Research Article

Determination of Atropine Sulfate in Human Urines by Capillary Electrophoresis Using Chemical Modified Electrode as Electrochemiluminescence Sensor

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A Ru(bpy)₃²⁺-based electrochemiluminescence (ECL) detection coupled with capillary electrophoresis (CE) was developed for the determination of atropine sulfate on the basis of an Eu-PB modified platinum electrode as the working electrode. The analyte was injected to separation capillary of 50 cm length (25 μm i.d., 360 μm o.d.) by electrokinetic injection for 10 s at 10 kV. Parameters related to the separation and detection were discussed and optimized. It was proved that 10 mM phosphate buffer at pH 8.0 could achieve the most favorable resolution, and the high sensitivity of detection was obtained by using the detection potential at 1.15 V and 5 mM Ru(bpy)₃²⁺ in 80 mM phosphate buffer at pH 8.0 in the detection reservoir. Under the optimized conditions, the ECL peak area was in proportion to atropine sulfate concentration in the range from 0.08 to 20 μg · mL⁻¹ with a detection limit of 50 ng · mL⁻¹ (3σ). The relative standard derivations of migration time and peak area were 0.81 and 3.19%, respectively. The developed method was successfully applied to determine the levels of atropine sulfate in urine samples of patients with recoveries between 90.9 and 98.6%.

1. Introduction

Atropine ((±)-hyoscyamine) is an active tropine alkaloid from solanaceous plants (as one of a traditional Chinese crude herb), and its chemical structure is shown in Figure 1. Atropine sulfate has been utilized clinically for many years as anticholinergic agent in premedication of anesthesia. However, a high dosage of atropine sulfate can stimulate the central nerve system and disrupt the human renal function leading to toxic reaction [1]. Therefore, it is necessary to establish sensitive and effective methods for the quantitation of atropine sulfate in the field of clinical medicine.

Up to date, different chromatographic methods including liquid chromatography (LC) [2], high-performance liquid chromatography (HPLC) [3, 4], thin-layer chromatography (TLC) [5], and gas chromatography (GC) [6] have been reported for the analysis of atropine. However, a drawback of chromatography appears to be time consuming due to

necessary extraction, concentration, and/or derivatization prior to the analysis. In addition, a bulk acoustic wave sensor has recently been fabricated and utilized for the determination of atropine sulfate in serum and urine [7]. Of the applied field of spectroscopic analysis, the atomic absorption method (AAS) [8], chemiluminescence method (CL) [9], and the electrochemiluminescence method (ECL) [10] have also been developed for atropine detection. However, these methods often encounter poor selectivity in the assay of complicated samples when coupled with no other separated techniques.

Capillary electrophoresis (CE) represents an interesting alternative as a powerful separation tool and has been applied for atropine analysis already. However, the present CE methods for atropine are usually coupled with UV-V detector, resulting in the restricted detection limits [11]. In recent years, the marriage of CE to Ru(bpy)₃²⁺-based ECL detection has proved to be a promising and efficient

FIGURE 1: Structure of atropine.

analytical technique in the field of biochemical analysis with high sensitivity and excellent separation efficiency [12–15]. However, the utilization of a bare metal platinum electrode in CE-ECL detection can deteriorate stability and sensitivity because of the poisoning effect of complicated matrices in real biological fluids [10].

In recent years, chemical modified electrodes have been widely used for the detection of trace amounts of real biological fluids since the poisoning effect of complex matrixes on the bare platinum electrode could deteriorate the stability and sensitivity [16]. Especially, Prussian Blue (PB), its analogue and related composite film modified electrodes have received more attention in wide range of electrochemistry because of inherent stability, highly reversible nature of the electrode reactions, ease of preparation, and low cost [17]. Unfortunately, the application of a PB analogue modified electrode with rare earth ions in CE-ECL systems has not been reported by other workers although some modified electrodes have also been used as solid-state ECL detectors in CE-ECL detection [16, 18]. It was proved in our previous works [19, 20] that only europium (III)-doped Prussian blue analogue film (Eu-PB) modified platinum microelectrode had good catalytic activity to the electrochemiluminescence of $\text{Ru}(\text{bpy})_3^{2+}$ -based system. Given this, a platinum electrode modified by Eu-PB is prepared and applied as a working electrode and a $\text{Ru}(\text{bpy})_3^{2+}$ -based CE-ECL method is developed for the direct determination of atropine sulfate in urine samples of patients in this paper. By this alternative, a wider linear range and significantly improved sensitivity for atropine sulfate has been obtained since the possible electrode fouling can be avoided.

2. Experimental

2.1. Reagents and Chemicals. Atropine sulfate was from Sigma (St. Louis, MO, USA) and freshly prepared by serial dilution with doubly deionized water just before use. Tris (2,2'-bipyridyl) ruthenium (II) chloride hexahydrate (98%) was from Aldrich (Milwaukee, Wis, USA). Sodium phosphate (pH 8.0, G. R.) was used as the buffer solution. All chemicals and reagents were of analytical grade except specific statements. Doubly deionized water was used throughout, and all solutions were filtered through a 0.22 μm pore-size membrane before use.

2.2. Apparatus. MPI-A multiparameter chemiluminescence capillary electrophoresis analysis system with self-compiled CE-ECL software (Xi'an Remax Electronic and Technological Co., China) was employed. Uncoated fused silica capillary (50 cm $\times$ 25 μm i.d.) was obtained from Yongnian Optical Fiber Factory (Hebei, China). The end-column ECL detection was installed with a three-electrode configuration, which was made up of a Eu-PB modified platinum disk (Φ = 0.5 mm) as a working electrode, an Ag/AgCl filled with saturated KCl as a reference electrode and a platinum wire as an auxiliary electrode.

A CHI832 electrochemical analyzer (Shanghai Chenhua Apparatus Corporation, China) was used for both modification of the working electrode and measurement of the differential pulse voltammograms (D.P.V.s).

2.3. Procedure. The schematic diagram of the CE-ECL detection system was the same as reported in the previous work [19]. A solution of 5 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 80 mM phosphate buffer (pH 8.0) was directly injected into the reaction reservoir. Running buffer solution was 10 mM phosphate buffer (pH 8.0). Samples were injected in an electrokinetic mode at 10 kV for 10 s. The separation voltage was 17 kV. The photomultiplier tube (PMT) was biased at -850 V. The capillary-to-working electrode distance was adjusted to about 150 μm . Fresh $\text{Ru}(\text{bpy})_3^{2+}$ was replaced every 3 h in order to obtain good reproducibility. Capillary was rinsed with the running buffer between two sample injections until the baseline was stable. The sample concentrations were quantified by ECL peak area.

2.4. Preparation of Eu-PB Modified Platinum Electrode. The modified composite film was prepared on a smooth and cleaning surface of a platinum electrode. A solution of 10.0 mL FeCl_3 , 10.0 mL $\text{K}_3\text{Fe}(\text{CN})_6$, 6.5 mL HCl, 5.0 mL EuCl_3 , and 5.0 mL potassium hydrogen phthalate (all concentration were 0.01 M) was directly added into the electrochemical cell. The Eu-PB film was gradually electrodeposited when cell potential was cyclically scanned from 0 to 1.3 V at a rate of 20 mV/s for twenty segments round (versus SCE reference electrode).

2.5. The Urine Sample Preparation. Fresh urine samples of patients were obtained from Lanzhou University Second Hospital and collected from two male patients after 3 h and 8 h when 0.5 mg atropine sulfate were injected, respectively. The urine samples were centrifugated at 2000 rpm for 10 min. Then the top layer was separated and diluted 20-fold with deionized water, followed by passing through a 0.22 μm membrane and being directly injected into the CE-ECL system and analyzed.

3. Results and Discussions

3.1. Effect of the Eu-PB Modified Platinum Working Electrode

3.1.1. Effect on Electrooxidation Characterization of $\text{Ru}(\text{bpy})_3^{2+}$. The electrooxidation characterization of $\text{Ru}(\text{bpy})_3^{2+}$ was tested before and after modification of

FIGURE 2: Differential pulse voltammograms of $\text{Ru}(\text{bpy})_3^{2+}$ in phosphate buffer (pH 8.0): (a) at bare Pt electrode; (b) at Eu-PB modified Pt electrode. Amplitude: 0.05 V; pulse width: 0.05 s; pulse period: 0.2 s. Concentrations: $\text{Ru}(\text{bpy})_3^{2+}$, 5 mM; phosphate buffer, 80 mM.

working electrode by differential pulse voltammetry. As shown in Figure 2, the peak current from the electro-oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ was enhanced significantly at the modified electrode, and the oxidation peak of $\text{Ru}(\text{bpy})_3^{2+}$ was observed at ca. 1.05 V (versus Ag/AgCl), with slight negative shift ca. 20 mV with respect to that obtained on the unmodified electrode. Consequently, ECL efficiency of $\text{Ru}(\text{bpy})_3^{2+}$ could be improved due to catalytic oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ and therefore more production of excited state of $\text{Ru}(\text{bpy})_3^{2+}$ in the prepared electrode.

3.1.2. Effect on the ECL Response of Atropine. Luminescence response of atropine was investigated in the prepared electrode as illustrated in Figure 3. It was found that the ratio of the change of ECL signal versus the change of atropine sulfate concentration ($\Delta I/\Delta C$) was significantly increased by using the Eu-PB modified platinum electrode as ECL sensor, indicating that the change of ECL signal was more sensitive to the change of atropine sulfate concentration. Consequently, the slope of calibration curve would be enhanced. In addition, it was also indicated in Figure 3 that the linear range would be broadened when the Pt electrode was modified. Thus, the prepared electrode would benefit from the improved sensitivity and linearity for atropine sulfate.

3.1.3. Antipoisoning Effect of the Modified Electrode. In order to investigate the Antipoisoning effect of the modified electrode, atropine sulfate in real urine samples of patients were determined by the CE-ECL method using a platinum electrode and the prepared electrode as ECL sensor, respectively. It was found that the use of unmodified electrode led to peak broadening and a considerably unstable measurement due to poisoning effect of electrode, and therefore was unfit for the

FIGURE 3: Luminescence response of atropine sulfate at bare Pt electrode and at Eu-PB modified electrode: separation capillary: 25 μm , i.d., 45 cm length; sample injection: 10 s at 10 kV; separation voltage: 17 kV; running buffer: 10 mM sodium phosphate, concentration in detection cell: 80 mM, all at pH 8.0.

assay of real urine samples. While it was found that uric acid, other matrices, and atropine metabolites in urine samples had little interference to the detection by use of the prepared electrode, and consequently the enhanced ECL signal, lower noise, better peak shape, and improved reproducibility were obtained. It was proved as well that the prepared electrode was stable enough for repetitive use in the detection system over one month with no need for electrode replacement. In a word, the modified electrode shows advantage of excellent Antipoisoning effect for real urine samples.

3.2. Conditions Optimization

3.2.1. Effect of $\text{Ru}(\text{bpy})_3^{2+}$ Concentration. Concentration of $\text{Ru}(\text{bpy})_3^{2+}$ had great effect on the ECL signal. The results showed that the ECL intensity increased markedly with increasing $\text{Ru}(\text{bpy})_3^{2+}$ concentration from 0.2 to 5.0 mM due to the acceleration of reaction rate. In this work, 5 mM $\text{Ru}(\text{bpy})_3^{2+}$ in 80 mM phosphate buffer was adopted due to concerned oversensitivity and economy in use of reagent.

3.2.2. Effect of Running Buffer. Determination of atropine was studied in different buffer systems including phosphate, acetate, Tris-HCl, citric acid-sodium citrate, and borate buffers. Finally, phosphate was chosen in terms of the stable baseline, lower noise, shorter analysis time, and better peak shape.

Further, pH effect of phosphate on the detection was investigated in a wide pH range of 4.5–10.0 at intervals of 0.5 pH units. As indicated in Figure 4, the ECL intensity increased with increase in pH value in the pH range 4.5–7.5 and reached a plateau at pH 7.5–8.5, above which it decreased a little. The possible reason was considered as

FIGURE 4: pH effect of running buffer on ECL intensity: other conditions, the same as in Figure 3.

the competitive reaction between $\text{Ru}(\text{bpy})_3^{2+}$ and OH^- ions produced at high pH value [21]. So pH 8.0 was selected for all the following work.

With fixed pH value at 8.0, the concentration of running buffer was changed from 5 to 20 mM. It was found that working at high buffer concentration allowed improved sensitivity and resolution. However, high buffer concentration would induce excessive heating caused by Joule effect, resulting in an unstable measurement. Hence, 10 mM phosphate at pH 8.0 was used as the running buffer.

3.2.3. Effect of Detection Potential. The ECL intensity depends on the efficiency of electroproduced $\text{Ru}(\text{bpy})_3^{3+}$ and substantially depends on the oxidation potential applied to the electrode. As seen in Figure 5, the increased production of $\text{Ru}(\text{bpy})_3^{3+}$ with a rise of potential resulted in an increased response and the highest value was obtained at 1.15 V. Above which, the response slightly diminished, implying that the efficiency of electroproduced $\text{Ru}(\text{bpy})_3^{3+}$ decreased as competitive reactions involving the buffer dominate. Thus, the optimal potential was 1.15 V.

3.2.4. Effect of Separation Voltage. Separation voltage simultaneously impacted on the ECL intensity and migration time. More analyte arrived in the diffusion layer of working electrode within a given time with the increasing separation voltage, leading to higher ECL signal. Also, increasing separation voltage shortened the migration time because of the increase of EOF. However, the inability of the system to remove excess Joule heat generated at high voltages resulted in peak broadening and a decrease in reproducibility. Finally, the best choice for separation voltage was 17 kV.

3.2.5. Effect of Injection Voltage and Injection Time. The effects of injection voltage and injection time were studied as well. The results demonstrated that it was difficult to obtain a favorable ECL intensity though a high column

FIGURE 5: Effect of detection potential on ECL intensity: other conditions, the same as in Figure 3.

efficiency could be achieved when the injection time was shortened and the injection voltage was diminished. Also, the reproducibility became worse when an excessive sample volume was introduced. Finally, the injection parameters of 10 s at 10 kV were recommended.

3.3. Calibration and Detection. Under the optimum conditions, the calibration graph of atropine sulfate concentration versus ECL peak area was linear in the range from 0.08 to $20 \mu\text{g}\cdot\text{mL}^{-1}$, which was wider than that obtained at a bare platinum electrode [22]. The regression equation could be expressed as: $\Delta S = 566.26 + 2256.75C/\mu\text{g mL}^{-1}$ with a correlation coefficient of 0.9994 ($n = 8$). The detection limit, defined as three times the S.D. for the reagent blank signal, was $50 \text{ ng}\cdot\text{mL}^{-1}$.

The precision of the proposed method was determined by reduplicate injections ($n = 6$) of $5.0 \mu\text{g}\cdot\text{mL}^{-1}$ atropine sulfate standard solution. The relative standard deviations (R.S.D.) of migration time and ECL peak area were 0.81 and 3.19%, respectively.

3.4. Applications. In order to examine the application for clinic analysis, the levels of atropine sulfate in real urine samples of two patients were determined by the proposed method. The electropherogram in Figure 6 showed that uric acid, other matrices, and atropine metabolites in urine samples had little interference to the detection by use of the prepared electrode. As seen in Figure 6, the peak at 267 s was identified to be from atropine sulfate by spiking the standards to the sample solution. The quantitative recovery was 98.6% ($n = 5$), and about 22.4% atropine sulfate was excreted from urine with unchanged form. In the measurement process, another peak at 375 s was always detected, which was estimated to be from a normal metabolite in human urine. Similarly, another urinary sample was analyzed as well and the recovery of 90.9% ($n = 5$) was obtained. The results were listed in Table 1.

FIGURE 6: Electropherograms of (a) the diluted urinary sample of a healthy male; (b) the diluted urinary sample of a male patient; (c) the diluted urinary sample of the male patient spiked with $5.0 \mu\text{g}\cdot\text{mL}^{-1}$ atropine sulfate standard solution; other conditions are the same as in Figure 3.

TABLE 1: The determination of atropine sulfate in patient urines ($n = 5$).

Sample	Found ($\text{mg}\cdot\text{mL}^{-1}$)	R.S.D. (%) for peak area	Added ($\text{mg}\cdot\text{mL}^{-1}$)	Recovered ($\text{mg}\cdot\text{mL}^{-1}$)	Recovery (%)
1	0.112	1.52	0.100	0.0986	98.6
2	0.0782	2.19	0.100	0.0909	90.9

Conditions were the same as Table 1. Sample 1 was from the patient who was operated for the polyp excision after ca. 3 h when atropine sulfate was injected; sample 2 was from the patient who was operated for uremia after ca. 8 h when atropine sulfate was injected.

4. Conclusion

This paper described a $\text{Ru}(\text{bpy})_3^{2+}$ -based ECL method coupling to CE technique for the determination of atropine sulfate. An Eu-PB modified platinum electrode was prepared and used as ECL sensor to replace the traditional bare Pt electrode since the adsorption and direct oxidation of electroactive species in real urine samples of patients on the surface of working electrode could be avoided as much as possible. Consequently, the stability and reproducibility in signals, the linear range, and the ratio of ECL signal to atropine sulfate concentration were all significantly improved. To sum up, the proposed method showed an excellent performance with respect to selectivity, sensitivity, linearity, and stability, and further it holds great promise for the determination of tropane alkaloids in body fluids.

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