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Determination of epinephrine based on its enhancement for electrochemiluminescence of lucigenin

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Abstract

Epinephrine was found to be able to strongly enhance the electrochemiluminescence (ECL) of lucigenin system by using the anodic potential sweep. Based on which, a novel ECL method for the determination of epinephrine was developed. Under the optimum condition, the enhanced ECL intensity was linear with the epinephrine concentration in the range of 4.0×10^{-8} to 2.0×10^{-7} mol L^{-1}. The detection limit (defined as S/N = 3) was 2.4×10^{-8} mol L^{-1}, and the relative standard deviation was 2.7% for 1.0×10^{-7} mol L^{-1} epinephrine (n = 11). The method was successfully applied to the determination of epinephrine in pharmaceutical samples with satisfactory results. In addition, the possible mechanism for the lucigenin ECL system in the presence of epinephrine has also been discussed.

Keywords: Electrochemiluminescence; Epinephrine; Lucigenin; Anodic potential sweep

1. Introduction

Epinephrine [1-(3,4-dihydroxyphenyl)-2-methyloaminoethanol], one of the well-known catecholamines biosynthesized in the adrenal medulla and sympathetic nerve terminals, plays important roles as a neurotransmitter. Pharmaceutically, it is widely used for the treatment of neural disorders [1]. A number of methods have been applied to determine epinephrine, such as spectrophotometry [2,3], fluorimetry [4], liquid chromatography (LC) coupled with various detection techniques [5,6], capillary electrophoresis (CE) coupled with amperometric detection [7,8], electrochemical detection with various modified electrodes [9–11], chemiluminescence (CL) [12,13] and electrochemiluminescence (ECL) [14,15].

CL is a sensitive and selective analytical technique for detection of the bioactive compounds, and the ECL was developed based on CL, in which an appropriate voltage is applied to an electrode to produce the light emission. However, so far to the best of our knowledge, only two ECL methods have been applied to determine epinephrine, one based on a luminol system was developed by Zheng et al. [14], another based on a $Ru(bpy)_3^{2+}$/tripropylamine system was developed by Li and Cui [15].

When reviewing the ECL analysis, only seven papers on ECL of lucigenin in aqueous media have been reported [16–22] and few analytical applications involving lucigenin have been proposed. One of the reasons might be that the previously reported ECL of lucigenin was obtained at cathodal potentials (−0.30, −0.67 and −0.83 V) where most species could not be reduced.

In this paper, the ECL behavior of lucigenin in neutral solution containing the non-ionic surfactant Triton X-100 has been investigated at anodic potential. As a result, we found that lucigenin also displayed a good ECL response at anodic potentials above 1.1 V (versus Ag/AgCl) and the ECL signal was greatly enhanced by epinephrine. Some influenced factors including the electrochemical parameters, the ECL reaction medium, the pH effect, and the surfactant effect were investigated in detail. A new, convenient and simple ECL method was developed for the determination of epinephrine and the analytical application of the

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ECL of lucigenin during the anodic potential sweep was explored.

2. Experimental

2.1. Reagents

Lucigenin (97+%) and epinephrine (98+%) were obtained from Sigma Chemical Co. (USA) and used without further purification. Triton X-100 was obtained from Shanghai Chemical Co.(China). The Britton–Robinson (B.R.) buffer (pH 2.0–12.0) was prepared by titrating a stock solution containing 0.04 mol L^{-1} acetic acid, 0.04 mol L^{-1} phosphoric acid, 0.04 mol L^{-1} boric acid with 0.2 mol L^{-1} sodium hydroxide to the desired pH value. A stock solution of 1.0×10^{-3} mol L^{-1} epinephrine was prepared in B.R. buffer solutions (pH 7.0) and stored at 4 °C in a refrigerator, to minimize exposure to light and air. Working solutions were made by dilution of these stock solutions. All other chemicals were of analytical reagent or better, double-distilled water was used throughout.

2.2. Apparatus

ECL intensity versus potential was detected by using a BPCL Ultra-Weak Chemiluminescence Analyzer controlled by a personal computer with BPCL program (Institute of Biophysics, Chinese Academy of Sciences) in conjunction with a CH Instruments model 660a Electrochemical Analyzer (Shanghai Chenghua Instrument Co., China). The electrochemical analyzer was used for controlling waveforms and potentials.

A conventional three-electrode system was used for the electrolytic system, including a glassy carbon electrode as a working electrode, a platinum wire as the counter electrode and Ag/AgCl (sat. KCl) electrode as the reference electrode. A commercial 5 mL cylindroid glass cell was used as ECL cell, and it was placed directly in the front of the photomultiplier tube. ECL spectra were measured on a Cary Eclipse Fluorescence Spectrophotometer (Varian, USA). The working electrode was pretreated before use by polishing their surfaces with aqueous slurries of alumina powders (average particle diameters: 1.0 μm and 0.3 μm α-Al_2O_3) on the polishing microcloth and rinsed with water to give a smooth electrode surface.

2.3. Procedure

2.3.1. Static ECL measurement

The working electrode was carefully polished and sonicated in ethanol and water successively. The ECL cell was washed with 0.2 mol L^{-1} nitric acid and water before use. One millilitre of sample solution, 1 mL of 6.0×10^{-5} mol L^{-1} lucigenin, 1 mL of 0.48% (v/v) of Triton X-100 were added successively to a 10-mL volumetric flask, and diluted with B.R. (pH 7.0) buffer to required volume, 2.5 mL of this solution was then transferred to the ECL cell. Differential pulse voltammetry with appropriate parameters (see Table 1) was performed and the ECL signal was recorded simultaneously.

Table 1
Differential pulse voltammetry parameters

Initial E(V)	Final E(V)	Increased E(V)	Amplitude	Pulse width (s)	Sampling width	Pulse period (s)
0.0	1.5	0.004	0.5	0.05	0.0167	0.1

The quantitative analysis was carried out based on the enhanced ECL intensity (ΔI), $\Delta I = I_s - I_0$, where I_0 was the ECL intensity for the blank of the lucigenin system, and I_s was the ECL intensity for the sample with addition of epinephrine. Hence, ΔI can be well related to the concentration of the injected sample.

2.3.2. Sample preparation

The epinephrine hydrochloride injection samples obtained from a drugstore in Fuzhou (Fuzhou Fuyao Pharmaceutical Co. Ltd, China, 1.0 mg mL^{-1}) were appropriately diluted with B.R. buffer and subjected directly to the ECL measurement.

3. Results and discussion

3.1. ECL of lucigenin at anodic potential and enhanced by epinephrine

The ECL of lucigenin was primarily examined at a glassy carbon electrode (GCE) by differential pulse voltammetry in B.R. buffer solution (pH 7.0). When an applied potential was scanned from 0 to 1.5 V, a broad ECL wave, which initiated at 1.0 V and reached a maximum value at 1.4 V was observed (see Fig. 1(a)). The ECL intensity of the system was greatly

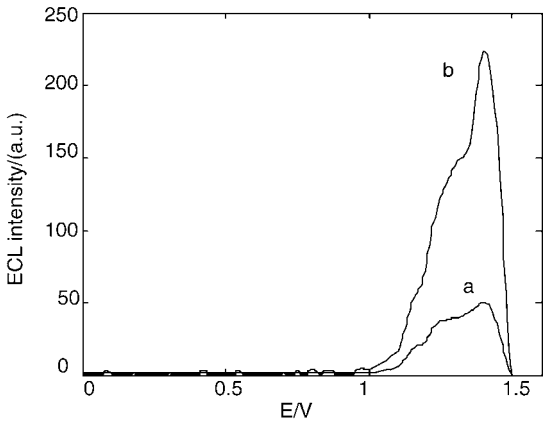

Fig. 1. ECL emission vs. potential curves. (a): 6.0×10^{-6} mol L^{-1} lucigenin + 0.048% Triton X-100; B.R., pH 7.0. (b): 6.0×10^{-6} mol L^{-1} lucigenin + 0.048% Triton X-100 + 1.0×10^{-7} mol L^{-1} epinephrine; B.R., pH 7.0. Conditions of DPV were the same as in Table 1.

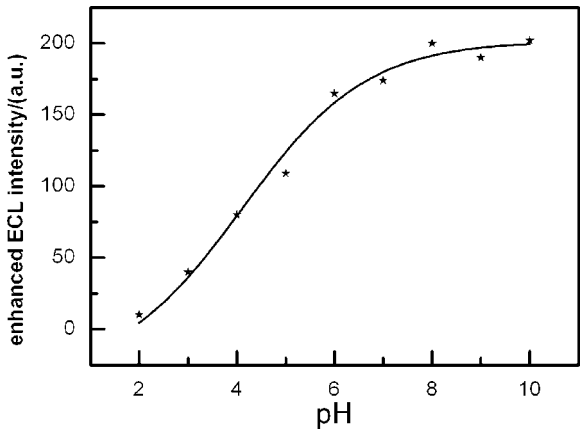

Fig. 2. Effect of pH on the enhanced ECL intensity. [Luc]: 6.0×10^{-6} mol L^{-1}, [Triton X-100]: 0.048%, [epinephrine]: 1.0×10^{-7} mol L^{-1}. Conditions of DPV were the same as in Table 1.

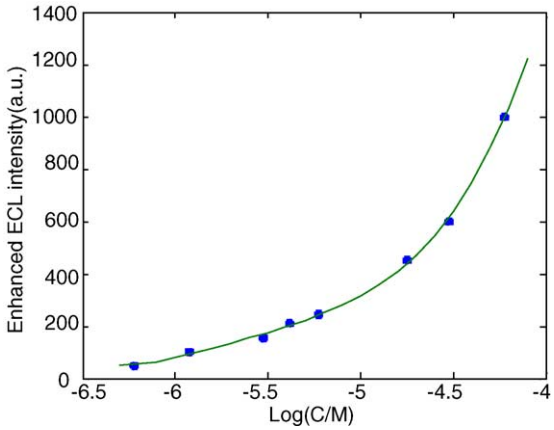

Fig. 3. Dependence of the concentration of Luc on enhanced ECL intensity. [Triton X-100]: 0.048%. [epinephrine]: 1.0×10^{-7} mol L^{-1}, B.R., pH 7.0. Conditions of DPV were the same as in Table 1.

enhanced by addition of epinephrine (see Fig. 1(b)). The ECL intensity at 1.4 V was selected for quantitative analysis of epinephrine because the maximum ECL intensity could be measured conveniently.

3.2. Selection of electrochemical parameters

The linear sweep voltammetry (LSV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) were selected to examine the effect of excitation waveform on the ECL signal. The result showed that the ECL emission occurred when SWV or DPV was applied, and the most stable ECL could be obtained by using DPV. The relationship of pulse potential with the ECL intensity will be studied in detail in the future. In the present work, DPV was selected for the subsequent studies.

To establish the optimal conditions for the determination of epinephrine, the luminescent intensity was measured as a function of pulse amplitude, pulse width and pulse period. Based on these experiments, all of these parameters affected the luminescent intensity to a certain extent. The appropriate pulse amplitude, pulse width and pulse period are beneficial, and the results are summarized in Table 1.

3.3. Selection of chemical reaction conditions

3.3.1. Selection of pH

Epinephrine was easily oxidized by ambient oxygen in the alkaline medium and the degree of oxidation depends on the pH. Therefore, pH value below 10.0 was used for further study. It was observed that the background ECL intensity of lucigenin was increased greatly with pH, and the net ECL intensity changes (ΔI) reached a maximum and constant in the range of pH 4.0–10.0. This result, as illustrated in Fig. 2, indicated that the ECL reaction of lucigenin was better carried out in a neutral or weak alkaline solution.

3.3.2. Selection of reaction medium

It is well known that the medium plays an important role in ECL reaction. The effect of the buffer components on the ECL was examined with acetate buffer, phosphate buffer, borate buffer and B.R.buffer under the same pH 7.0. The result obtained was shown in Table 2. It was found that the maximum ratio of enhanced ECL signal of epinephrine to blank ECL signal of lucigenin could be obtained in B.R. buffer. So B.R. buffer at pH 7.0 was selected in subsequent work.

3.3.3. Effect of lucigenin concentration

The concentration of the lucigenin also has an effect on the ECL intensity. As shown in Fig. 3, the enhanced ECL intensity was increased with the concentration of lucigenin from 6.0×10^{-7} to 6.0×10^{-5} mol L^{-1}. But the blank ECL intensity was quite high when the lucigenin concentration was higher than 1.0×10^{-5} mol $L^{-1}$and a significant increase in the noise amplitude of the base line was observed, which would increase the detection limit. On the other hand, a problem for lucigenin ECL is the low solubility of its reductive product, which may precipitate onto the electrode surface when the lucigenin concentration is higher. Therefore, 6.0×10^{-6} mol L^{-1} lucigenin was used for all experiments.

Table 2
Effect of reaction medium

Reaction medium	Borate buffer (0.02 mol L^{-1})	Acetate buffer (0.02 mol L^{-1})	Phosphate buffer (0.02 mol L^{-1})	B.R.buffer (0.02 mol L^{-1})
Enhanced ECL signal	179 ± 5.1	92 ± 4.5	153 ± 4.0	174 ± 4.3
Blank ECL signal	68 ± 3.3	53 ± 2.9	63 ± 3.5	50 ± 2.9

$n = 5$; 6.0×10^{-6} mol L^{-1} lucigenin, 0.048%Triton X-100, 1.0×10^{-7} mol L^{-1} epinephrine; B.R., pH 7.0. Conditions of DPV were the same as in Table 1.

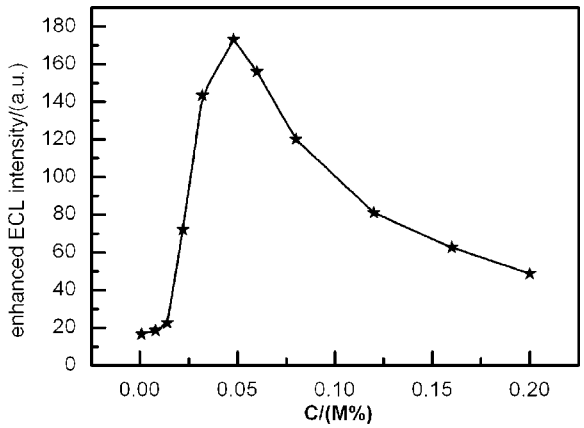

Fig. 4. Effect of Triton X-100 on enhanced ECL intensity. [Luc]: 6.0×10^{-6} mol L^{-1}, B.R., [epinephrine]: 1.0×10^{-7} mol L^{-1}, pH 7.0. Conditions of DPV were the same as in Table 1.

3.3.4. Effect of surfactants

Differential surfactants have been added into the ECL system of lucigenin. The effects of two neutral surfactants (Triton X-100, Tween 40), one cationic surfactant (Cetyltrimethylammonium Bromide) and two anionic surfactants (Sodium Dodecylbenzene Sulfonate and Sodium Dodecyl Sulfonate) on the ECL reaction of lucigenin were investigated. The effect of Triton X-100 concentration on the enhanced ECL intensity is shown in Fig. 4. Similarly, significant ΔI increase was observed for increase of Triton X-100 concentration in the range of 0.03–0.05%, and slow down for the further increase. Therefore, 0.048% of Triton X-100 was chosen as the optimal value.

It is known that the ECL of lucigenin was derived from the decomposition of a dioxetane-type intermediate formed by the radical–radical coupling reaction between the nascent oxygen [O] and lucigenin [22]. The adsorption of Triton X-100 on the electrode surface [23–26] could prevent the adsorption of the ECL product (i.e., *N*-methylacridone) on the electrode surfaces. In addition, the hydrophobic domain of Triton X-100 formed in the vicinity of electrode surfaces may contribute to an increase in the lifetime of [O] in aqueous solutions [27,28], and may become a suitable reaction area for the radical–radical coupling reaction between [O] and lucigenin. These effects in the micellar solution of Triton X-100 would enhance the stability of ECL.

3.4. Linear response range, detection limit and precision

Under the optimum conditions, the enhanced ECL intensity was linear with the concentration of epinephrine in the range of 4.0×10^{-8} to 2.0×10^{-7} mol L^{-1}. The regression equation was,

$$\Delta I_{ECL} = 16.02 + 162.0 \times C/10^{-7}\ \text{mol L}^{-1} \quad R = 0.9950$$

where I_{ECL} is the enhanced ECL intensity; C is the concentration of epinephrine. The relative standard deviation for 1.0×10^{-7} mol L^{-1} epinephrine was 2.7% (n = 11). The detection limit (defined as the concentration that could be detected at the signal-to-noise ratio of 3) was 2.4×10^{-8} mol L^{-1}.

Table 3
Tolerable concentration ratios with respect to epinephrine for some interfering species (<5% error)

Substance	Tolerable concentration ratio
Na^+, K^+, SO_4^{2-}, NO_3^-, glucose, sucrose, lactose, amylum	>1000
Ac^-, HPO_4^{2-}, Cl^-, polyethylene glycol, citric acid, ethanol,	500
L-Glutamic acid, formaldehyde	50
Mg^{2+}, Ba^{2+}, Fe^{3+}	10
Zn^{2+}, DL-α-amino acid	5
Pb^{2+}, Cd^{2+}, Ca^{2+}, Mn^{2+}	2
Vitamin C, Pb^{2+}	1
Co^{2+}, Cu^{2+}	0.1

3.5. Interference

In order to apply the proposed method to the determination of epinephrine in pharmaceutical formulations samples, the interference of some possible co-existing ions was examined. A foreign ion was considered not to interfere if it caused a relative error <5% during the determination of 1.0×10^{-7} mol L^{-1} epinephrine solution. The results are listed in Table 3. Because of the interference produced by these compounds, it is necessary to perform a separation pretreatment for epinephrine before analytical measurement when the antioxidants (sodium bisulfite, ascorbic acid or formaldehyde) are present in sample.

3.6. Application

The proposed method was further applied to the analysis of certain injection samples containing epinephrine. The samples were diluted appropriately with B.R. buffer before measurement. The determination results are shown in Table 4. The values obtained by the calibration method, as well as the standard addition method, were in excellent agreement with the reference values. Therefore, the method could be used to determinate epinephrine in commercial samples.

3.7. Possible mechanism

The ECL of the lucigenin system seemed to be similar to the ECL behavior of acridinium esters, which was studied by Fang and co-workers [29]. Their result showed that acridinium ester displayed a good ECL response at anodic potentials above 2.0 V, and the ECL intensity was increased greatly with the increasing of potential until it reached a plateau at the potentials >3.5 V. However, we could observe the ECL of lucigenin at anodic potential above 1.0 V and it reached the maximum value at 1.4 V. The use of different electrochemical excitation modes might be the main reason for this difference.

In order to elucidate the mechanism of this ECL system, voltammetry and luminescence experiments were carried out.

Table 4
Determination of epinephrine in injection samples

Sample number	Epinephrine found (mg mL^{-1})		R.S.D. (%)[a]	Added value ($\times 10^{-7}$ mol L^{-1})	Recovery	
	This method	Reference value			Value ($\times 10^{-7}$ mol L^{-1})	Percentage
021102	1.04	1.00	2.14	1.00	0.914	91.4
021302	1.02	1.00	1.80	1.00	0.980	98.0

[a] $n = 7$.

Cyclic voltammetry of lucigenin in neutral solution experiment showed no electrochemical signal for lucigenin over the range of 0.0–2.0 V (versus Ag/AgCl). The result proved that it was impossible for lucigenin to be oxidized directly at electrode surface to emit light.

We examined the ECL spectrum of lucigenin in the absence of epinephrine (see Fig. 5(a)), which was obtained at 1.4 V (versus Ag/AgCl) after potential scanning and the wavelength scan speed of the spectrophotometer was 10 nm^{-1} s. As shown in Fig. 5(a), the maximum wavelength of the ECL emission was 430–440 nm when a potential of 1.4 V was applied. All these results suggested that the emitter was *N*-methylacridone, the oxidization product of lucigenin.

Bruice and co-workers [30] has reported the mechanism of the chemiluminescence of lucigenin by hydroxide and peroxide, and some people [31] have reported hydroxide and peroxide would be produced upon the oxidization of water. So we proposed that the ECL of lucigenin might be related to the highly reactive OH radicals and fresh atomic oxygen [O], which was produced upon the oxidization of water. The reaction mechanism would be proposed as follow:

$$2H_2O \xrightarrow{-2e,-2H^+} 2\,[OH^\bullet] \xrightarrow{-e,-2H^+} [O_2{}^{\bullet -}]$$

$$2H_2O \xrightarrow{-4e,-4H^+} 2\,[O]$$

$$Luc \xrightarrow{[O]\ \text{or}\ [O_2{}^{\bullet -}]} NMA^* \rightarrow NMA + h\nu$$

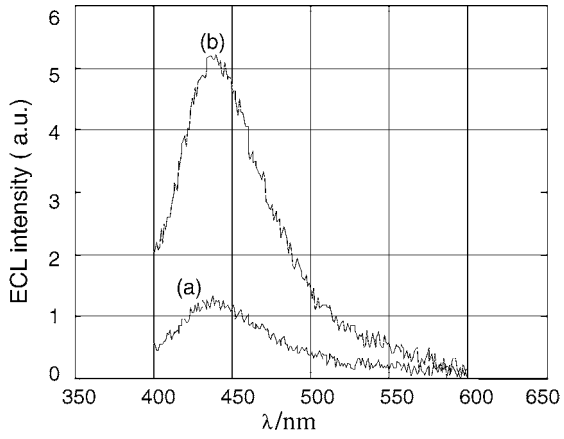

Fig. 5. ECL spectra of Lucigenin in the present or absence of epinephrine. (a): In the absence of epinephrine. (b): In the presence of epinephrine. [Lucigenin]: 6.0 × 10^{-6} mol L^{-1}, [Triton X-100]: 0.048%, [epinephrine]: 1.0 × 10^{-7} mol L^{-1}, B.R., pH 7.0. Conditions of DPV were the same as in Table 1.

The reaction intermediate, dioxetane, decomposes to an exited NMA*. Some of the NMA* can transfer their energy to lucigenin forming excited lucigenin. Both the excited lucigenin and NMA* species return to their ground states simultaneously and give a mixed broad light emission [30]. However, in our experiment, the concentration of lucigenin is so low that we could not observed the ECL emission of excited lucigenin in the ECL spectrum.

We also investigated the ECL spectrum of lucigenin in the presence of epinephrine (see Fig. 5(b)) and the cyclic voltammetry experiment of epinephrine in B.R. buffer (pH 7.0). The ECL spectrum of lucigenin in the presence of epinephrine was the same as that in the absence of epinephrine and the maximum light emission was 430–440 nm. The result indicated that the emitter was still *N*-methylacridone.

From the cyclic voltammogram of epinephrine, one irreversible oxidation peak corresponding to the oxidation of epinephrine was observed at ca. 0.7 V versus Ag/AgCl, which is lower than 1.4 V. As with other catecholamines, epinephrine has electroactive groups, the oxidation process to quinone has been widely studied [32]. Based on our experimental results and previous literatures of enhanced chemiluminescence of lucigenin with epinephrine [33], the enhanced effect should be caused by the oxidation product of the epinephrine (epinephrine quinone and adrenolutin).

4. Conclusion

Epinephrine has been found to be able to enhance the ECL of lucigenin by using the anodic potential sweep, based on which a novel ECL method for determination of epinephrine was developed. Compared with the previous methods [2–15] for the determination of epinephrine, the method in this paper is simple, rapid and convenient and can also be well applicable for pharmaceutical sample. Though its selection is not more satisfied than the traditional CL and ECL methods [13–15], the potential application of lucigenin at anodic potential was explored and it is beneficial to the further study of lucigenin. The preliminary mechanism of the ECL generated by lucigenin at anodic potential was proposed as the result of the chemiluminesence of lucigenin by the highly reactive OH radicals and fresh atomic oxygen [O] generated electrochemically, and the enhanced effects should be caused by the oxidation product of the epinephrine.

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