marked decrease of CL signals (Fig. 2), to be attributed to the absorption of the CL emission by the excess Ce(IV) ions.

Some fluorescing compounds have been studied for use in energy transfer CL reactions of Ce(IV) with sulphite [21–23]. Based on these experiments, trials were performed with respect to the enhancement of the measured CL signals by some fluorescing substances. The results showed that rhodamine B is a good sensitizer which increases the CL efficiency of the system about 9-fold; therefore, this dye was used for subsequent studies. The optimum concentration range of rhodamine B for this purpose is 1.0–2.0 μM.

Under the selected experimental conditions for rhodamine B described above, the calibration graph was linear over the 0.1–6.0 μM captopril range and the regression equation was: $I_{CL} = 0.019C + 0.001$ ($r = 0.998$). The detection limit was 37 nM of starting captopril solution. The RSD for 1.0 μM captopril: 3.12% ($n = 10$).

Applications

In order to apply these methods for the analysis of a pharmaceutical dosage form, the influence of commonly used excipients and tablet additives for captopril were investigated. Captopril solutions of 5 μM were used for these experiments. No interferences could be observed when up to a 10-fold excess of lactose, saccharose, glucose, cellulose or starch was added to the original sample to be extracted, for either of the methods. The results for the determination ($n = 10$) of captopril in the pharmaceutical dosage form are shown in Tables 1 and 2. As can be seen, there are no significant differences between the labelled value and those obtained by the proposed methods.

Conclusion

Two analytical CL reactions for captopril determination are proposed in this paper, applying the alkaline luminol–H_2O_2–Cu(II) and the acid Ce(IV) system. The reaction of Ce(IV) with captopril in acidic medium proves to be 10-fold more sensitive than that with luminol–H_2O_2–Cu(II) in alkaline environment. The linear range of captopril using the luminol–H_2O_2–Cu(II) system was also limiting as compared with the Ce(IV) method, since the former is based on CL quenching effects.

Table 1
Determination of captopril in a pharmaceutical preparation by CL reaction with the luminol–H_2O_2–Cu(II) system

Sample	Amount (mg)		Added (mg)	Recovered (mg)	Recovery (%)
	Label	Found ± SD ($n = 10$)			
Capoten^R (tablets)	25.0	24.91 ± 0.73	20.0	44.37	98.6
			40.0	67.67	104.1

Table 2
Determination of captopril in a pharmaceutical preparation by CL reaction with the Ce(IV) system in acidic medium

Sample	Amount (mg)		Added (mg)	Recovered (mg)	Recovery (%)
	Label	Found ± SD ($n = 10$)			
Capoten^R (tablets)	25.0	26.2 ± 1.15	10.0	33.4	95.4
			20.0	44.8	99.5

Both methods could be applied to the assay of captopril in a commercial formulation.

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