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Original Research Article

A chemiluminescence reagent free method for the determination of captopril in medicine and urine samples by using trivalent silver

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ABSTRACT

A novel flow-injection chemiluminescence (FI-CL) method free of CL reagent was developed for the determination of captopril based on its enhancing effect on the CL derived from diperiodatoargentate(III)-sulfuric acid system. Compared with the conventional CL system, the CL system based on trivalent silver was characterized of good selectivity for the absence of CL reagent. The CL mechanism was discussed through CL spectra and UV–vis absorption spectra. The conditions of the FI-CL system were investigated and optimized. Under the optimal conditions, the relative CL intensity was linear with the captopril concentration in the range of 0.3–15.0 μg/mL. The detection limit for captopril was 0.05 μg/mL, and the relative standard deviation (n=11) was 2.0% for 5.0 μg/mL captopril. The proposed method was applied to the analysis of captopril in tablet and human urine with the recoveries of 83.1%–112.5%, and the relative standard deviations of 0.5%–4.4%. The results obtained by the proposed method agreed well with those obtained from HPLC method. The proposed method is fast, convenient, and cost-effective for the determination of captopril in medicine and biological samples.

1. Introduction

Captopril, 1-(2S)-3-mercapto-2-methyl propionyl-L-proline (Fig. 1), is an angiotensin converting enzyme (ACE) inhibitor, which reduces peripheral resistance and lowers blood pressure [1,2]. It is widely utilized in the treatment of hypertension and some types of congestive heart failure [3,4]. However, improper dosage or overdose of captopril would have side effects such as dizziness, nausea, and intense coughing [5].

Various methods available for the determination of captopril include titrimetry [6], high-performance liquid chromatography (HPLC) [7,8], capillary electrophoresis [9], spectrophotometry [10–12], surface-enhanced raman spectroscopy [13], and voltammetry [14]. However, they have some disadvantages. For example, captopril has weak ultraviolet absorption located at the end of the ultraviolet spectrum, and has no fluorescent property. Therefore, determination methods such as spectrophotometry methods with UV detector or fluorescence detector generally require some extra derivatization work before the detection to enhance the sensitivity of the method. For example, El-Didamony [10] reported UV methods for the indirect detection of captopril. It was based on the bromination of captopril with a solution of excess brominating mixture in hydrochloric acid medium. Vicentini et al. [12] introduced a flow injection-spectrophotometric method for the determination of captopril, which was based on the formation of silver mercaptide between the captopril and Ag(I). However, deviation makes the operation tedious and may produce errors. And some of the above-mentioned methods may have shortcomings such as laborious and time-consuming operation, and requirement of special instrument and skilled operators.

Chemiluminescence (CL) method has received much more attention for analysis of organic and inorganic species owing to its low detection limit, high sensitivity, wide linear dynamic range, short response time and relatively simple instrument. It has been extensively applied in different fields of analytical chemistry [15–17]. Determination of captopril based on CL had been reported previously; however, most of the conventional CL methods involved expensive or poisonous CL reagents such as luminol [18,19], bipyridine ruthenium [20], and rhodamine 6G [21]. The instability of some CL reagents may cause some errors and reduce the selectivity and sensitivity of the methods [22]. Therefore, it is still necessary to develop a new CL system with relatively cheaper and greener reagents.

The polydentate chelates of transition metals in the superior oxidation state are powerful oxidants of high stability in certain

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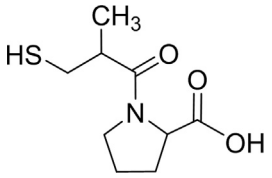

Fig. 1. The structure of captopril.

conditions and may produce free radicals in the process of the chemical reaction. The special character enables them to be regarded as CL oxidant. Diperiodatoargentate(III) (DPA, $Ag(HIO_6)_2^{5-}$), one kind of the superior oxidation state complexes, can exist stably in alkaline media by suitable multi-tooth ligand complexing generally [23,24]. DPA is a powerful oxidant in alkaline medium with the reduction potential of 1.74 V owing to the strong versatile nature of the two electron oxidants [25,26]. It has been widely used in the study of reaction kinetics and oxidation mechanism [27,28]. In recent years, DPA has been reported to be applied in CL for its strong oxidizability and catalytic properties [29,30]. The DPA-based CL system has been developed for the detection of 10-hydroxycamptothecin [30], penicillin antibiotics [31], fluoroquinolones synthetic antibiotics [32] and so on. It contains two types with one is related to CL reagents such as luminol [33] and the other is absent of CL reagents [29]. With DPA involved as CL oxidant, the CL reaction free of CL reagents possesses high selectivity and high sensitivity.

In this work, a flow injection (FI) method free of CL reagents for the determination of captopril was developed. The weak CL of DPA in acid condition can be greatly enhanced in the presence of captopril without CL reagent. Based on this principle, a novel CL method was established for the determination of captopril by combining with FI technique. The method was successfully applied for the determination of captopril in pharmaceutical formulations and human urine. The possible CL reaction mechanism was also investigated through UV–visible spectroscopy and CL spectrum.

2. Experimental

2.1. Materials

Captopril was purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium periodate (KIO_4), potassium persulfate ($K_2S_2O_8$) and argentum nitricum ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Potassium hydroxide (KOH) and concentrated sulfuric acid (H_2SO_4) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). All of the chemicals were of analytical reagent grade without further purification. Deionized water was used throughout the work.

The DPA complex was prepared by oxidizing Ag(I) compound in an alkaline medium according to the known literature [34]. In brief, KIO_4 (3.24 g), $AgNO_3$ (1.36 g), $Na_2S_2O_8$ (3.0 g) and KOH (8.0 g) were added into 200 mL of deionized water. The mixture was heated to boil and reflux on a hot plate with constant stirring. The mixture turned red and the boiling was continued for another 20 min until completion of the reaction. The mixture was then cooled and filtrated. The obtained DPA complex was stored under refrigeration and shielded from light, thus being stable for several months. After two months of storage, the appearance of DPA stock solution (3.2 mol/L) was checked and its concentration was remeasured with a UV/visible spectrophotometer. The captopril stock solution was 1.0 mg/mL, prepared by dissolving appropriate amount of standard substance with deionized water. The captopril working standard solutions were prepared from the stock solution by corresponding dilutions. The complex was characterized by the UV–visible spectrum, which exhibited a brood band at 361 nm with the molar absorptive coefficient (ε) of 1.26×10^4 L/(mol·cm).

2.2. Apparatus

The FI-CL system used in this work is shown in Fig. 2. Two peristaltic pumps (HL-2D, Shanghai B Qingpu Huxi Instruments Factory, China) were used to deliver all chemicals. PTFE flow tubes (0.8 mm i.d.) were used to connect all components in the system. A six-port injection valve (CIVI, German), equipped with a loop of 100 μL, was used for sample introduction. The CL signal was monitored by a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China), consisting of a flat coil glass flow cell facing the window of the photomultiplier tube (PMT). Data acquisition and treatment were performed with BPCL software. The UV-absorbance was detected with the UV-2600 spectrophotometer (Shimadzu Ltd, Japan). The CL spectrum was obtained with the RF-5301PC fluorospectrophotometer (Shimadzu Ltd, Japan).

2.3. Procedures

FI-CL method: The FI system was easy to operate. As shown in Fig. 2, the peristaltic pump propelled the DPA solution, H_2SO_4 solution and analyte (the standard captopril solution or a sample containing captopril) through the system, respectively. When the injection valve was set to the load position, the H_2SO_4 solution was merged with DPA solution at "M" point and ran through the whole system until a stable baseline was recorded. When the injection valve was switched to the inject position, the H_2SO_4 carried the sample solution in the reagent loop (100 μL captopril solution), and ran directly through the flow cell, producing CL emission. The CL signal was recorded simultaneously. The relative CL intensity ΔI, defined as the difference of CL intensity with or without captopril solution respectively, was proportional to the corresponding concentration of captopril.

2.4. Sample preparation

Tablet and urine were collected for analysis. The average tablet weight was calculated from the weight of 20 tablets. Firstly, the homogenized powder sample equivalent to about 50 mg captopril was accurately weighed. It was then shaken for 15 min with 50 mL water

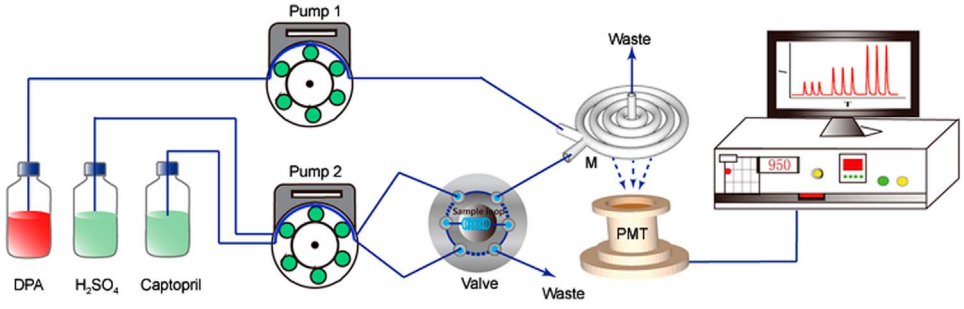

Fig. 2. Schematic diagram of FI-CL flow system. Pump: peristaltic pump, Valve: a six-port valve, M: mixing point, PMT: photomultiplier tube.

then. The resulting mixture was filtered and the filtered product was diluted with water into a calibrated 100 mL flask for further sample analysis. Urine sample was taken from a healthy male person. In order to remove the reducing substances such as ascorbic acid and glutathione, 50 mL urine sample (containing 0.1 g Na_2CO_3) was heated at 60 ℃ for 10 min. An appropriate amount of standard captopril solution was added into the 10 mL treated urine sample. The spiked samples were extracted with CH_2Cl_2 for three times after 30 min. standing. Then the distilled water was added for analysis after the organic phases were collected and dried. The spiked urine samples with concentration of 0.5, 2.0, and 5.0 μg/mL were directly analyzed.

3. Results and discussion

3.1. Kinetics curve of the CL reaction of DPA-H_2SO_4-captopril

Before the FI method was carried out, kinetic characteristics of the proposed CL reaction were studied by using the batch method. In the batch mode, the experimental parameters were kept constant, and the intensity-time curve of DPA-H_2SO_4-captopril was recorded to study the kinetic characteristic of the CL reaction. As shown in Fig. 3, this CL system responded quickly. The CL intensity peak appeared within 0.4 s since the DPA solution was injected and decreased instantly to baseline within 15 s. The obtained result indicated that captopril could enhance the weak luminescence of DPA in H_2SO_4. Thus the CL method is rapid and sensitive enough to perform determination of captopril.

3.2. Optimization of the experiment procedure

3.2.1. The effect of DPA solution

The CL was emitted from the oxidation reaction of captopril by DPA in acid medium. As the only oxidant, the concentration of DPA affected not only the sensitivity, but also the linear range for the assay. The influence of DPA concentration on the CL intensity was tested in the range 3.0×10^{-6}–1.5×10^{-3} mol/L, and as demonstrated in Fig. 4, the CL intensity increased with the increase of the DPA concentration in a low-concentration range, and reached the maximum at 3.0×10^{-4} mol/L. When the concentration was over 3.0×10^{-4} mol/L, the decrease of CL intensity probably resulted from self-absorption of brunet DPA with higher concentration. Hence 3.0×10^{-4} mol/L was the optimal concentration and was selected in the subsequent work.

3.2.2. The effect of acid medium

The CL intensity involved DPA in acid medium could be enhanced in the presence of captopril. The concentration of the acid used in the reaction significantly influenced the intensity of the CL emission. The effect of H_2SO_4 concentration, ranging from 0.01 mol/L to 2.0 mol/L, was tested. As shown in Fig. 5, CL intensity of the DPA- H_2SO_4-captopril system increased with the increase of H_2SO_4 concentration in a range of 0.01–0.5 mol/L and reached the maximal value at 0.5 mol/L. Further increase of the H_2SO_4 concentration led to decrease of CL emission. The observation may attribute to the enhanced baseline noise that resulted from released heat when H_2SO_4 was at higher concentrations. Thus 0.5 mol/L H_2SO_4 solution was used in the following experiments.

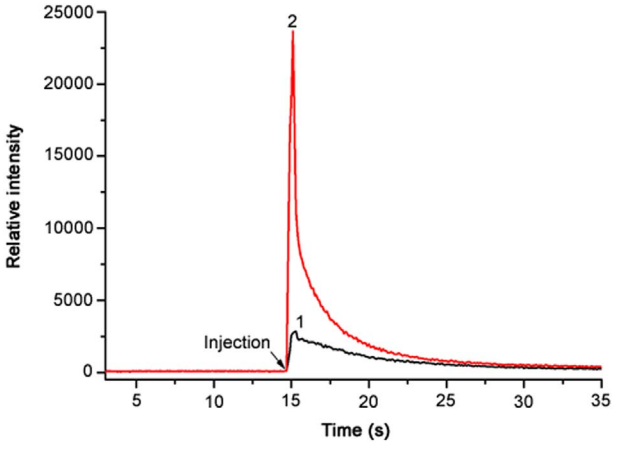

Fig. 3. Kinetic curves of DPA-H_2SO_4-captopril system. (1) 50 μL DPA injected into the mixture of H_2SO_4 and water; (2) 50 μL DPA injected into the mixture of H_2SO_4 and captopril. DPA: 1.0×10^{-3} mol/L; H_2SO_4: 0.5 mol/L; captopril: 1.0 μg/mL; high voltage: −950 V.

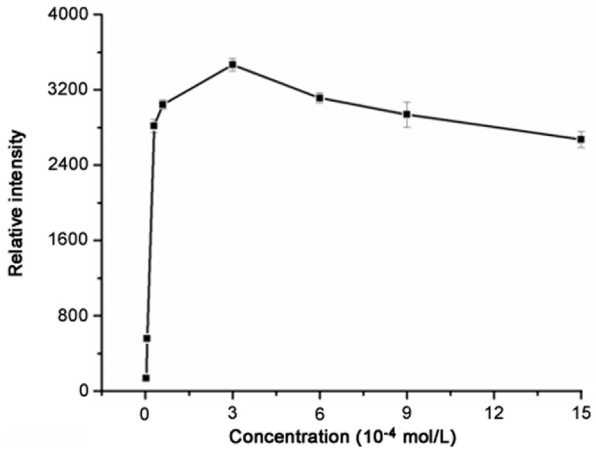

Fig. 4. Effect of DPA concentration on CL intensity. H_2SO_4: 0.5 mol/L; captopril: 1.0 μg/mL; high voltage: −850 V; flow rate: 2.5 mL/min.

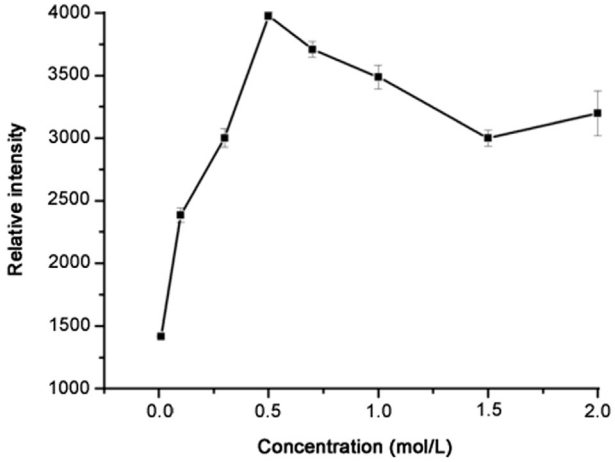

Fig. 5. Effect of H_2SO_4 concentration on CL intensity. DPA: 3.0×10^{-4} mol/L; captopril: 1.0 μg/mL; high voltage: −850 V; flow rate: 2.5 mL/min.

3.2.3. The effect of high voltage and flow rate

The high voltage affected the sensitivity of the CL system, the high voltage corresponding to the maximal signal/noise(S/N) value was regarded as optimum condition generally and was used in the whole experiment. The influence of high voltage on CL intensity was tested in the range from −800 to −960 V. The S/N increased with the increase of the high voltage. So high voltage of −960 V was used in the following experiments.

The CL system was investigated within a given range of flow rate of 0.2–2.5 mL/min. The result showed that the relative CL intensity increased with the increase of flow rate in the range of 0.2–2.5 mL/min. In consideration of reproducibility and sensitivity, we used 2.5 mL/min in the whole experiment.

Table 1
Comparison of the proposed method with analytical methods reported in literatures.

Method	Matrix	Linear range (μg/mL)	Detection limit (μg/mL)	Reference
Spectrofluorimetric method	Tablet	0.1–1.3	0.05	[10]
Spectrophotometry	Tablet	0.4–6.0	0.021	[11]
SERS	Tablet, human serum	–	0.03	[13]
CL: hypochlorite-luminol	Tablet	4.35–32.6	0.44	[18]
CL: bipyridine ruthenium-Ce(IV)	Tablet	0.4–32.6	0.2	[20]
Spectrophotometry	Tablet	0.6–21.73	0.2	[38]
HPLC-UV	Tablet, human serum	30–300	0.2	[39]
CL: DPA-sulfuric acid	Tablet, human urine	0.3–15.0	0.05	The proposed method

3.3. Analytical characteristics of the FI-CL method

An FI-CL method based on DPA and sulfuric acid was developed for the analysis of captopril. In order to investigate the practicality of the developed method, the linearity, precision and limit of detection were obtained under the optimized conditions. The linear ranges were 0.3–15.0 μg/mL with the limit of detection of 0.05 μg/mL (3σ). The regression equation was $\triangle I = 4056.24c - 506.16$ (c being the captopril concentration, μg/mL, $R^2 = 0.9966$), and the relative standard deviation was 2.0% for 5.0 μg/mL captopril ($n = 11$). The result was comparable with most of those obtained from the other methods reported in literature, as shown in Table 1.

3.4. Influence of coexisting foreign species

Under the selected experimental conditions, the influence on CL intensity of some common excipients in drugs, metal ions in the human body and several organic compounds were examined. The experiments were carried out by comparing the intensities obtained with and without the potentially interfering substances added. The results are listed in Table 2. The results indicated that under the experimental conditions of 2.0 μg/mL captopril and a given relative error (< 5%), some interferences cannot be ignored. So it was necessary to do pretreatment work before detection of urine sample.

3.5. Analytical applications in the determination of real sample

According to the procedure detailed in the experimental section, the proposed method was applied to the determination of captopril in tablet and human urine. And recovery studies were carried out on real samples for the evaluation of the validity of the proposed method. The results in Table 3 were further compared with those obtained by HPLC method. As shown in Table 3, the recovery values for the two samples were in the range of 83.1%–112.5% with RSDs of 0.5%–4.4%. The HPLC value agreed well with the results obtained by the proposed method.

Table 2
The interference of some common foreign species on the determination of 2.0 μg/mL captopril.

Foreign species	Tolerance limits
Cl^-	0.05
CO_3^{2-}	500
NO_3^-	500
SO_4^{2-}	500
Co^{2+}	2
NH_4^+	500
Fe^{3+}	25
Fe^{2+}	5
Mg^{2+}	50
Cu^{2+}	0.05
PO_4^{3-}	0.5
Uric acid	2
Glucose	2
Lactose	0.5
Ascorbic acid	0.5
L-methionine	0.5
L-cysteine	0.05
Polyvinylpyrrolidone	50
Al^{3+}	500
Ni^{2+}	25
Zn^{2+}	500
Cr^{3+}	1

Table 3
The analytical results of captopril in pharmaceutical tablet (25 mg) and human urine samples (n=3).

Sample	Detected	Added	Found	Recovery (%)	RSD (%)	HPLC method
Captopril tablet (mg)	26.1	12.5	39.8	110.0	0.5	24.8
		25.0	54.3	112.5	2.2	
		50.0	71.7	91.3	1.1	
Urine (μg/mL)	N.D.	0.50	0.42	83.1	2.3	N.D.
		2.00	2.10	105.1	0.8	
		5.00	5.43	108.6	4.4	

N.D.: Not detected.

3.6. Mechanism studies of the CL reaction

To explore the mechanism of the CL reaction, the UV-visible absorption spectra were recorded. The UV-visible absorption spectra of DPA-H_2SO_4-captopril are shown in Fig. 6. DPA had a characteristic absorption peak at 357 nm. After adding sulfuric acid to DPA solution,

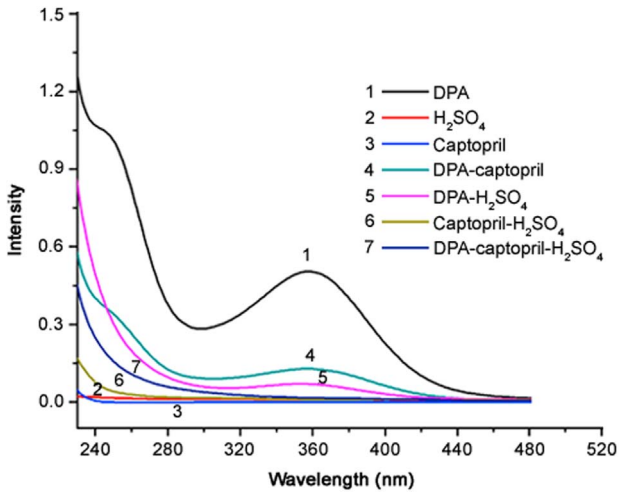

Fig. 6. The UV-vis spectrum of the CL system. DPA: 3.0×10^{-5} mol/L; H_2SO_4: 0.5 mol/L; captopril: 10.0 μg/mL.

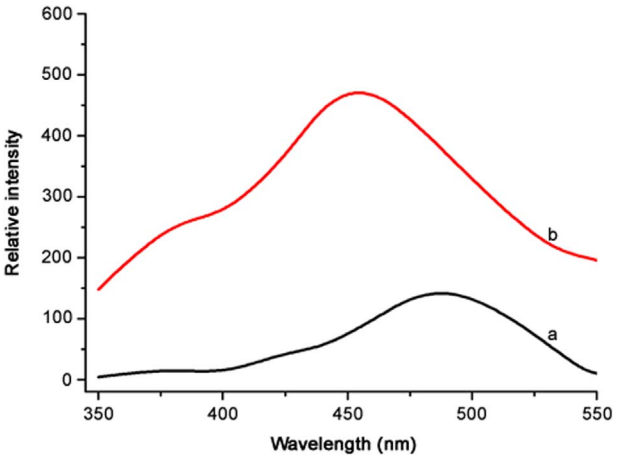

Fig. 7. CL spectrum of DPA-H_2SO_4 (a) and DPA-H_2SO_4-captopril (b). DPA: 3.0×10^{-4} mol/L; H_2SO_4: 0.5 mol/L; (a) High voltage: −1200 V; (b) captopril: 10.0 μg/mL, and high voltage: −1000 V.

the color of DPA gradually faded away, and intensity of the characteristic absorption at 357 nm decreased, which demonstrated that reactions took place between sulfuric acid and DPA. Captopril has weak absorption in ultraviolet region. When captopril was mixed with DPA, the intensity of the characteristic absorption of DPA at 357 nm decreased, indicating that reactions took place between captopril and DPA. It was obvious that reactions took place between captopril and DPA. When DPA, sulfuric acid and captopril were mixed together, the characteristic absorption peak of DPA almost disappeared, which proved that intensive reaction had taken place among the three compounds.

The CL spectra of the present system were measured by means of a series of high-energy optical filters in the range of 350–550 nm, as shown in Fig. 7. It was found that a CL peak at 490 nm was observed for DPA-sulfuric acid system, and a new CL peak at 460 nm was obtained for the DPA-sulfuric acid-captopril system. The CL emission at 490 nm might be caused by the excited state $(O_2)_2$* [35]. Otherwise, the CL emission at 460 nm might be produced through an intermolecular energy transfer.

Shi et al. [36] showed that DPA has the forms of $Ag(HIO_6)_2^{5-}$ and $Ag(HIO_6)(OH)(H_2O)^{2-}$, and the latter could be an active center and complex reaction could occur. H_2SO_4 supply H_3O^+, $Ag(HIO_6)(OH)(H_2O)^{2-}$react with H_3O^+ to produce $O_2^{\cdot-}$. Otherwise, the observed CL spectra at 490 nm for $Ag(HIO_6)_2^{5-}$-H_2SO_4 system suggest that $O_2^{-\bullet}$ is produced in the reaction systems. The recombination of part of $O_2^{-\bullet}$ may generate energy-rich precursors of excited molecules $(O_2)_2$* [37]. The captopril molecule received excitation energy from part $(O_2)_2$*, and then decayed to its ground state, producing the CL emission at 460 nm.

Based on the above discussions, a possible reaction mechanism was suggested as follows:

$$Ag(HIO_6)_2^{5-} + H_2O \rightarrow Ag(HIO_6)(OH)(H_2O)^{2-} + H_2IO_6^{3-}$$

$$Ag(HIO_6)(OH)(H_2O)^{2-} + H_3O^+ \rightarrow Ag^+ + H_5IO_6 + O_2^{\cdot-}$$

$$2\ O_2^{\cdot-} \rightarrow (O_2)_2*$$

$$(O_2)_2* \rightarrow 2O_2 + h\nu$$

$$Ag(HIO_6)(OH)(H_2O)^{2-} + H_3O^+ + \text{Captopril} \rightarrow \text{Captopril complex} + H_2O$$

$$(O_2)_2* + \text{Captopril complex} \rightarrow 2O_2 + \text{Captopril complex}*$$

$$\text{Captopril complex}* \rightarrow \text{Captopril} + h\nu$$

4. Conclusion

In this study, an FI-CL method free of CL reagents was developed for the determination of captopril based on its enhancing effect on the CL derived from DPA-sulfuric acid system with satisfactory results. The linear range was 0.3–15.0 μg/mL. The detection limit for captopril was 0.05 μg/mL, and the relative standard deviation (n=11) was 2.0%. The proposed method was successfully applied for the determination of captopril in pharmaceutical samples and human urine. The reaction mechanism of the chemiluminescence system was discussed briefly. The proposed method is fast, convenient, and cost-effective for the determination of captopril in medicine and biological samples.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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