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Short-capillary electrophoresis with electrochemiluminescence detection using porous etched joint for fast analysis of lidocaine and ofloxacin

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Abstract

Fast analysis of ofloxacin and lidocaine, as bactericide and analgesic or anesthetics, is of clinic importance for understanding the patient's medical process. This paper presented a high throughput, simple analysis method of lidocaine and ofloxacin by capillary electrophoresis coupled with electrochemiluminescence (ECL) using porous etched joint. To shorten the analysis time and to improve the analytical performance, a capillary with 10 cm in length was used as the separation channel. The cyclic voltammograms of $Ru(bpy)_3^{2+}$ with different capillary length at same field strength showed that the porous etched joint eliminated the effect of electrophoretic current on the ECL detection. Following micro total analysis systems (μTAS), some advantages of which this approach has, the fabrication of channel in chip was not needed. Compared with capillary electrophoresis with 40-cm-long capillary, the high sample throughput and low zone broadening may be the main advantage of the present system. Under optimal condition, the detection limits of lidocaine and ofloxacin based on peak height were 3.0×10^{-8} and 5.0×10^{-7} mol L^{-1} and a 60 h^{-1} of sampling frequency was obtained. The precision (R.S.D.) of the migration time and the peak height for five replicate injections of a mixture of lidocaine (1.0×10^{-6} mol L^{-1}) and ofloxacin (4.0×10^{-6} mol L^{-1}) were 3.2–3.9% and 4.7–5.3%, respectively.

Keywords: Capillary electrophoresis; Electrochemiluminescence; Lidocaine; Ofloxacin

1. Introduction

Micro total analysis systems (μTAS), also called "lab on a chip", are growing rapidly since its naissance [1,2]. The advantages of μTAS including high performance, short analysis time, portability, disposability, and consumption of minute sample and reagent have become obvious [1,2]. Most researchers hope this instrument platform contains many functions, such as sample preparation, separation, and detection, encompassed within it [1,2]. For separation, capillary electrophoresis (CE) or CE-based separation techniques are highly suitable for implementation on the microchip format. Electrokinetic control of fluid transport eliminates the need for external components such as pumps and valves. Electrophoresis in planar chips, as a simple and versatile μTAS, included successfully injection, separation, and detection within the same device and attracted much attention in the separation field [1,2]. The main reason for miniaturization was initially to enhance the analytical performance of the whole device by integrating much part together, but as indicated previously [3], not one of the systems currently on market is an entire laboratory on a chip, and microfluidic merely indicated microstructures carrying fluid [3]. So, conventional capillary electrophoresis with suitably decreased dimension can be used with some advantages of μTAS, while the fabrication of μTAS is not needed.

Firstly, capillary with 25 and 50 μm using in conventional CE has the similar cross-section area to that of the channel used in some chip CE. Although capillaries longer than

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20 cm in length were utilized in most of CE separation, there are some works using short capillary for some purpose, such as study of conjugation reaction of protein cytochrome *c* with sodium dodecyl sulfate [4] and fast analysis of DNA [5,6], free acid [7], or antibacterial isothiazolones [8]. Those works showed that higher sensitivity was obtained in a short capillary than that in a long one because the peak broadening decrease and peak sharpening increased because of the short electromigration distance [4]. The increased sample throughput is evident within short capillary separation. This advantage is similar to that in μTAS whereas the fabrication of chip is eliminated.

But the choice of the capillary length of CE separation is detector-dependent. The capillaries with longer than 100 cm in length were used to couple CE with inductively coupled plasma mass spectrometry (ICP-MS) just because of the complex construction of ICP-MS detector [9,10]. The so-called short capillary in CE on-column detection such as laser-induced fluorescence (LIF) [5] and fluorescence [7] is only to decrease the effective length of the capillary. Linear charge-coupled device (CCD) combined with whole-column imaging can be used in real short-capillary electrophoresis [4]. Electrochemiluminescence (ECL) based on tris(2,2′-bipyridine)ruthenium (II) ($Ru(bpy)_3^{2+}$), the process where electrochemically generated reactants undergo a high energy electron transfer reaction to generate an excited state, is proved to be a powerful analytical tool combining the simplicity of electrochemistry and the inherent sensitivity and wide linear range of chemiluminescence (CL) method [11–13]. In principle, while the marriage of ECL to CE using short capillary enjoys the whole advantage of short-capillary electrophoresis, such as fast separation and high sensitivity, it enhances increasingly the sensitivity of the whole system by using the high sensitive ECL detection. In previous work [14], we fabricated successfully a CE–ECL system by using porous etched joint, which eliminated the effect of CE high-voltage field on the ECL procedure and made the CE–ECL system versatile [14]. Contrary to the other CE–ECL systems [15–20], the simple instrumental setup of the system makes the using of short capillary possible. Huang et al. [21] designed a pseudo-chip CE end-column ECL detection system, where a short capillary, a falling-drop sample introduction interface for coupling to the FI system and an end-column reservoir was integrated on a glass slide. A sample throughput of 50 h^{-1} was obtained with the system [21].

Lidocaine, an amide synthesized from cocaine, is one of the most extensively used local anesthetics and peripheral analgesic, which is effective to reduce pain [22]. Lidocaine toxicity primarily affects the cardiovascular and central nervous system and over-dosed intake may result in ventricular arrhythmia. Less than 10% of lidocaine is excreted unchanged by the urine [22–24]. While ofloxacin, as a synthetic fluoroquinolone derivative, has demonstrated broad-spectrum activity against many pathogenic gram-negative and gram-positive bacteria. The bactericidal action of ofloxacin results from interference with enzyme DNA gyrase that is needed for the synthesis of bacterial DNA [25,26]. And thus, fast analysis of both ofloxacin and lidocaine, which are bactericide and analgesic or anesthetics, is of clinic importance for understanding the patient's medical process.

In the present work, an attempt was made to fast analysis of lidocaine and ofloxacin by coupling CE with ECL using short capillary and thus shortening the analysis time, augmenting the sample throughput. The effect of capillary length on ECL detection, optimization of CE separation, ECL detection, and analytical performance of the developed technique were described and discussed in detail.

2. Experimental

2.1. Instrumentation

A homemade CE setup with ECL detector was used in this work. High-voltage power supply was from Shanghai Nucleus Institute (Shanghai, China). The power supply was operated in voltage-controlled mode. Fifty micrometers i.d. uncoated fused-silica capillaries (Yongnian Optical Fiber Co. Ltd., Hebei, China) with different length were used as separation channel to examine the effect of capillaries on ECL with a 350 V/cm of field strength.

New capillaries were conditioned by flushing with methanol, 0.1 M HCl, double distilled water (DDW), 0.1 M NaOH for 10 min, respectively. Before separation, the capillary were flushed with running buffer for 5 min. In all experiments, the sample was introduced electrokinetically.

The electrochemical measurement for ECL experiments was carried out with Model CH800 Voltammetric Analyzer (CH Instruments, Austin, TX, USA). A three-electrode system was employed with Pt wire as counter electrode, Ag/AgCl as reference electrode, and 300-μm diameter Pt disk as working electrode. The newly prepared working electrode was polished with 0.3-μm α-alumina powder slurry before using. Once fouling, a simple cyclic voltammetric scanning between −0.5 and 0.0 V or repolishing was performed to activate the work electrode. The ECL emission was detected with a Model MCDR-A Chemiluminescence Analyzer Systems (Xi'An Remax Science & Technology Co. Ltd., Xi'An, China). The voltage of photomultiplier tube (PMT) used in Chemiluminescence Analyzer was set at 850 V in the process of detection.

The CE–ECL setup used in this work is as described previously [14]. Briefly, a porous etched joint was used to achieve electrical connection at a distance of 7 mm from the capillary outlet. Because the porous capillary wall allowed CE current to pass through and there was no electric field gradient beyond that section, the influence of CE high-voltage field on the ECL detection was eliminated. The separation capillary can be separated two sections: the part in light-tight chamber (5 cm), among which 2 cm was inserted in the main body of the CE–ECL system and the part out of the light-tight chamber (5 cm). The distance between the working

electrode and the end of capillary maintained 100 μm and the volume of $Ru(bpy)_3^{2+}$ solution reservoir was about 300 μl. The $Ru(bpy)_3^{2+}$ solution is replaced every half of the day to keep a reproducible signal. After CE separation using phosphate solution as separation electrolytes, the analytes were in contact with and reacted with $Ru(bpy)_3^{2+}$ at the electrode surface, and hence an electropherogram was obtained because the enhancement of analytes to the $Ru(bpy)_3^{2+}$ ECL.

2.2. Reagents

All the reagents employed were at least of analytical grade and DDW was used throughout. Ofloxacin and tris(2,2′-bipyridine)ruthenium (II) chloride pentahydrate ($Ru(bpy)_3Cl_2{\cdot}5H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 10 mmol L^{-1} $Ru(bpy)_3^{2+}$ was dissolved in DDW as a stock solution and stored in refrigerator at 4 °C. The working solution was prepared daily by diluting the stock solutions with 0.1 mol L^{-1} phosphate salt solution (pH 8.2) just before use. Ofloxacin and lidocaine (Beijing Yongkang Pharmaceutical factory, Beijing, China) were dissolved in DDW directly at concentration of 1 mmol L^{-1} as stock solution, respectively.

Disodium hydrogen phosphate (Beijing Chemicals Co., Beijing, China) was used to prepare the separation buffer solution. The pH of the buffer solution was adjusted with 0.1 mol L^{-1} NaOH and 0.1 mol L^{-1} HCl. Different concentrations and pH of phosphate buffer solution were used to investigate the effect of buffer solution on electrophoretic separation.

3. Results and discussion

3.1. The effect of capillary length on ECL procedure

In previous work [14], the investigation of the influences of CE separation voltage, buffer concentration, and capillary inner diameter indicated that the porous etched joint can eliminate the effect of the CE field strength on ECL procedure completely. The effect of high-voltage with different capillary lengths at same electric field strength was examined with 10 mmol L^{-1} phosphate buffer (pH 8.0) for investigation of the effect of capillary length on ECL procedure. For this purpose, the capillaries with different length were filled with the separation buffer to obtain electrophoretic current and then cyclic voltammograms (CVs) were recorded. Fig. 1 shows the CVs scanned from 0.5 to 1.3 V (versus Ag/AgCl) at a scan rate of 50 mV s^{-1} in no electrophoretic current and the electrophoretic current obtained from the different capillary lengths with same electrophoretic field strength of 350 V/cm. As given in Fig. 1, no shift of redox potential of $Ru(bpy)_3^{2+}$ between no CE voltage and same electrophoretic field strength in different capillary lengths. The decreased oxidizing current of $Ru(bpy)_3^{2+}$ in CVs may be due to the dilution to $Ru(bpy)_3^{2+}$ solution near the working electrode surface by electrophoretic capillary effluent. The nearly consistent oxidizing current shows the similar electroosmotic flow (EOF) between the capillaries with different length, which is thinkable because of the same field strength.

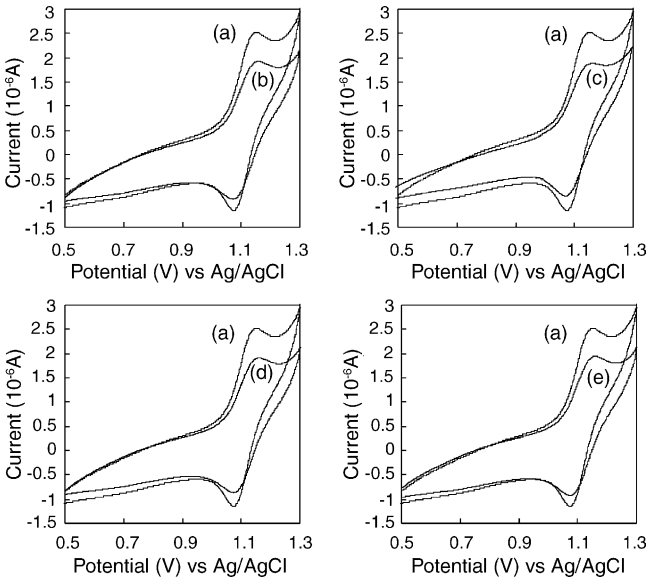

Fig. 1. CV of $Ru(bpy)_3^{2+}$ affected by CE current with same field strength and different capillary lengths: (a) Without CE current; (b) 40 cm capillary length; (c) 30 cm capillary length; (d) 20 cm capillary length; and (e) 10 cm capillary length. Field strength: 350 V/cm.

3.2. CE separation

The pH of the CE buffer plays an important role in controlling CE separation. It affects the CE separation by influencing the mobility of analytes and the characteristic of capillary inner surface and thus EOF. The influence of the pH of CE buffer on the separation of both analytes was investigated using 10 mmol L^{-1} phosphate buffers at different pH. The baseline separation of the two species was achieved at all of the tested pH value of 7.0, 7.5, 8.0, 8.5, and 9.0, but the best separation efficiency was obtained at a pH of 8.0, which is also similar to that of the detection buffer and thus affects the detection much less.

The effect of the concentration of phosphate buffer on the separation was tested at a pH of 8.0 in the buffer solution. Over the concentration range examined (3–20 mmol L^{-1} phosphate), the two species were baseline separated. However, the migration time of each individual species increased with the increase of the buffer concentration. But the solutions lower than 10 mmol L^{-1} phosphate buffers give deficient buffer capacity. Based on the above results, a 1 ml solution of 10 mmol L^{-1} phosphate at pH 8.0 was employed as the CE buffer, which has buffer capacity enough used at least 3 h of operation without affecting the separation reproducibility. Fig. 2 shows the electropherograms of a mixture of the two analytes standards in DDW or spiked in urine under the optimal conditions.

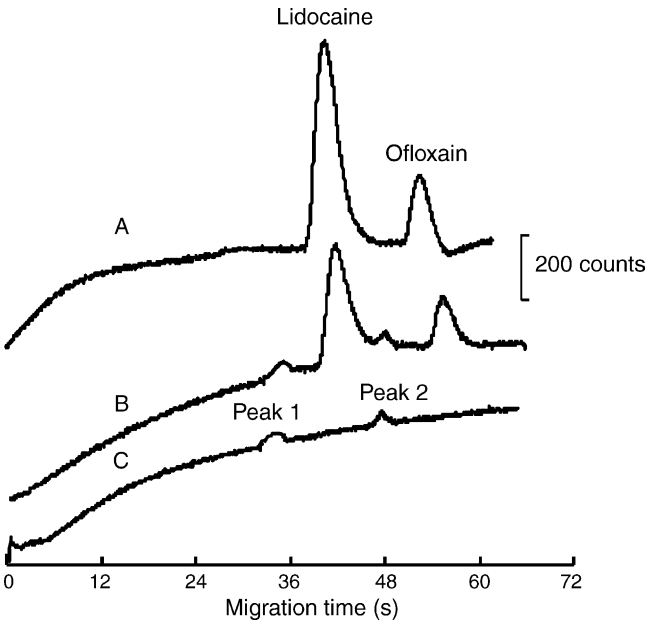

Fig. 2. Electropherograms of (A) the standard solution; (B) the spiked urine sample; and (C) the urine sample. ECL solution: 0.05 mol L^{-1} phosphate at pH 8.2; capillary length: 10 cm; separation buffer: 10 mmol L^{-1} phosphate at pH 8.0; injection: electrokinetic injection for 3 s at 3500 V.

3.3. ECL detection

The pH value of ECL solution is an important factor affecting the ECL intensity [27,28]. The previous work [27,28] indicated that the efficiency was markedly affected by both reaction pH and the analyte molecular structure. The structures of the two analytes are given in Fig. 3. The deprotonation step of analyte in ECL reaction should be the important factor to affect the ECL intensity, and therefore, the effect of the pH on ECL intensities of the two analytes was tested from pH 5 to 9 of 50 mmol L^{-1} phosphate buffer. As illustrated in Fig. 4, the ECL intensity of ofloxacin reached maximum at pH 7.0 and then level off within the pH range tested, while that of lidocaine increased with increase of pH from 5 to 9 significantly. The tendency of ECL intensity versus pH is coincident with the previous report [27]. The results demonstrated that the ECL intensity is dependent on the structures of analytes and the detection buffer pH. As the results shown above, the solution at pH 8.2 was chosen with a compromise.

Investigation of the effect of the concentration of $Ru(bpy)_3^{2+}$ on the two analytes ECL intensity from 1 to 10 mmol L^{-1} indicated that the two analytes had similar profile of ECL intensity versus concentration of $Ru(bpy)_3^{2+}$. Although the responses of the two analytes increased with increasing in the concentration of $Ru(bpy)_3^{2+}$, higher concentrations of $Ru(bpy)_3^{2+}$ caused higher baseline noise, and thus, poor reproducibility. Low concentration of $Ru(bpy)_3^{2+}$ probably gave deficient $Ru(bpy)_3^{3+}$ oxidizing the analytes to produce ECL emission. To maintain high sensitivity and low baseline noise, a 5 mmol L^{-1} $Ru(bpy)_3^{2+}$ contained in the 50 mmol L^{-1} phosphate salt solution at pH 8.2 was used as the ECL reagents.

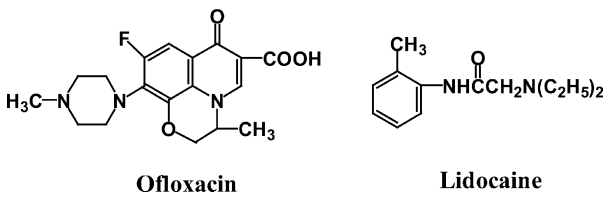

Fig. 3. The molecular structure of ofloxacin and lidocaine.

The influence of applied potential on the analyte ECL signals was tested by changing the potential from 0.5 to 1.3 V (versus Ag/AgCl). When the applied potential was lower than 1.0 V, few light emission was observed because $Ru(bpy)_3^{2+}$ was not oxidized on the working electrode. While two analytes gave the constant ECL intensity ranging from 1.15 to 1.25 V, the decreased signals above 1.25 V may be due to the interference from the oxidation of water as the increased oxidizing current shown in Fig. 1.

3.4. Injection time

By electrokinetical injection, a fixed 3500 V of injection voltage consistent to the separation voltage was used to investigate the effect of injection time ranged from 1 to 7 s on the ECL intensity and separation efficiency using capillary with 10 cm in length. The results indicated that the peak height of both analytes increased with the increase of the injection time from 1 to 3 s, and then, from 3 to 7 s, the peak height did not increased significantly and only the peak became broadened. Although an injection time of 5 s or more was often used in conventional capillary electrophoresis system [28], the present short-capillary electrophoresis showed lower sample load. Sample overloading was observed when injection time was beyond 3 s in present system. The injection volume can be calculated as following [29]:

$$v = \frac{(\mu_{ep} + \mu_{eo})\pi r^2 Vt}{L} = \frac{\mu_{ap}\pi r^2 Vt}{L}$$

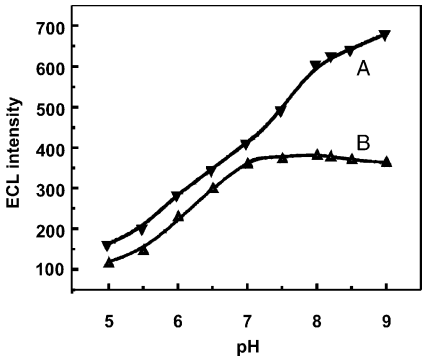

Fig. 4. Effect of the detection buffer pH on the ECL intensity (peak hight) of lidocaine ((A) 1.0×10^{-6} mol L^{-1}) and ofloxacin ((B) 4.0×10^{-6} mol L^{-1}). The other conditions as shown in Fig. 2.

where v is the injection volume, V the injection voltage, t the injection time, r the capillary radius, L the capillary length and μ_{ep}, μ_{eo}, and μ_{ap} are the electrophoretic mobility of the analytes, electroosmotic mobility and apparent mobility of the analytes, respectively. According to the equation above, the injection volume of lidocaine and ofloxacin is about 15 and 11 nl, respectively, that is much more than that in traditional μTAS and facilitates the enhanced sensitivity.

3.5. Validation of the short-capillary electrophoresis electrochemiluminescence system

Two blank urine samples were provided by a healthy male volunteer and a healthy female volunteer. The samples were diluted four-fold after being filtered through 0.45 μm membrane for the spike experiment. The accuracy of the present short-capillary electrophoresis ECL system was checked by analyzing the two human urine samples spiked with 1umol L^{-1} lidocaine and 4 umol L^{-1} ofloxacin. The typical electropherogram of a standard solution was given in Fig. 2A, while Fig. 2B and C were the electropherograms of the male volunteer urine with and without spiked the analytes. As given in Fig. 2B and C, the two analytes were separated from the interferences of urine (peak 1 and peak 2). It was found that the migration time and peak height of each analyte spiked in the urine samples and that of the standard mixture were different to a certain extent, maybe due to their different ion strengths. Accordingly, the content was decided by using a standard addition calibration for the analysis of these spiked samples. The analysis results are shown in Table 1 and the recoveries of the spikes ranged from 90.9 to 111%.

3.6. Analytical performance

It was indicated that not one of the systems currently on the market is an entire laboratory on a chip, but "micro total analysis system instruments are being manufactured with moderate success" due to smaller sample volumes, faster and easier procedure, and in some case better data [3]. The advantages of μTAS were obtained by using capillary electrophoresis system in the present work. The simple setup of ECL detection allowed the use of short capillary, and thus, a fast, high sensitivity of analysis of two analytes was achieved. As shown in Fig. 2A, a separation time less than 1 min was achieved with about 10 nl of sample consumption. About 10 nl of sample injection is higher than that in μTAS that is facilitated to improve the detection sensitivity. At the same time, the fabrication of the micro channel of μTAS is not needed. On the other hand, the high sample throughput and low zone broadening may be the main advantage over the tradition CE with 40-cm-long capillary. Fig. 5 is a comparison of the separation of the two compounds between 10- and 40-cm-long capillary. While the 10-cm-long capillary given short separation time, the resulted peaks were much sharper may be due to the small diffusion from the short separation time.

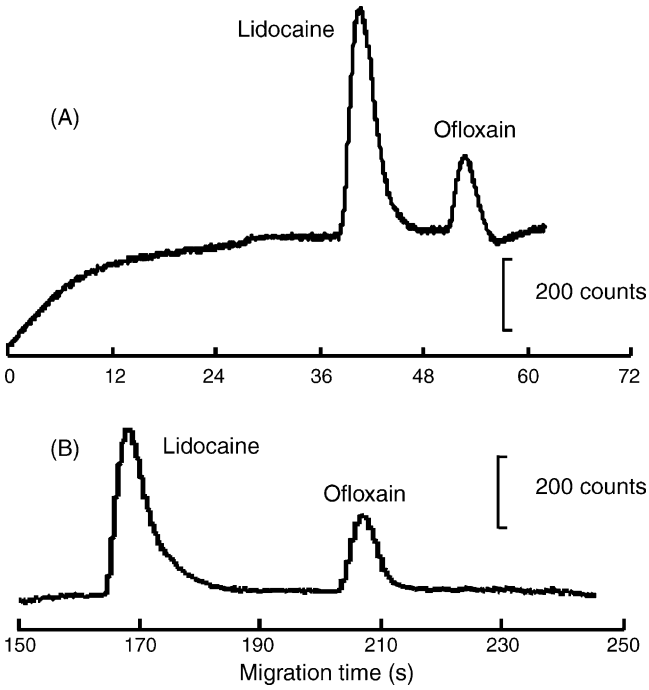

Fig. 5. Effect of the capillary length on the electrophoresis separation. (A) 10-cm-long capillary and (B) 40-cm-long capillary. The other conditions as shown in Fig. 2.

Analytical characteristic data of the present system are summarized in Table 1. The precision (R.S.D.) of the migration time and the peak height for five replicate injections of a mixture of lidocaine (1.0×10^{-6} mol L^{-1}) and ofloxacin (4.0×10^{-6} mol L^{-1}) were 3.2–3.9% and 4.7–5.3%, respectively. The detection limits based on signal-to-noise of 3 of lidocaine and ofloxacin for peak height measurement were 3.0×10^{-8} and 5.0×10^{-7} mol L^{-1}, respectively. A 60 h^{-1} of sampling frequency was obtained with present system.

Table 1
Characteristic performance data for the short-capillary electrophoresis electrochemiluminescence system

	Precision (R.S.D. (%), n = 5)		Detection limits[a] ($\times 10^{-8}$ mol L^{-1})	Recovery (%)		Sampling frequency (h^{-1})
	Migration time	Peak height		Male urine	Female urine	
Lidocaine	3.2	4.7	3.0	90.9	93.7	60
Ofloxacin	3.9	5.3	50	96.4	111	

[a] Based on S/N = 3.

As shown in Table 1, the sensitivity of ofloxacin is low than that of lidocaine although ofloxacin has three tertiary amine groups. Because the reaction between amine and $Ru(bpy)_3^{2+}$ on the electrode surface involves amine radical intermediates formation, Knight and Greenway [30] found the resonance stabilization of the radical intermediates reduce the reactivity of the intermediate, and hence the ECL activity. So, the tertiary amines neighboring aromatic cycle and carbon–carbon double-bond in ofloxacin are weak ECL response or non-ECL response. The discrimination of electrokinetic injection may also contribute the poor sensitivity of ofloxacin [28].

4. Conclusion

This paper presents a fast, simple, and reproducible determination method of lidocaine and ofloxacin based on capillary electrophoresis electrochemiluminescent detection. Following μTAS, its some advantages, such as short analysis time and low sample consumption in present system, were achieved, while the fabrication of chip-based separation channel and integration of detector to chip were eliminated. The high sample throughput and low zone broadening is the advantage of the present system over the capillary electrophoresis with long capillary.

Acknowledgements

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