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Simultaneous Determination of Tramadol and Diltiazem in Waterbodies by Capillary Electrophoresis with End-Column Electrochemiluminescence Detection

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A novel and sensitive method was proposed for determination of tramadol and diltiazem concentrations in water using capillary electrophoresis coupled with electrochemiluminescence. The effects of experimental conditions were investigated. Under optimized conditions, the calibration plot was linear over tramadol concentrations in the range 6×10^{-6} to 3×10^{-4} mol/L ($r \geq 0.997$), with a detection limit (S/N = 3) of 3.012×10^{-8} mol/L, diltiazem concentrations in the range 5×10^{-7} to 2×10^{-4} mol/L ($r \geq 0.986$), with a detection limit of 1.038×10^{-8} mol/L (S/N = 3). For the determination of tramadol and diltiazem concentrations in waterbodies, the relative standard deviation (RSD) of electrochemiluminescence intensity and migration time were and 4.55 % to 4.58 %, 1.73 % to 2.01 %, 0.72 % to 1.39 % and 0.97 % and 1.11 %, respectively. The proposed method was simple, rapid, sensitive and also had the merits of good reproducibility without external interference, on small samples. It was applied to determine tramadol and diltiazem concentrations in lake water samples which showed higher recoveries thereof. The new set-up was able to detect a matrix of samples both directly and simultaneously.

Keywords: Capillary electrophoresis, Diltiazem, Electrochemiluminescence, Tramadol.

INTRODUCTION

Drugs have played a positive role in human health and livestock production. However, some substances are not absorbed or fully utilized. By organisms excreting metabolites, background values of medication in water have increased bringing harm to ecosystems and human health. This so-called drug environmental pollution problem was therefore worth study to find a sensitive, reliable, easy analytical method of detecting drug residues in water, protecting the aquatic environment and contributing to ecological safety.

Tramadol hydrochloride is a new type of potent non-morphine analgesic, which acts on the central nervous system and the specific body-part associated with pain. Current measurement methods include: high performance liquid chromatography (HPLC) [1], capillary gas chromatography [2], voltammetry [3,4] and UV spectrophotometry [5]. Hydrochloride diltiazem - a calcium channel blocker - can effectively expand the epicardial and subendocardial coronary arteries, alleviating spontaneous or ergonovine-induced coronary artery spasm angina pectoris. The current measurement methods include: spectrophotometry [6], NIR-FT-Raman spectroscopy [7], adsorption voltammetry [8] and mobile amperometric methods [9]. The existing methods are complicated, time-consuming and often not sufficiently sensitive.

Electrochemiluminescence analysis has the advantages of both electrochemical analytical methods and chemiluminescence analyses. So capillary electrophoresis coupled with electrochemiluminescence (CE-ECL) is an efficient separation, high-sensitivity detection method [10] which is used for the separation and detection of drugs such as tramadol and etamsylate lidocaine [11,12], as well as diltiazem hydrochloride combined with human serum protein binding points and binding constants [13]. This research established a new capillary electrophoresis separation method based on a ruthenium [$Ru(bpy)_3^{2+}$] electrochemiluminescence system (CE-ECL) for simultaneous separation and detection of tramadol hydrochloride and diltiazem hydrochloride in water. The results showed that this method was simple, rapid and reliable.

EXPERIMENTAL

Tramadol hydrochloride standard (China Pharmaceutical and Biological Products); hydrochloride diltiazem (Shanghai Bo Yun Biological Technology Co., Ltd.); terpyridine ruthenium chloride hexahydrate (Sigma-Aldrich Corporation United States); and sodium phosphate buffer solution salt system (Na_2HPO_4/NaH_2PO_4) were all chemical reagents of analytical grade purity. The water was Milli-Q ultrapure water; samples before injection were passed through a 0.22 μm acetate cellulose membrane filter.

The MPI-A capillary electrophoresis electrochemiluminescence detector (Xi'an Remax Science & Technology Co. Ltd., Xi'An, China); uncoated fused silica capillary (25 μm i.d, 360 μm o.d, 40 cm effective length, Yongnian Optical Fibre Factory); Lida PHS-3C pH meter (Shanghai LIDA Instrument Factory, Shanghai, China); AUW220D Shimadzu analytical balance (Shimadzu Corporation, Kyoto, Japan) and the Milli-Q ultrapure water system (Millipore, Bedford, MA, USA) were used throughout.

Experimental set-up: The electrochemiluminescence detection cell was placed in a luminescence detector cassette in the centre of the light transmission window of a photomultiplier tube. The detection cell was filled with 5 mmol/L $Ru(bpy)_3^{2+}$ in phosphate buffer (pH 8.0); the phosphate buffer was used as an electrophoresis separation buffer. The sample was electrokinetically injected. The potential difference across the photomultiplier tube was set to 800 V. All solutions were filtered by the aforementioned cellulose acetate membrane before injection [14]. The capillary inlet end was inserted into the buffer and the light emitting electrochemical analyzer then opened. The capillary injection end was not inserted into the sample solution until a stable baseline was achieved by sample electromigration injection. The injector-side to the buffer solution was switched and then a separation potential applied. The electrophoretic separation and sample detection were thereby achieved. The $Ru(bpy)_3^{2+}$ in the solution reservoir was replaced once every 3 h to reduce the influence of reaction product impurities and solution evaporation on the detection.

RESULTS AND DISCUSSION

Electrochemiluminescence behaviour of tramadol and diltiazem: To study the effect of tramadol hydrochloride and diltiazem hydrochloride on the behaviour of the electrochemiluminescence of $Ru(bpy)_3^{2+}$, the cyclic voltammetric behaviour of 5 mmol/L $Ru(bpy)_3^{2+}$ in phosphate buffer solution was investigated. Adding tramadol hydrochloride and diltiazem hydrochloride to the previous $Ru(bpy)_3^{2+}$ system respectively, the cyclic voltammetric behaviour is shown in Fig. 1A and 1B. The curve **a** in these two figures represented $Ru(bpy)_3^{2+}$ in the phosphate buffer solution. The electrochemiluminescence signal was weak and from curve **b** it was seen that with the addition of tramadol hydrochloride and diltiazem hydrochloride to the $Ru(bpy)_3^{2+}$ system, the electrochemiluminescence intensity increased. The maximum voltage occurred at 1.17 V, which matched the oxidation potential of $Ru(bpy)_3^{2+}$. It transpired that tramadol hydrochloride and diltiazem hydrochloride may be used as co-reactants and thus participated in oxidation of $Ru(bpy)_3^{2+}$, making the electrochemiluminescence intensity increase [15,16].

Optimization of separation conditions

Detection potential options: The applied potential difference across the working electrodes, as the electrochemiluminescent reaction detection potential, was an important factor affecting the electrochemiluminescence intensity. The effect of detection potential on electrochemiluminescence is shown in Fig. 2. Electrochemiluminescence reached its highest value at 1.2 V and then declined when the scan potential increased with detection potential changing over the range 1.0 to 1.40 V.

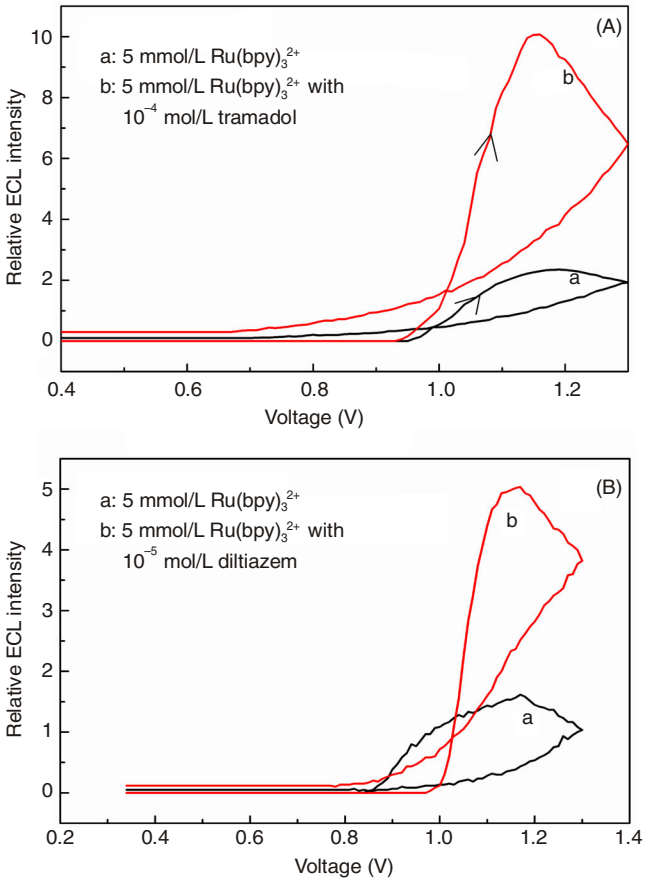

Fig. 1. Effects of tramadol (A) and diltiazem (B) on electrochemiluminescence intensity of $Ru(bpy)_3^{2+}$ in phosphate buffer solution (pH 7.8) at bare Pt electrode. Scan rate: 100 mV s^{-1}, scan range: 0~1.3V

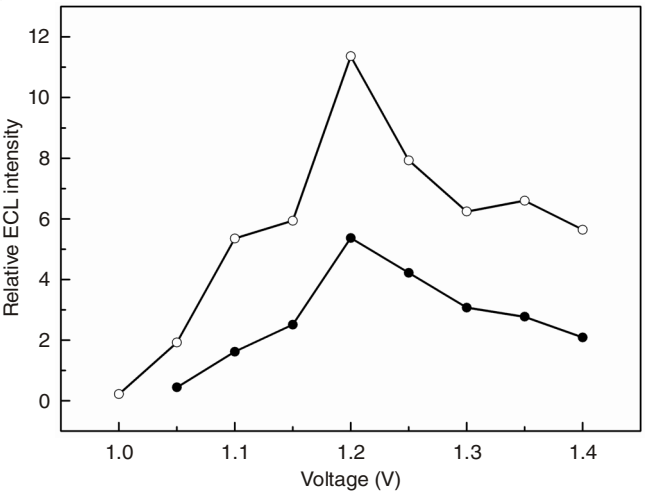

Fig. 2. Effect of detection potential on electrochemiluminescence intensity. Condition: 1.55 × 10^{-4} mol/L tramadol (○); 5.39 × 10^{-5} mol/L diltiazem (●); 20 mmol/L (pH 7.50) buffer and 5 mmol/L $Ru(bpy)_3^{2+}$ in detection cell; electrokinetic injection 10 s × 10 kv

When the potential was less than 1.2 V, with the potential rising, the concentration of $Ru(bpy)_3^{3+}$ generated in the system increased accordingly, electrochemiluminescence intensity increased rapidly. However, when the potential was higher than 1.2 V, the light intensity decreased, this may have been because the water in the system was oxidized and thus having an impact on the emission intensity [17]. Therefore, 1.2 V was chosen as the detection potential for subsequent experiments.

Influence of buffer solution: Buffer type, concentration (ionic strength) and pH not only affected the electro-osmotic flow, but also influenced the electrophoretic behaviour of the sample solutes, as well as the separation efficiency and analysis time for the capillary electrophoresis, which was of importance when selecting options for the separation conditions. This work investigated several buffer systems including: H_3PO_4/NaH_2PO_4, NaH_2PO_4/Na_2HPO_4, sodium citrate/citric acid, CH_3COOH/CH_3COONa. The results showed that 20 mmol/L Na_2HPO_4/NaH_2PO_4 buffer solution had advantages of a smooth baseline, low noise, shorter separation time and a better defined peak shape. So, the chosen optical electrophoresis buffer was 20 mmol/L Na_2HPO_4/NaH_2PO_4.

The phosphate buffer pH in the detector cell influenced the electrochemiluminescence intensity. Under the optimum detection potential, keeping the concentration of $Ru(bpy)_3^{2+}$, phosphate buffer and the standard running buffer constant, only the phosphate buffer pH into which $Ru(bpy)_3^{2+}$ dissolved was changed to assess the electrochemiluminescence intensity of tramadol and diltiazem caused by aqueous acidity [18] (Fig. 3). As can be seen from Fig. 3, when the pH was set to between 4 and 11, the maximum electrochemiluminescence intensity for the two analytes occurred at pH 7.5. In this experiment, a phosphate buffer (pH 7.5) was selected as the best running buffer.

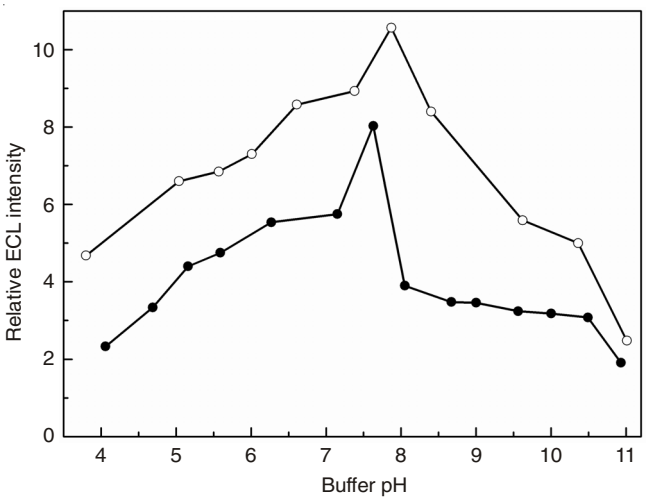

Fig. 3. Effect of the buffer pH on electrochemiluminescence intensity. Condition: 1.55×10^{-4} mol/L tramadol (○); 5.39×10^{-5} mol/L diltiazem (●); detection potential 1.2 V; electrokinetic injection 10 s × 10 kv

Effects of injection time and injection voltage: Under electrokinetic injection mode, the injection volume of tramadol and diltiazem will be simultaneously affected by the injection time and injection voltage. The longer the injection time, a higher injection voltage will lead to more analytes flowing into the detection cell. When the chemiluminescent reactions occurred, electrochemiluminescence strength was mainly determined on the concentration of analyte measured on the working electrode surface. More materials spread into the diffusion layer as a result of the electrochemiluminescence intensity increasing [19]. However, this may cause zone broadening, peak broadening and furthermore, the separation efficiency decreased. On the other hand, although a shorter injection time and a lower injection voltage can result in higher column efficiency, it was difficult to achieve the optimal electrochemiluminescence intensity, even to the extent of affecting the detection sensitivity for each sample.

The experiments were investigated to examine the effect of injection time and injection voltage on the electrochemiluminescence intensity of tramadol and diltiazem. The influence of sampling time was shown in Fig. 4A. The electrochemiluminescence intensity of tramadol increased as the injection time changed over the range 2 to 16 s. However, the electrochemiluminescence intensity of diltiazem increased with as the injection time changed over the range 4 to 10 s; thereafter the chemiluminescence intensity decreased significantly. After comprehensive consideration, the injection time was selected as 10 s. The effects of injection voltage on electrochemiluminescence were shown in Fig. 4B. When the injection voltage reached 10 kV, both tramadol and diltiazem had their peak electrochemiluminescence intensity, followed by a slow decline at higher voltages. Therefore, 10 kV was chosen as the optimum injection voltage.

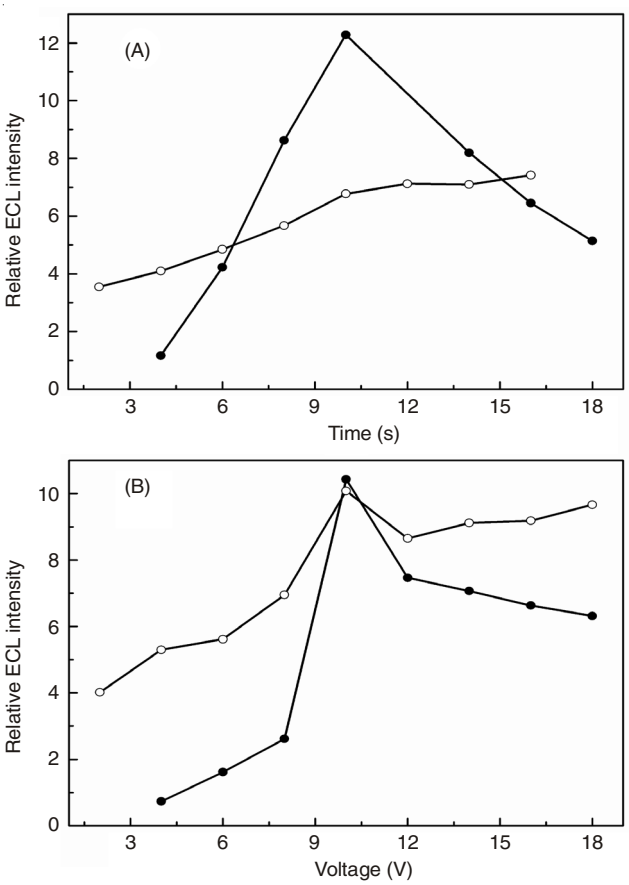

Fig. 4. Effect of injection time (A) and injection voltage (B) on electrochemiluminescence intensity. Condition: 1.55×10^{-4} mol/L tramadol (○); 5.39×10^{-5} mol/L diltiazem (●); detection potential 1.2 v; 20 mmol/L (pH 7.50) buffer and 5 mmol/L $Ru(bpy)_3^{2+}$ in detection cell

Impact of separation voltage: Changing the separation voltage affected the electrochemical luminous intensity, migration time, column efficiency and resolution. By increasing the separation voltage from 7 to 19 kV, the corresponding changes in electrochemiluminescence intensity and resolution were

examined. As shown in Fig. 5, tramadol hydrochloride and diltiazem hydrochloride electrochemiluminescence intensities increased as the separation voltage increased. The electrochemiluminescence reached its highest value at 15 kV and then declined as the voltage increased further, which was attributed to Joule heating in the capillary when the separation voltage was greater than 15 kV. As a result, baseline noise increased and more analytes flowed into the detection cell from the capillary tube: the $Ru(bpy)_3^{2+}$ concentration at the electrode surface decreased, as did the luminous efficiency [18]. When the separation voltage reached 15 kV, the relative resolution between tramadol hydrochloride and diltiazem hydrochloride was 1.82. Therefore, 15 kV was selected by considering sensitivity, separation efficiency, migration time and other factors.

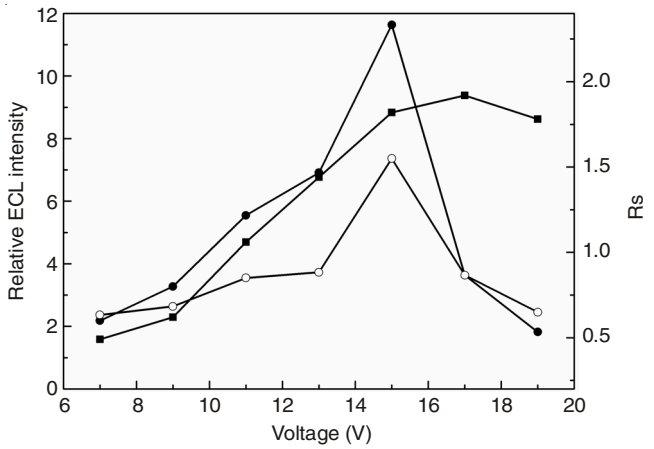

Fig. 5. Effect of separation voltage on electrochemiluminescence intensity, resolution (Rs, ■) of diltiazem and tramadol. Condition: 1.55×10^{-4} mol/L tramadol (○); 5.39×10^{-5} mol/L diltiazem (●); detection potential 1.2 V; 20 mmol/L (pH 7.50) buffer and 5 mmol/L $Ru(bpy)_3^{2+}$ in detection cell, electrokinetic injection 10 s × 10 kv

Analytical performance of CE-ECL and its application: Under optimum conditions, the electrochemiluminescence for tramadol and diltiazem with different concentrations (mol/L) was tested to examine their respective relevance. A value of 1×10^{-5} mol/L was chosen for standard solutions of each component: eight-fold successive injections were used to examine its reproducibility [20]. The linear range of the components, the linear correlation coefficient, the relative standard deviation of the migration time and electrochemical luminescence intensity and the detection limit based 3 values are listed in Table-1.

Samples were taken from local rivers and lakes. After centrifugation, standing and 0.22 μm cellulose acetate membrane filtration, no tramadol hydrochloride and diltiazem hydrochloride response signal was found under optimal experimental conditions. To investigate the practical application of CE-ECL, some 5.39×10^{-5} mol/L diltiazem hydrochloride and 1.55×10^{-6} mol/L tramadol hydrochloride standard solution mixtures were added to the sample (Fig. 6a). The electrophoretic spectrum showed two peaks. To verify the attributes of the peaks, 4.66×10^{-6} mol/L tramadol hydrochloride standard solution was added to the mixed solution. It was found that one of them increased with an increased tramadol concentration (Fig. 6b). Thus, it was concluded that the chromatogram peaks that appeared initially were representative of tramadol hydrochloride: those appearing latterly represented diltiazem hydrochloride. High recovery was realized for standard additions to the sample. Consequently, CE-ECL technology could detect the tramadol hydrochloride and diltiazem hydrochloride in water with a complete separation thereof, without interference from other impurities in the sample.

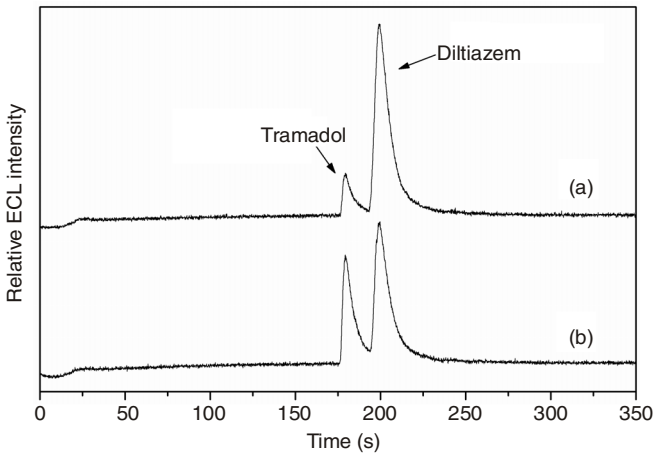

Fig. 6. Typical electropherograms of: (a) 5.39×10^{-5} mol/L diltiazem and 1.55×10^{-6} mol/L tramadol solution; (b) 4.66×10^{-6} mol/L tramadol was spiked to "a"

Conclusion

In summary, a sensitive method for the study of drug residues in water, based on CE-ECL, was reported. The proposed method was used to separate and detect tramadol hydrochloride and diltiazem hydrochloride in water. Their electrophoretic separation conditions were optimized. Under these optimized experimental conditions, two analytes reached baseline separation in 204 s. The experiments also studied the reproducibility, precision and accuracy of the new method. It was believed that the CE-ECL based approach bestowed advantages in this area because it was simple, rapid, sensitive, accurate and precise. It could thus be applied to the analysis of drug residue concentrations in water pollution scenarios.

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TABLE-1
LINEAR RANGE, DETECTION LIMIT AND REPRODUCIBILITY OF PROPOSED METHOD

Components	Linear range (mol/L)	Correlation coefficient	Detection limit (mol/L)	RSD % (ECL intensity)	RSD % (migration time)
Tramadol	6×10^{-6} to 3×10^{-4}	0.997	3.012×10^{-8}	4.55-4.58	0.72-1.39
Diltiazem	5×10^{-7} to 2×10^{-4}	0.986	1.038×10^{-8}	1.73-2.01	0.97-1.11

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