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ORIGINAL RESEARCH

Flow injection chemiluminescence determination of captopril based on its enhancing effect on the luminol–ferricyanide/ferrocyanide reaction

Jianxiu Du, Yinhuan Li and Jiuru Lu*

Department of Chemistry, School of Chemistry and Material Science, Shaanxi Normal University, Xi'an 710062, People's Republic of China

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ABSTRACT: A new flow injection chemiluminescence method is described for the determination of captopril. It is based on the enhancing effect of captopril on the chemiluminescence reaction of luminol with potassium ferricyanide in alkaline solution in the presence of potassium ferrocyanide. The method allows the determination of captopril over 0.1–40 μg/mL range, with a relative standard deviation (SD) of 1.0% for the determination of 0.5 μg/mL captopril solution in 11 repeated measurements. The method was satisfactorily applied to the determination of captopril in commercial captopril tablets. The possible reaction mechanism is also discussed briefly. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: chemiluminescence; flow injection; captopril; pharmaceutical analysis

INTRODUCTION

Captopril, 1-[(2s)-3-mercapto-2-methylpropionyl]-L-proline, an orally active inhibitor of the angiotensin-converting enzyme, has been widely used for the treatment of hypertensive diseases and heart failure. Various methods have been reported for the determination of captopril in pharmaceutical preparations. These include titrimetry (1), spectrophotometry (2), electroanalytical method (3), chromatography (4, 5) and capillary electrophoresis (6). Chemiluminescence (CL) is an attractive detection method for analytical determination because of the low detection limit and wide linear working range that can be achieved while using relative simple instrumentation. Recently, several CL methods based on the cerium (IV) reaction have been reported for the determination of captopril in pharmaceutical preparations (7, 8).

In this work, it was found that captopril could react with luminol to generate strong CL in alkaline solution in the presence of potassium ferricyanide and potassium ferrocyanide, which has ever been used as an enhancer for the CL reaction of luminol with some reducing agents (9, 10). Based on this find, a new CL system, the luminol–ferricyanide/ferrocyanide–captopril system, has been proposed and a new flow injection CL method has been developed for the determination of captopril. The method was applied to the determination of captopril in pharmaceutical preparations and compared satisfactorily with the pharmacopoeia method (1). A brief discussion on the reaction mechanism was also undertaken.

*Correspondence to: J. Lu, Department of Chemistry, School of Chemistry and Material Science, Shaanxi Normal University, Xi'an 710062, People's Republic of China.
Email: xzb4@snnu.edu.cn

MATERIALS AND METHODS

Apparatus

A schematic diagram of the flow system used for the determination of captopril is shown in Fig. 1. A peristaltic pump was used to deliver all solutions at a flow rate of 1.4 mL/min on each line. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Injection was made using a six-way injection valve equipped with a 40 μL sample loop. The CL signal produced in the flow cell was detected by a R_{456} photomultiplier tube (Hamamatsu) and recorded with an IBM-compatible computer employing an IFFL-D flow-injection chemiluminescence analysis system software (Xi'an Ruike Electronic Equipment Corporate, Xi'an, China).

Reagents

All chemicals were of analytical grade and doubly deionized water was used for the preparation of solutions. The 1.0 mg/mL standard solution of captopril was prepared by dissolving 100.0 mg captopril (Institute of Pharmaceutical and Bio-material Authentication of the People's Republic of China) in water and diluting to 100 mL with water; when not in use it was stored at about 4°C in a dark bottle. The 1.0×10^{-3} mol/L luminol

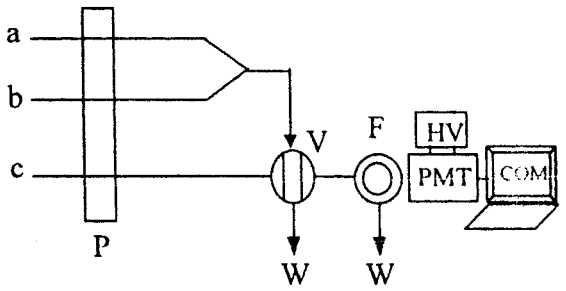

Figure 1. Schematic diagram of the flow system for captopril determination. a, luminol solution; b, potassium ferricyanide solution; c, sample/standard solution; P, peristaltic pump; V, six-way injection valve; F, flow cell; PMT, photomultiplier tube; HV, high voltage; COM, computer; W, waste solution.

solution was prepared in 0.5 mol/L sodium hydroxide solution and contained 0.25 mol/L potassium ferrocyanide (Xi'an Chemical Reagent Factory). The 0.02 mol/L potassium ferricyanide solution was prepared by dissolving 6.58 g potassium ferricyanide (Xi'an Chemical Reagent Factory) in water and diluting to 1000 mL with water.

Preparation of samples

The average tablet weight was calculated from the weight of 30 tablets. They were finely powdered, homogenized and a portion of the powder, equivalent to about 50 mg captopril, was accurately weighed and shaken for 15 min with 50 mL water. The resulting mixture was filtered and the filtrate was diluted with water into a calibrated 100 mL flask for further sample analysis.

Procedure

As shown in Fig. 1, lines a, b and c were inserted into luminol solution, potassium ferricyanide solution and sample/standard solution, respectively. Reagents and samples were pumped at a constant speed until a stable baseline was recorded. Then, the mixture (40 μL) of luminol and potassium ferricyanide was injected into standard/sample stream, producing chemiluminescence (CL). The concentration of captopril was quantified by CL intensity.

RESULTS AND DISCUSSION

Optimum of experimental conditions

A series of experiments were conducted to achieve the optimum conditions for the chemiluminescence determination of captopril.

The influence of flow rate on the CL reaction was examined in the range 0.7–3.5 mL/min. The maximum signal was obtained at the flow rate of 1.4 mL/min and this flow rate was used in the experiments.

The influence of luminol concentration on the CL reaction was examined in the 5×10^{-4}–5×10^{-3} mol/L range. The most suitable luminol concentration is 1×10^{-3} mol/L because it gave the maximum signal: blank ratio, therefore 1.0×10^{-3} mol/L luminol was chosen in the experiments.

The concentration of sodium hydroxide in luminol solution was examined within the 0.3–0.6 mol/L range. A maximum signal:blank ratio was obtained around 0.5 mol/L sodium hydroxide and this was used in the experiments.

It was observed that potassium ferricyanide can enhance the CL reaction of captopril with luminol in alkaline solution when the concentration of potassium ferricyanide was higher than 1.0×10^{-2} mol/L. However, the blank signal was very large in the absence of potassium ferrocyanide, because potassium ferricyanide can also react with luminol to give a strong CL in alkaline solution. Sherlin and Neufeld (11) have reported that the CL reaction of luminol with potassium ferricyanide could be inhibited by addition of potassium ferrocyanide. Therefore, if there was an appropriate concentration of potassium ferricyanide and potassium ferrocyanide in the reaction, not only would the CL signal from the reaction of captopril with luminol be efficiently enhanced, but also the blank signal from the CL reaction of luminol with potassium ferricyanide would be greatly decreased. The effect of 0.015–0.025 mol/L potassium ferricyanide and 0.10–0.30 mol/L potassium ferrocyanide on the CL reaction was examined. The signal:blank ratio of the CL reaction reached maximum when 0.02 mol/L potassium ferricyanide and 0.25 mol/L potassium ferrocyanide was used.

Calibration curve and precision

Under the selected conditions, the concentration of captopril is proportional to the CL intensity in the range of 0.1–40 μg/mL. The regression equation is $I = 18.92\ C[\mu g/mL] + 7.32$ and the correlation coefficient is 0.9985 ($n = 13$). The relative standard deviation is 1.0% (0.5 μg/mL captopril, $n = 11$). The determination of captopril could be performed in 40 s including sampling and washing, giving about 90 samples/h.

Interference

In order to apply the method to the analysis of commercial formulations of captopril, the effect of some common additives used in pharmaceutical preparations was studied for the determination of 0.5 μg/mL captopril. The tolerance limit was taken as the amount that caused an error of ±5% in peak height. No interference could be found when including up to a 100-fold weight concentra-

Table 1. Determination of captopril in some captopril tablets (mg/tablet)

		This method		Pharmacopoeia method (1)	
Samples	Claimed	Found[#]	R.S.D. (%)	Found[#]	R.S.D. (%)
990503	25.0	25.1	1.9	25.2	1.1
990602	25.0	25.2	1.7	24.8	1.0
991023	25.0	24.9	2.0	25.1	0.9

Average of five measurements.

tion of glucose, sucrose, fructose, lactose, cyclodextrin and glycine and 1000-fold weight concentration of starch.

Application

The method was applied to the determination of captopril in commercial captopril tablets (Captopril tablets were purchased from Changzhou Pharmaceutical Plant of China). The results are shown in Table 1. As can be seen, no significant differences between the proposed method and the pharmacopoeia method (1) have been found.

Mechanistic studies

The standard solution, the luminol solution and the potassium ferricyanide solution were purged with nitrogen or oxygen for 5 min. When the solutions were purged with oxygen for 5 min, the CL intensity increased 35%; in contrast, while dissolved oxygen was driven out by the flow of nitrogen for 5 min, the CL intensity decreased 40%. The results indicate that the dissolved oxygen plays an important role in the CL reaction.

It was reported that superoxide radical can be produced from the photochemical reaction of riboflavin in alkaline solution (12), and the produced superoxide radical can oxidize alkaline luminol to generate CL (13). It was found that the CL signal from the reaction of the riboflavin (UV-irradiation)–luminol system was strongly increased in the presence of potassium ferricyanide and potassium ferrocyanide. The results indicated that the CL signal from the reaction of luminol with superoxide radical in alkaline solution could be effectively enhanced by potassium ferricyanide and potassium ferrocyanide.

It was also reported that some thiol (–SH) compounds can reduce dissolved oxygen to superoxide radical in alkaline solution (14). Therefore, it is assumed that captopril having a–SH group can also reduce dissolved oxygen to superoxide radical in alkaline solution. However, no CL signal could be detected when luminol mixed with captopril in alkaline solution in the absence of potassium ferricyanide and potassium ferrocyanide. This was possibly because the thermodynamic equilibrium constant of reaction of captopril with dissolved oxygen was smaller, which led to a low yield of superoxide radical. In the presence of potassium ferricyanide and potassium ferrocyanide, the CL signal from the reaction of luminol with superoxide radical was enhanced, and the reaction equilibrium of captopril with dissolved oxygen was also destroyed. These all give a strong CL signal.

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