Flow Injection Chemiluminescence Determination of Captopril with In Situ Electrogenerated Mn^{3+} as the Oxidant

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Abstract
By designing a novel flow-through electrolytic cell, a new flow-injection electrogenerated chemiluminescence method for the determination of captopril is described. It is based on the direct chemiluminescence oxidation of captopril by nascent Mn^{3+} which was in situ electrogenerated on the near surface of the platinum flake electrode by electrochemical oxidizing $MnSO_4$ in sulfuric acid medium. The proposed procedure has a linear application range of 3.0×10^{-7}–1.0×10^{-4} mol/L for captopril ($r=0.9998$) and with a detection limit of 8.0×10^{-8} mol/L original concentration. Designing of the flow-through electrolytic cell as well as some experimental conditions for the determination of captopril are optimized and the possible reaction mechanism is also discussed. The method was successfully used for the determination of captopril in a pharmaceutical preparation.

Keywords: Captopril, In situ electrogenerated Mn^{3+}, Chemiluminescence, Flow-injection analysis, Pharmaceutical analysis

1. Introduction

Captopril, 1-[(2s)-3-mercapto-2-methylpropionyl]-L-proline (Figure 1), is an orally active inhibitor of the angiotensin-converting enzyme and is widely used for the treatment of hypertensive disease on its own or in combination with other drugs [1]. This compound can also be used to treat congestive heart failure [2]. Although several analytical methods, including spectrophotometry [3], chromatography [4–8] and capillary zone electrophoresis (CZE) [9], have been reported for the quantitative determination of captopril, these methods suffer from poor sensitivity, time-consuming and requiring expensive instrumentation.

Chemiluminescence (CL) analysis offers high sensitivity, wide linear range and simple instrumentation [10–15] and has been successfully applied to the determination of captopril by using Ce^{4+} and rhodamine B or rodamine 6G as analytical reagents [14–15]. But the reported CL methods for captopril suffer from the expensive reagent (Ce^{4+}) consumption. In recent years, electrogenerated chemiluminescence (ECL) analysis have received much attention and many analytical applications have appeared in the literature [16–21]. In ECL analysis, a chemiluminescent reaction is often produced in the vicinity of an electrode when a potential is applied to it. Reagents required, such as highly redox intermediates, can be produced in situ from passive precursors in the carrier stream, and the CL detector can be designed for the maximum sensitivity. Moreover, the ECL reaction cell can be designed to achieve effective detection of the rapid ECL signal since the all ECL reaction procedure, including the initial step of electrogenerated reagent and the subsequent CL reaction procedure, could be restricted to the near surface of the electrode. Compared with CL analysis, the ECL analysis not only retains the advantages of the conventional CL analysis, but also possesses some special advantages over the conventional CL analysis. Such as the better sensitivity by electrogenerating in situ the highly active CL reagents [22], wider application fields by electrochemically modified analyte into the CL active species to achieve its ECL analysis [19, 23, 24]. However, some disadvantages such as the electrode fouling, poor application of linear range due to the smaller area of the electrode etc., still existed and limit the wide application of ECL analysis [25]. For resolving these problems, many schemes, including the pretreatment of the electrode with electrochemical or a ultrasonic method, designing different structure electrolytic flow-through cells etc. have been proposed for this purpose [26–28], but the ECL analysis suffers from time-consuming, poor linear range, poor repeatability and the complicated structure of ECL cell.

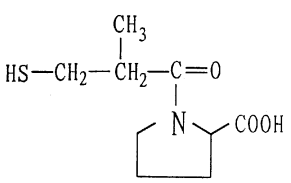

Fig. 1. Chemical structure of captopril.

In this article, by designing a novel and simple flow-through electrolytic cell (FEC), the nascent Mn^{3+}, a useful coulometric titrant [29] and an effective CL oxidant in some MnO_4^--based CL systems [30], can be easy, quantitative generated in the near surface of the platinum flake electrode by galvanostatic oxidizing $MnSO_4$ in H_2SO_4 medium. Then, it was found that the spatial zone of the nascent Mn^{3+} concentration distribution in the near surface of the working electrode could be easily adjusted by the flow-injection technique, and the subsequent CL reaction procedure of nascent Mn^{3+} with analyte injected could be made in the farther place with respect to the surface of the electrode to avoid the electrode fouling problem. Thereafter, it was further found that the nascent Mn^{3+} could oxidize captopril, which was injected into the flow cell, to generate strong and rapid CL signals. Based on this observation, a new, sensitive and rapid flow-injection ECL method for the determination of captopril is proposed and the disadvantages, which the conventional ECL method has, such as smaller emission area, electrode fouling etc. was effectively improved in the proposed flow-injection ECL system.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-reagent grade materials with distilled, deionized water. Stock solution of

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captopril (1.0×10^{-3} mol/L) was prepared by accurately weighing the pure compound obtained from Sigma Chemical Company into a 50 mL calibrated flask and diluting to volume with water. When not in use, the stock solution was kept at about 4 °C in a dark bottle. Testing standards solution was prepared daily by appropriate dilution of the stock solution with water. 0.050 mol/L $MnSO_4$ solution was obtained by dissolving 8.5 g of $MnSO_4$ (Xi'an Chemical regeants factory, China) in 5.0 mol/L sulfuric acid and diluted to 1 L with the same concentration of sulfuric acid.

2.2. Apparatus

Figure 2 is a schematic diagram of the proposed FIA-ECL system for the determination of captopril. An R456 photomultiplier tube (PMT) (Hamamatsu) was used for the detection of ECL emission signal, the constant current applied for electrolysis was achieved with a JH2C potentiostat/galvanostat (Shanghai Second Component Factory, China). The flow-through electrolytic cell was made with a transparent glass tube (length: 2.5 cm, i.d.: 0.5 cm) and utilized a conventional two-electrode setup (as Figure 3); 0.50 cm^2 Pt flake and a stainless steel tube (length 2 cm, i.d.: 3 mm) were used as the working electrode and counter electrode, respectively. The flow-cell was enclosed in a light-tight box and placed directly in front of a PMT. The output signal was recorded using an XWT-204 recorder (Shanghai Dahua Instrument and Meter Plant). All reagents were delivered by two peristaltic pumps and a six-way injection valve equipped with an 80 μL injection loop was used to introduce the sample into carrier stream. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system.

2.3. Procedures

The electrode was cleaned prior to the first experiment by washing it in 3 mol/L HNO_3, followed by washing with distilled water. All solutions were pumped by two peristaltic pumps. After 10 mA electrolytic current was applied to the flow-through electrolytic cell for 3 min electrolysis, 80 μL of the captopril sample solutions was injected into the carrier stream, the ECL signal was recorded and the concentration of captopril was quantified by the peak height of the ECL intensity.

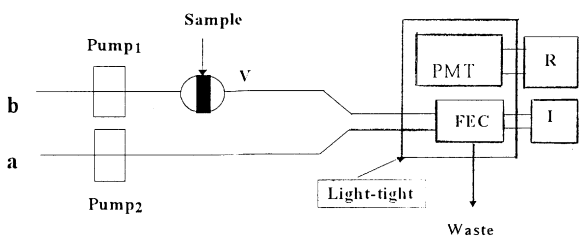


Fig. 2. ECL Flow-injection manifold for captopril determination. a) Electrolyte stream; b) H_2O carrier stream. I: JH2C potentiostat; FEC: flow-through electrolytic cell; V: injection valve; Pump1 and pump2: peristaltic pump; PMT: R456 photomultiplier tube; R: recorder.

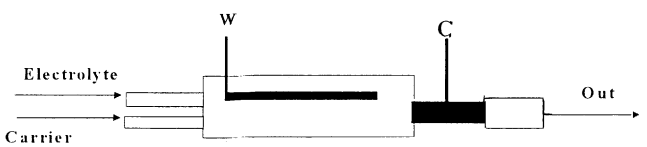


Fig. 3. The construction of the flow-through electrolytic cell. W: working electrode; C: counter electrode.

3. Results and Discussion

3.1. Design of the Flow-Through Electrolytic Cell

Although many kinds of FECs, including three-electrode setup [31] relating to constant potential electrolytic method and two-electrode setup [32] relating to constant current electrolytic method, for ECL analysis have been reported and have been widely used in ECL analysis, the former had the electrode fouling problem and the latter had the active lowering of the electrogenerated reagent.

In order to either retain the nascent state CL reacting active of in situ electrogenerated Mn^{3+} or make the subsequent CL reaction of Mn^{3+} with analyte injected in the farther place relative to the surface of the electrode to avoid the electrode fouling problem, we found that the combination of a two-electrode setup FEC, in which the working electrode was located on the front of the CL detector (PMT) and the counter electrode was down stream, with the constant current electrolytic method offer the better ECL analytical performance for captopril and the structure of the two-electrode setup was the key factor. For obtaining the best ECL analytical performance of this FEC for captopril, some important factors were studied and stated as following:

First, it was found that the distance between the working electrode and the counter electrode was one of the important parameters for the ECL analysis of captopril and the main reason may be that the electrolytic efficiency of electrogenerating Mn^{3+} was more depended on this distance. While the areas of the two electrodes, $MnSO_4$ concentration, captopril concentration and other experimental conditions were fixed, respectively, the results investigated showed that the ECL emission intensity increased with the decreasing of this distances in the range of 12–5 cm and nearly was constant in the range of 5–3 cm. Below 3 cm, the ECL emission intensity signal was unstable. Thus, 4 cm was selected as the optimum distance between the two electrodes.

Secondly, the ECL reaction cell should offer enough Mn^{3+} distributing zone in the near surface of the platinum flake electrode to have abundant spatial interfaces of Mn^{3+} to react with captopril. For this purpose, both the diameter and the volumes of the FEC were found to be the important factors. Further results showed that the electrolytic cell, which diameter and length were 4 mm and 2 cm, respectively, presented the best analytical performance.

In addition, to avoid the electrode fouling from the injection of sample solution, the distance between the surface of the working electrode and the pathway, which the carrier stream pass through the electrolytic cell, was the key factor. The results investigated showed that while the pathway of the carrier stream in the cell is about 3 mm below the surface of the working electrode, the stable and the strongest ECL signals for the determination of 2 μmol/L captopril can be observed. So the parallel inserting mode flow cell (as shown Figure 3) was chosen for further experimentation.

3.2. Optimization of Experimental Variables

A series of experiments was conducted to establish the optimum analytical variables. The parameters optimized included

electrolytic currents used for generating Mn^{3+} and the components of electrolyte and some physical variables, including the total flow rate.

3.2.1. *Effect of Electrolytic Current Used for Electrogenerating Mn^{3+}*

In this flow system, the Mn^{3+} concentration used for the CL reaction was on-line adjusted by the varying of both the electrolytic current and flow rate of electrolyte.

The effect arising from the Mn^{3+} concentration, which was controlled by the electrolytic current, on the ECL intensity of detecting 1.0×10^{-6} μmol/L captopril was investigated when the flow rate of the electrolyte was fixed at 2.0 mL/min. As shown in Figure 4, while the electrolytic current changed in the range of 2–8 mA, the ECL emission intensity increased dramatically with the increasing values of the electrolytic current. This may be due to the concentration of the ECL oxidant (Mn^{3+}) being increased with the increasing of electrolytic current. Above 8 mA, the ECL emission intensity was not only nearly constant, but also reached its maximum values. One possible reason is that this value of electrolytic current can produce suitable Mn^{3+} concentration to make the subsequent CL reaction. Thus, 10 mA electrolytic current was selected in subsequent experiments.

3.2.2. *Effect of the Components of Electrolyte*

The initial test results showed that the oxidation of captopril by Mn^{3+} was accompanied by the ECL emission signal only when the ECL reaction was made in acidic medium. Different acids contribute differently to ECL intensity since the nature and concentration of the acid added to the reaction solution influence the potential and effective concentration of Mn^{3+}. The effect of four common acids, H_2SO_4, HNO_3, H_3PO_4, HAc along with $HClO_4$ on the ECL intensity were studied, the ECL intensity from the oxidation of captopril by Mn^{3+} electrogenerated with 9 mA electrolytic current in the available concentration range of various acids were made, respectively. The results indicated that the sulfuric acid medium was the best one.

Based on both the initial results above and the conditions of coulometric titration with Mn^{3+} proposed by Selim et al. [29], the electrogenerating Mn^{3+} concentration is greatly affected by not only the electrolytic currents, but also the concentrations of both $MnSO_4$ and H_2SO_4 in the electrolyte. So the components of electrolyte would have great effects on the ECL intensity for the determination of captopril. These effects were investigated, respectively.

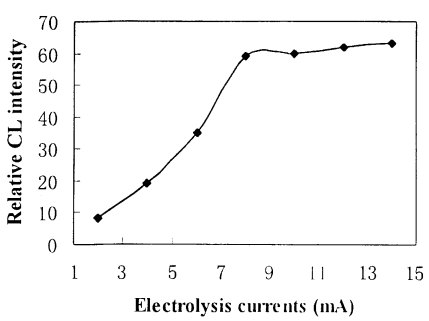


Fig. 4. Effect of electrolysis currents for generating Mn(III) on CL intensity. $MnSO_4$: 0.10 mol/L; H_2SO_4: 5.0 mol/L; concentration of captopril: 4 μmol/L; flow rate: 2.0 ml/L.

The effect of $MnSO_4$ concentration was first investigated. The results were as follows: When the $MnSO_4$ concentration is lower than 0.020 mol/L, the electrogenerated Mn^{3+} concentration is too small to be used for the determination of captopril; when the $MnSO_4$ concentration is changed from 0.020 to 0.050 mol/L, the ECL intensity increases with increasing $MnSO_4$ concentration. Above 0.050 mol/L $MnSO_4$, the ECL intensity rapidly decreased. The possible reason is that the ECL emission spectrum is partly overlapped with the absorbed spectrum of Mn^{2+}. Thus, 0.050 mol/L $MnSO_4$ was selected for subsequent studies.

The concentration of H_2SO_4 in the electrolyte controlled not only the Mn^{3+} effective concentration [29], but also the ECL reaction medium. To examine the influence of the H_2SO_4 concentration on the ECL intensity of 1.0×10^{-6} mol/L captopril, all other reagents concentrations and other parameters were held at their optimum values, the concentration of H_2SO_4 in the electrolytic solution was changed (range: 1.0–6.0 mol/L), the results showed that the ECL intensity was increased over the range 1.0–4.0 mol/L H_2SO_4. The possible reason is that the effective Mn(III) concentration and its oxidizing ability increased with increasing H_2SO_4 concentration [29]. Above 4.0 mol/L, the ECL emission intensity was nearly constant and reached its maximum values, the possible reason is that 4.0–6.0 mol/L H_2SO_4 acid medium may not only be the better ECL reaction acidic condition but also can provide enough effective Mn(III) to make CL reaction. Thus 5.0 mol/L H_2SO_4 was selected as the acid medium of electrolyte.

3.2.3. *Effect of Flow Rate*

Based on the principal of FIA and our preliminary testings, we found that the flow rate of electrolyte not only controlled the Mn^{3+} concentration in the nearby surface of the electrode but also had an important effect on the ECL intensity when the electrolytic currents and flow rate of the carrier stream were fixed at 9 mA and 2.0 mL/min, respectively. The effect of the flow rate of the electrolyte on the ECL signal was studied in the range of 0.5–3.0 mL/min and the results showed that the ECL signal increased with the increasing of the electrolyte flow rate from 0.5 to 2.0 mL/min and reached its maximum at 2.0 mL/min, above which the ECL signal decreased with increasing electrolyte flow rate. 2.0 mL/min was selected as the optimum flow rate of the electrolyte.

3.3. Performance of ECL Flow System for Captopril Measurements

Under the selected conditions given above, the response to captopril concentration was linear in the range of 3.0×10^{-7}–1.0×10^{-4} mol/L with a detection limit of 8.0×10^{-8} mol/L (signal/noise = 3). The regression equation was $I = 6.99 + 6.89 \times C$ (where I is relative CL intensity and C is the captopril concentration, unit was μmol/L). The correlation coefficient was 0.9998.

3.4. Interference

In order to apply the suggested method to the analysis of a pharmaceutical dosage form or a serum sample, the interference of commonly used excipients and additives or the other component was investigated for determination of 4 μmol/L

Table 1. Recovery of 4 μmol/L of captopril from various additives used as excipients.

Additive	*Additive/captopril concentration ratio*	*Recovery (%) (n = 3)*
Starch	1000	98.4
Lactose	1000	101.1
Galactose	1000	103.5
Sucrose	500	99.5
Cellulose acetate	1000	97.8
Ca^{2+}	1000	99.6
Mg^{2+}	1000	102.3
Na^{+}	1000	100.2
K^{+}	1000	102.4
Cl^{-}	1000	100.4
PO_4^{3-}	1000	101.6
Lactic acid	500	98.2
Uric acid	500	99.8
Cholesterol	500	103.2
Tartrate	500	100.5
Citric acid	200	102.2
Oxalate	200	101.4
Protein (BSA)	200	99.5

captopril solutions. A substance was considered not to interfere when the variation in captopril peak height was less than 5 %. The results are shown in Table 1.

3.5. Application

The method was applied to the determination of captopril in tablet form. The results are shown in Table 2. In order to evaluate the validity of the proposed method for the determination of captopril in pharmaceuticals, recovery studies were carrierd out on samples to which known amounts of captopril were added. As for the standard UV absorption based *I-C* determinations, recoveries above 97 % were found.

3.6. Discussion of the ECL Reaction Mechanism

In order to explain the possible ECL mechanism of this ECL reaction and propose a possible emitter, a series of studies for this purpose were made and the results were shown as following:

First, when no electrolytic currents were applied to the working electrode, no ECL signal was observed when 4 μmol/L captopril was injected into the FEC, and a stronger ECL signal was detected when the electrolytic currents were applied to the FEC. At the same time, the absorbed spectrums of the on-line products electrogenerated were obtained with a UV-spectrophotometer Model-752 (Shanghai Third Analytical Instrument Plant), the result (as shown in Figure 5) agreed well with that of Selim et al. [29], and also suggested that the electrogenerating Mn^{3+} was obtained by the FI system proposed in this article. These results suggested that the electrogenerated product, Mn^{3+}, was the CL oxidant of captopril.

Table 2. Determination of captopril in a pharmaceutical preparation by proposed CL method.

	Amount (mg)				
Sample	*Label*	*Found ± SD (n = 7)*	*Added (mg)*	*Recovered (mg)*	*Recovery (%)*
Captopril Tablets	25.0	24.7 ± 1.12	10.0 20.0	35.2 44.7	97.4 101.2

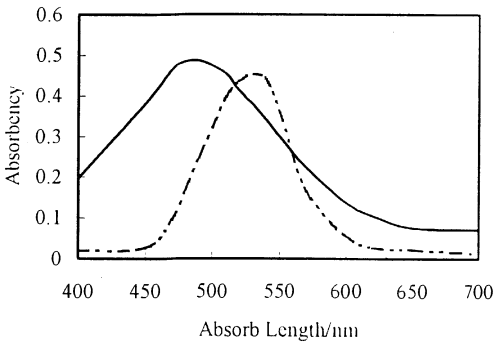
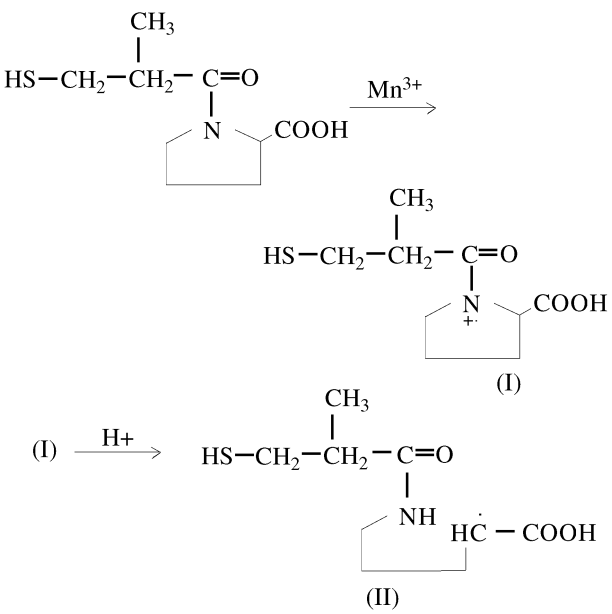


Fig. 5. Absobent spectrum of Mn^{3+} in H_2SO_4 medium. The Mn^{3+} was obtained with 10 mA electrolytic current, the medium was 5.0 mol/L H_2SO_4, – - - –) 5×10^{-4} mol/L MnO_4^-, —) Mn^{3+}.

Secondly, when nitrogen or oxygen was bubbled through solutions used, the ECL signal decreased with nitrogen and increased with oxygen. These results suggested that the dissolved oxygen took part in this ECL reaction procedure.

From previous electrochemical and chemical studies, the oxidation of tertiary amines is understood to produce a strongly reducing intermediate [33–35]. Because the Mn^{3+} possesses the stronger oxidizing ability ($E^0_{Mn^{3+}/Mn^{2+}} = 1.50$ V), the tertiary amine group in the molecule of captopril may be oxidized by the Mn^{3+} to produce a strongly reducing intermediate by a similar way to that of other tertiary amines with electrochemical oxidation [33–35]. At the same time, this strongly reducing intermediate could further reduce the dissolved O_2 to generate the $HO_2^{\bullet}$ free radicals in acidic medium, and the $HO_2^{\bullet}$ could attack each other, giving a molecular pair $(O_2)_2^*$, the possible emitter [36, 37], and the light emitted when the excited state O_2^* went to its ground state. On the other hand, when some scavenging agent of the hydroxyl free radical, such as ascorbic acid, benzoic acid and sulourea was added to the captopril solution, respectively, the relating ECL signals decreased sharply. These results also suggested that the hydroxyl free radical may be produced in the proposed ECL reaction. So we proposed the possible ECL pathway would be as following reactions:

$$Mn^{2+} - e \longrightarrow Mn^{3+} \text{ (electrochemically oxidation)}$$



$$(II) + O_2 + H^+ \longrightarrow HO_2^*$$

$$2HO_2^\bullet \longrightarrow (O_2)_2^*$$

$$(O_2)_2^* \longrightarrow 2O_2 + \text{Light}$$

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5. References

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