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Determination of ketotifen fumarate by capillary electrophoresis with tris(2,2′-bipyridyl) ruthenium(II) electrochemiluminescence detection

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ABSTRACT: On the basis of an europium (III)-doped Prussian blue analog film modifying platinum electrode as the working electrode, a $Ru(bpy)_3^{2+}$-based electrochemiluminescence (ECL) assay coupled with capillary electrophoresis has been first established for the determination of ketotifen fumarate (KTF). Analytes were injected onto a separation capillary of 50 cm length (50 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 15 mM phosphate buffer at pH 8.0 could achieve the most favorable resolution, and the highest sensitivity of detection was obtained using the detection potential at 1.25 V and 5 mM $Ru(bpy)_3^{2+}$ in 100 mM phosphate buffer at pH 8.0 in the detection reservoir. Under the optimized conditions, the ECL intensity was in proportion to KTF concentration over the range from 3.0×10^{-8} to 5.0×10^{-6} g mL^{-1} with a detection limit of 2.1×10^{-8} g mL^{-1} (3σ). The relative standard deviations of the ECL intensity and the migration time were 0.95 and 0.26%, respectively. The developed method was successfully applied to determine KTF contents in pharmaceuticals and human urine with recoveries between 99.5 and 107.0%. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: capillary electrophoresis; electrochemiluminescence; chemical modified electrode; ketotifen fumarate; human urine

Introduction

Ketotifen fumarate (KTF; Fig. 1) is a H_1-receptor blocking agent with anti-allergic effect and has been widely utilized clinically to treat allergic rhinitis, asthma, conjunctivitis and urticaria for many decades (1). KTF is almost completely absorbed from the gastrointestinal tract following oral administration, but its bioavailability is reported to be only about 50% due to hepatic first-pass metabolism (2). It is therefore of interest to develop sensitive and selective methods for quantification of KTF in pharmaceuticals and especially in biological fluids. Until now, analytical methods for the detection of KTF have mainly been based on liquid chromatography (LC) (2–4) and gas chromatography (GC) (5,6). However, most of the methods mentioned above need expensive instrumentation coupled with mass spectrometry, limiting their application in common laboratory. Of the applied field of spectroscopic analysis, spectrophotometry (7) and atomic absorption spectroscopy (8) have been developed as well. In addition, Khater and co-workers (9) prepared a PVC membrane electrode for the determination of KTF in pure samples and its pharmaceutical preparations with ketotifen tetraphenylborate as ion exchanger.

In recent years, chemiluminescence (CL), including electrochemiluminescence (ECL) analysis, has become popular in various fields for its high sensitivity, simple instrumentation and relatively low detection limits of the order of nanogram per milliliter level. Determination of KTF utilizing a CL approach has also been reported occasionally using of calcein or luminol as a CL reagent (1,10). However, CL methods often encounter poor selectivity for biological samples when coupled with no other separated techniques. Capillary electrophoresis (CE) represents an interesting alternative as a powerful separation tool, and the marriage of CE to CL or ECL has proved to be a promising and efficient analytical technique with high sensitivity and excellent separation efficiency (11–14). However, as far as we know, such CE-CL or CE-ECL procedure has not been reported for the determination of KTF.

In this paper, a CE-ECL method based on $Ru(bpy)_3^{2+}$ system has been developed for the determination of KTF in pharmaceuticals and human urine. A platinum microelectrode modified with europium (III)-doped Prussian blue analog film (Eu-PB) was prepared and applied as the working electrode. By this alternative, the possible electrode fouling was avoided and the detection sensitivity for KTF was significantly improved.

Experimental

Reagents and chemicals

All reagents and chemicals used were commercially available and of analytical grade. Tris(2,2′-bipyridyl) ruthenium(II) chloride

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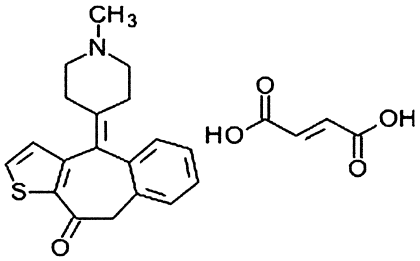

Figure 1. The structural formula of ketotifen fumarate (KTF).

hexahydrate (98%) was obtained from Aldrich (Milwaukee, WI, USA). KTF was purchased from the Chinese Pharmaceutical and Biological Test Institute (Beijing, China) and freshly prepared with water under light-proof conditions just before use. KTF tablet was obtained from the Jiangsu Yun'yang Pharmaceutical Co. Ltd. (Jiangsu, China). Nasal drops of KTF were taken from Guangxi Bo'ke Pharmaceutical Co. Ltd. (Guangxi, China). Double-distilled water was used throughout. All solutions for CE were stored in the refrigerator at 4°C and filtered through a 0.22 μm membrane prior to injection.

Apparatus

A MPI-A multi-parameter chemiluminescence capillary electrophoresis analysis system with self-compiled CE-ECL software (Xi'an Remax Electronic and Technological Co., China) was employed. An uncoated fused-silica capillary (50 cm × 50 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 an Eu-PB modifying platinum disk (diameter = 0.5 mm) as a working electrode, Ag–AgCl filled with saturated KCl as a reference electrode and platinum wire as an auxiliary electrode. A CHI832 electrochemical analyzer (Shanghai Chenhua Apparatus Corporation, China) was used for modification of the platinum working electrode.

Electrophoresis conditions

The design of the ECL detection cell was the same as that in the literature (15). A solution of 5 mM $Ru(bpy)_3^{2+}$ in 100 mM phosphate buffer (pH 8.0) was directly injected into the reaction reservoir. Running buffer solution was 15 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 −800 V. The capillary-to-working electrode distance was adjusted to about 150 μm. Prior to experiments every day, the capillary was sequentially rinsed with 0.01 M NaOH for 3 min at first, then with double-distilled water for 3 min and finally equilibrated with the running buffer for 5 min so as to maintain an active and reproducible inner surface. Fresh $Ru(bpy)_3^{2+}$ 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 intensities (16).

Preparation of Eu-PB modifying 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 $K_3Fe(CN)_6$, 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^{-1} for 20 segments around (vs SCE reference electrode).

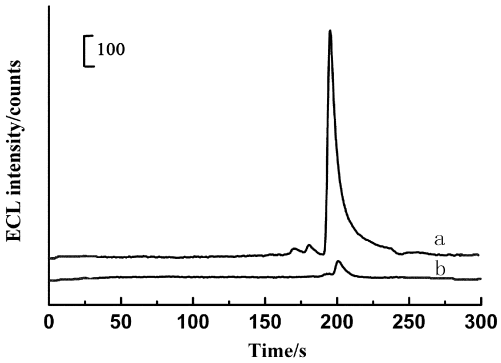

Figure 2. Electropherogram of 4.0×10^{-7} g mL^{-1} KTF standard solution: (a) at Eu-PB modifying pt electrode; (b) at bare Pt electrode. Separation capillary, 50 μm i.d., 50 cm length; sample injection, 10 s at 10 kV; separation voltage, 17 kV; detection potential, 1.25 V; running buffer, 15 mM phosphate buffer (pH 8.0); phosphate in the detection cell, 100 mM at pH 8.0.

Urine sample preparation

A urine sample was obtained from a healthy volunteer and spiked with 4.0×10^{-7} g mL^{-1} KTF standard solution. The synthetic urine sample was centrifugated at 2000 rpm for 10 min. Then the top layer was separated, following by passing through a 0.22 μm membrane, then directly injected into the capillary electrophoresis system and analyzed.

Results and discussion

Effect of the Eu-PB modifying platinum working electrode

Luminescence response of KTF was investigated in the Eu-PB modifying working electrode. As illustrated in Fig. 2, the ECL signal of KTF in the prepared electrode was 10 times high than that in a bare platinum electrode. The main reason was contributed to the increase of peak current for electrooxidation of $Ru(bpy)_3^{2+}$ and consequently more production of excited state of $Ru(bpy)_3^{2+}$ in the prepared electrode, as reported in the published work (15,17). It was also found that excipients and additives in pharmaceuticals, uric acid and other matrices in urine samples had little interference in the detection with the use of the present electrode. In addition, the prepared electrode was stable enough for repetitive use in the detection system over one month with no need for electrode replacement. In summary, the present electrode minimized electrode fouling and provided significant improvement in detecting KTF.

Effect of $Ru(bpy)_3^{2+}$ concentration

$Ru(bpy)_3^{2+}$ was used as the ECL reagent in the system and its concentration had a great effect on the ECL signal. The results showed that the ECL intensity increased markedly with increas-

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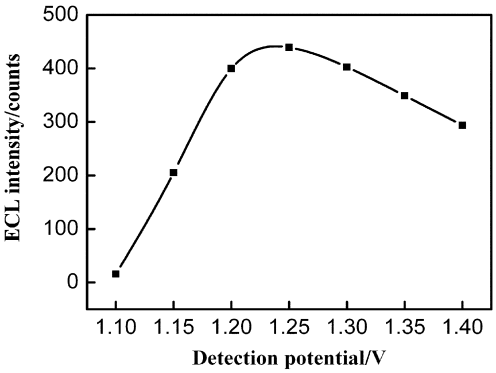

Figure 3. Effect of detection potential on ECL intensity. Other conditions are the same as in Fig. 2.

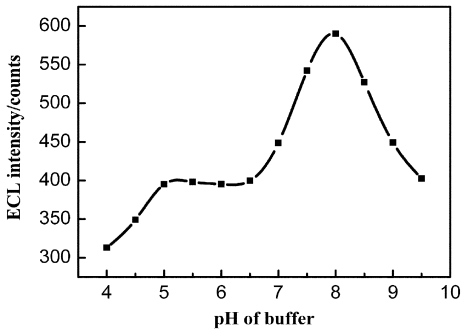

Figure 4. Effect of running buffer pH on ECL intensity. Other conditions are the same as in Fig. 2.

ing $Ru(bpy)_3^{2+}$ concentration from 0.2 to 5.0 mM due to the acceleration of reaction rate. In this work, 5 mM $Ru(bpy)_3^{2+}$ in 100 mM phosphate buffer (pH 8.0) was adopted due to concern over sensitivity and economy in the use of reagents.

Effect of detection potential

ECL intensity of KTF depends on the efficiency of electro-produced $Ru(bpy)_3^{3+}$, and substantially depends on the oxidation potential at the working electrode. The results in Fig. 3 showed that the increased production of $Ru(bpy)_3^{3+}$ with a rise of potential led to an increased response and the highest value was obtained at 1.25 V. Above this, the ECL response weakened slightly, implying that the efficiency of electro-produced $Ru(bpy)_3^{3+}$ decreased because of the oxidation of chloride ion and other impurities on the working electrode. Hence, the applied potential was set at 1.25 V.

Effect of running buffer

Determination of KTF 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, ECL intensity was influenced by the concentration of phosphate. When the running buffer concentration was augmented gradually from 5 to 30 mM, ECL intensity reached a stable maximum at the buffer concentration range of 10–20 mM and decreased on either side of this range. Meantime, high buffer concentration brought about excessive heating caused by the Joule effect, following an increased background signal and resulting in an unstable measurement.

With fixed phosphate concentration at 15 mM, the pH effect of phosphate was investigated as well in a wide pH range of 4.0–9.5 at intervals of 0.5 pH units. As illustrated in Fig. 4, the highest ECL intensity was observed at pH 8.0. Above this, ECL intensity decreased gradually. The possible reason was considered to be the competitive reaction between $Ru(bpy)_3^{2+}$ and OH^- ions produced at high pH value (18). As a result, 15 mM phosphate at pH 8.0 was selected as the running buffer.

Separation voltage

Separation voltage simultaneously impacted on ECL intensity and analysis time. More analyte arrived in the diffusion layer of the working electrode within a given time since the electroosmotic flow increased with the increasing separation voltage, making for a higher ECL signal. Meanwhile, increasing separation voltage shortened the analysis time. However, the inability of the system to remove excess Joule heat generated at high voltages resulted in peak broadening and a decrease in reproducibility. All in all, the best choice for separation voltage was 17 kV.

Effect of injection voltage and injection time

The effects of injection voltage and injection time on ECL intensity and resolution were studied. The theoretical plate numbers were calculated to evaluate resolution according to the following equation: $N = 5.54(t_m/W_{1/2})^2$, where N is the number of theoretical plates, t_m is the migration time, and $W_{1/2}$ is the width at half height of the electrophoretic peak. As seen in Fig. 5(A, B), it was difficult to obtain a favorable ECL intensity, although a high column 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.

Calibration and detection

Under the optimum conditions ascertained above, the calibration graph of KTF concentration vs ECL intensity was linear in two ranges and the correlation coefficients were fitted, respectively, as listed in Table 1. The detection limit of 2.1×10^{-8} g mL^{-1} was also calculated by the signal-to-noise ratio of 3 ($S/N = 3$). The results showed that the proposed method took advantage of a wider linear range over two orders of magnitude with respect to most of methods mentioned above (1–8,10).

Precision

Under optimized conditions, a standard solution containing 4.0×10^{-7} g mL^{-1} KTF was injected consecutively five times to determine the reproducibility of the proposed method based on peak height and migration time. The relative standard deviations (RSD) of ECL intensity and migration time were 0.95 and 0.26%, respectively. The high reproducibility indicates that this approach is accurate for detection of KTF.

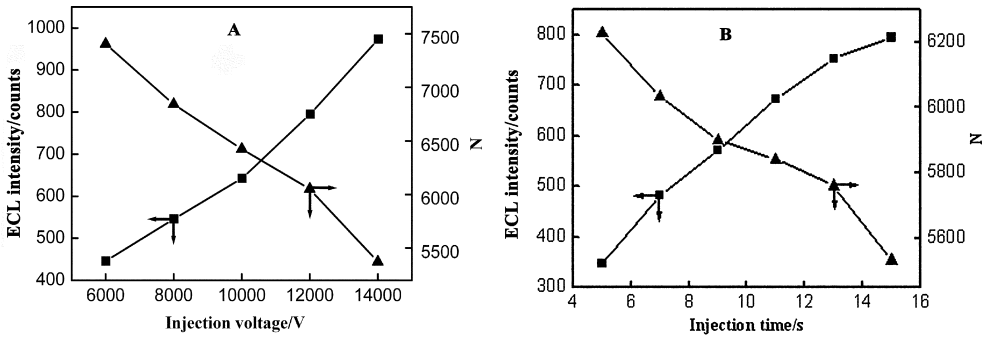

Figure 5. (A) Effect of injection voltage on the ECL intensity (■) and on the number of theoretical plates (▲). (B) Effect of injection time on the ECL intensity (■) and on the number of theoretical plates (▲). Other conditions are the same as in Fig. 2.

Table 1. Linearity and detection limit of the method

Linear range (g mL^{-1})	Regression equation	Correlation coefficient	Detection limit (g mL^{-1})
3.0×10^{-8} to 2.0×10^{-6}	$\Delta I = -39.80 + 1.95 \times 10^{9}\, C$	0.9999	2.1×10^{-8}
2.0×10^{-6} to 5.0×10^{-6}	$\Delta I = 1441.13 + 1.09 \times 10^{9}\, C$	0.9995	

Table 2. Determination of ketotifen fumarate in pharmaceuticals

Samples	Labeled (mg mL^{-1})	Present method[a] (mg mL^{-1})	RSD (%) (n = 5)	Added (mg)	Total found (mg mL^{-1})	Recovery (%) (n = 5)
Tablet	1[b]	0.97[c]	2.8	1.0	1.96[d]	99.5
Nasal Drop	1.5	1.48	0.5	1.0	2.54	102.4

[a]Measured by standard curve. [b–d]Represented as mg per tablet.

Applications

To examine the application for practical analysis, the CE-ECL method was applied to the determination of KTF in its pharmaceuticals and human urine under the optimized conditions. Certain amounts of pharmaceuticals were dissolved and diluted directly to a suitable concentration for the assay. The results obtained by the present method were in good agreement with the labeled values, as shown in Table 2. The proposed method has also been applied to analyze a synthetic urine sample containing 4.0×10^{-7} g mL^{-1} KTF by standard addition methods. The electropherogram is shown in Fig. 6. The average recovery of 107.0% (n = 5) was obtained and the RSD was 2.5%.

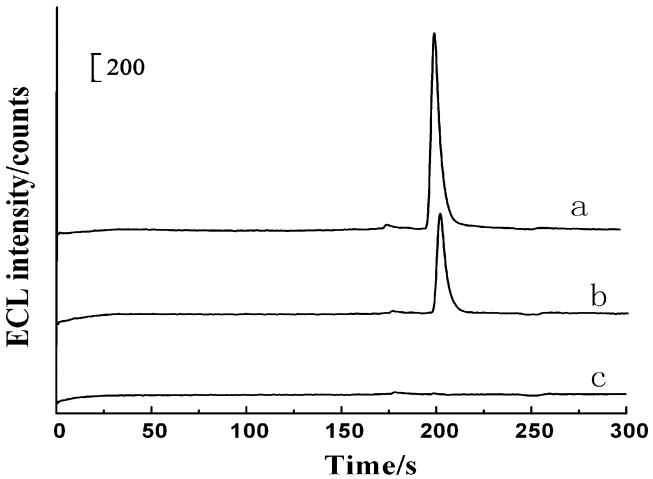

Figure 6. Electropherogram of blank human urine (c), spiked urine samples with 4.0×10^{-7} g mL^{-1} ketotifen fumarate (b) and with 6.0×10^{-7} g mL^{-1} ketotifen fumarate (a). Other conditions are the same as in Fig. 2.

Mechanism

The Ru(bpy)$_3^{2+}$-based ECL mechanism in the Eu-PB modifying platinum electrode has been briefly discussed in our previous work (15). Although both the current response and ECL intensity were enhanced by use of the Eu-PB modifying platinum electrode, no convincing evidence has been observed to indicate the existence of a new ECL mechanism until now. Thus, the ECL mechanism of the Ru(bpy)$_3^{2+}$/KTF system is also considered to be similar to the pathway of the Ru(bpy)$_3^{2+}$–TPA system at a bare platinum electrode (19). In the present study, the alkyl tertiary amine group in the molecular structure of KTF reacted with electro-produced Ru(bpy)$_3^{3+}$, resulting in the production of excited state of Ru(bpy)$_3^{2+}$ and light emission. To sum up, the possible mechanism can be expressed as follows:

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$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+}$$

$$KTFH^+ - e^- \rightarrow KTF\bullet$$

$$Ru(bpy)_3^{3+} + KTF\bullet \rightarrow [Ru(bpy)_3^{2+}]^* + products$$

$$[Ru(bpy)_3^{2+}]^* \rightarrow Ru(bpy)_3^{2+} + h\nu\,(\lambda = 620\,nm)$$

Conclusion

A new analytical procedure based on CE–ECL was introduced to detect KTF for the first time. KTF acted as a co-reactant to enhance $Ru(bpy)_3^{2+}$-based ECL intensity on an Eu-PB modifying platinum electrode. The proposed method offers the merits of rapid analysis speed (<4 min), wide linearity of over two orders of magnitude, improved sensitivity, reliable stability and good prospects with respect to performance for identification and determination of other antihistamine drugs.

Acknowledgments

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