Asian Journal of Chemistry Vol. 20, No. 5 (2008), 3833-3848

Separation and Detection of Chlorpromazine Hydrochloride and Promethazine Hydrochloride Using Electrochemiluminescence Followed by Capillary Electrophoresis

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Phenothiazine drugs, chlorpromazine hydrochloride (CPZ) and promethazine hydrochloride (PMZ), were determined with $Ru(bpy)_3^{2+}$ electrochemiluminescene by the capillary electrophoresis (CE-ECL). It was found that both CPZ and PMZ could produce an intermediate that acted as coreactants to react with $Ru(bpy)_3^{2+}$ to produce excited states which were capable of emitting light. This CE-ECL detection method had high sensitivity, good selectivity and reproducibility for CPZ and PMZ determination. Under the optimized conditions: ECL detection at 1.15 V, 3.0×10^{-2} mol L^{-1} phosphate buffer at pH 4.0, 5.0×10^{-3} mol L^{-1} of $Ru(bpy)_3^{2+}$ and 5.0×10^{-2} mol L^{-1} of phosphate buffer at pH 7.5 in the detection reservoir, the ECL intensity was linear with three orders for CPZ and two orders for PMZ and the detection limits (S/N = 3) were 1.0×10^{-8} mol L^{-1} for CPZ and 1.0×10^{-6} for PMZ mol L^{-1}, respectively. The CE-ECL method was applied to analyze CPZ and PMZ in real samples including tablets and injections and satisfactory results were obtained without interference from samples matrix. The method was successfully applied to the determination of CPZ and PMZ in human urine.

Key Words: Separation, Electrochemiluminescence, Capillary Electrophoresis, Chlorpromazine hydrochloride, Promethazine hydrochloride.

INTRODUCTION

Electrochemiluminescence (ECL) is a means of converting electrical energy into radiative energy. The species that easily form excited states are generated at electrode surfaces *via* electron-transfer reactions by an applied voltage. A photon of light is produced when the excited molecule decays to the ground state. *Tris*(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate

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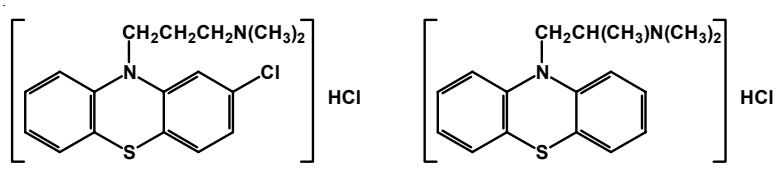
($Ru(bpy)_3^{2+}$) is a well-known ECL reagent with stability in aqueous solutions and highly efficient photon emission. $Ru(bpy)_3^{2+}$ ECL has been developed as an analytical tool and utilized widely in life sciences, clinic and pharmaceutical fields in recent years[1-5]. Most studies and applications of $Ru(bpy)_3^{2+}$ ECL are based on oxidative-reduction electrochemiluminescence mechanism in which an electrochemical oxidative coreactant is involved in the processes. For example, $Ru(bpy)_3^{2+}$ ECL can be used to analyze numerous amine-containing analytes, such as trimethylamine, triethylamine, tripropylamine, *etc*[6].

Owing to its high sensitivity, wide linear range and simplicity, $Ru(bpy)_3^{2+}$ ECL has been coupled with liquid chromatography (LC) and flow-injection analysis (FIA)[5,7]. In the past decade, $Ru(bpy)_3^{2+}$ ECL integrated with capillary electrophoresis (CE)[8-13] as a sensitive and efficient analytical technique has been achieved. The method has many advantages of simplicity, rapidity, low expense and many analytes can be directly detected without derivatization.

Phenothiazines are a group of basic drugs used generally as antipsychotics, neuroleptics and antihistamines. These drugs can decrease the activity of dopamine and block the dopamine receptors of the central nervous system[14,15]. Chlorpromazine hydrochloride (CPZ) and promethazine hydrochloride (PMZ) belong to phenothiazine family and their structures have phenazine ring (Fig. 1). CPZ has strong sedative, local anesthetic and moderate antiemetic effects. It has wide clinical application for the treatment of schizophrenia and other psychiatric disorders. PMZ is an antihistamine drug that can be predominantly used as an antiemitic or to prevent motion sickness. CPZ and PMZ are made in pharmaceutical formulations such as coated tablets and liquid injections. Though strong antipsychiatric and antihistamine effects, CPZ and PMZ also exert effects upon muscarinic and a1-adrenergic receptors, which may be a possible reason for many side effects associated with the treatment. Massive overdose of CPZ and PMZ may cause an indifference to the surroundings, coma, respiratory depression or other undesirable reaction, even death[14]. The monitoring of such compounds is important for quality assurance in pharmaceuticals, while minimizing the risk of toxicity and obtaining optimum therapeutic concentrations.



Chlorpromazine hydrochloride **Promethazine hydrochloride**

Fig. 1. Chemical structure of CPZ and PMZ

Several methods for the determination of CPZ and PMZ have been reported, including spectrophotometric method[16,17]; titrimetric methods[18,19]; gas chromatography-mass spectrometric (GC-MS)[20]; voltammetry[21,22]; luminol chemiluminescence (CL)[23]. Liquid chromatography (LC) and flow-injection analysis (FIA) with various detection systems have been developed to analyze CPZ and PMZ[24-29]. Moreover, some papers have reported the application of the CE with UV detection or end-column amperometric detection to analyze CPZ and PMZ[30-34]. Recently, electrochemiluminescence detection (ECL) using $Ru(bpy)_3^{2+}$ has been used to analyze CPZ[35,36]. CPZ can be accumulated and detected at the lauric acid-modified carbon paste electrode.

In this paper, we used electrochemiluminescence-capillary electrophoresis (CE-ECL) technology as an alternative for the determination of CPZ and PMZ, which were expected to be the efficient coreactants of $Ru(bpy)_3^{2+}$ during the electrochemical reaction. In order to achieve good resolution and high sensitivity, both separation and detection parameters were optimized.

EXPERIMENTAL

All the reagents were of analytical grade and were used without further purification. *Tris*(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate (98 %), chlorpromazine hydrochloride (CPZ) and promethazine hydrochloride (PMZ) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). And PMZ tablets were made by Shanghai Jiufu Pharmaceutical Co. (Shanghai, China). CPZ injections were produced by Tianjin Xinzheng Pharmaceuticals Co., Ltd. (Tianjin, China). PMZ injections were manufactured by Wuxi Seventh Pharmaceuticals Ltd. (Wuxi, Jiangsu, China). Standard solution of 1.0×10^{-2} mol L^{-1} CPZ and PMZ were prepared with doubly distilled water weekly and were protected from light. Prior to analysis, the required sample solutions were freshly prepared by serial dilution of the stock solutions with doubly distilled water. The buffer was prepared from Na_2HPO_4 and NaH_2PO_4. Electrochemical (EC) behaviours were operated in 0.1 mol L^{-1} phosphate buffer of pH 7.5. Various buffer pH values in the detection cell and in the capillary were adjusted by adding directly the suitable amount of either 1.0×10^{-2} mol L^{-1} H_3PO^4 or 10^{-1} mol L^{-1} NaOH. Added volumes did not influence the overall concentrations of reactants inside the cell and capillary. Doubly distilled water was prepared by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

In pharmaceutical preparations, 10 pieces of PMZ tablets were finely ground into powder and an accurately weighed portion was completely dissolved in doubly distilled water. For the determination of CPZ and PMZ injections, an accurately volume of each was appropriately dissolved in

doubly distilled water. Before analysis, these stock solutions were further diluted to the required concentration in the respective working range. All these solutions were filtered through a 0.22 μm cellulose ester membrane filter (Xinya Decontamination Apparatus Factory, Shanghai, China) before use and stored in the refrigerator at 4 ºC.

Fresh urinary samples were taken from a female volunteer and were filtered through a membrane (0.22 μm). After that, the samples were diluted 10-fold with double-distilled water. The filtrate was injected into the CE-ECL system and analyzed.

A CE-ECL system, namely a high-voltage power supply, an electrochemical potentiostate, a multifunction chemiluminescence detector and a multichannels data collection analyzer, was made in Xi'an Remex Electronic Sicence-Tech Co., Ltd. (Xi'an, China). The potential of the photomultiplier tube (PMT) was set at 800 V. The output ECL intensity was amplified and recorded with CE-ECL software by a computer.

A home-made CE-ECL detection cell was the same as reported[37]. The end-column ECL detection was measured in a conventional three-electrode system using a 500 μm-diameter disk Pt as working electrode, a Ag|AgCl as reference electrode and a Pt wire as counter electrode. Before experiments, the ECL detection cell was filled with 300 μL of 5.0×10^{-2} mol L^{-1} phosphate electrolyte (pH 7.5) containing 5.0×10^{-3} mol L^{-1} $Ru(bpy)_3^{2+}$. In order to eliminate the change of $Ru(bpy)_3^{2+}$ concentration, we replaced the solution in the ECL detection cell every 2 h during the experiments.

A 45-cm length of uncoated fused-silica capillary (25 μm i.d., 360 μm o.d.) was used (Yongnian Optical Fiber Factory, Hebei, China). Before the first use, the capillary was flushed with 0.1 mol L^{-1} sodium hydroxide solution for 12 h by a syringe. Then it was rinsed with doubly distilled water and running buffer for 10 min, respectively. In order to obtain better reproducibility, between 2 runs, the capillary was flushed with doubly distilled water and running buffer for 2 and 3 min, respectively. Samples were electrokinematically injected at 10 kV for 3 s. A voltage of 15 kV was applied. The measurements were carried out at least in triplicate to ensure reproducibility. Peak identification was conducted by spiking with the analytes.

Electrode treatment: A 500 μm-diameter Pt-disk working electrode was aligned carefully at the outlet of the capillary. In order to obtain good reproducibility, the working electrode was cleaned by scanning the potential in the range of -0.5–0.0 V (*vs.* Ag|AgCl) for 10 cycles at 100 mV/s between runs. By this electrochemical cleanup, the Pt working electrode could eliminate surface contamination without removal from the CE-ECL system.

RESULTS AND DISCUSSION

Tertiary amines can be determined by their electrochemiluminescence reaction with $Ru(bpy)_3^{2+}$. The chemical structure of CPZ and PMZ contains two tertiary amine groups, one is in the tricyclic structure and the other is in the aliphatic side chain. The nitrogen and sulfur atoms in the tricyclic structure and aliphatic side chain are easily oxidized[15]. So it is possible that CPZ and PMZ can be efficiently and sensitively detected by ECL of $Ru(bpy)_3^{2+}$. Similar to the mechanism of the ECL of the $Ru(bpy)_3^{2+}$/ tripropylamine (TPrA) system, CPZ and PMZ undergo oxidation at proper positive potential at the electrode surface, which leads to the formation of a short-life radical cation intermediate. Then it reacts with oxidized $Ru(bpy)_3^{3+}$ to form the excited $Ru(bpy)_3^{2+}$* and light will be obtained when the excited $Ru(bpy)_3^{2+}$* releases energy to return[1,6] to $Ru(bpy)_3^{2+}$.

Electrochemical (EC) and ECL behaviour of $Ru(bpy)_3^{2+}$, CPZ and PMZ: The cyclic voltammograms of $Ru(bpy)_3^{2+}$, CPZ and PMZ in 0.1 mol L^{-1} phosphate buffer of pH 7.5 at a 500 μm-diameter Pt electrode were recorded in the range of 0.0-1.3 V (*vs.* Ag|AgCl). As shown in Fig. 2A, **curve a** was the cyclic voltammogram of 0.1 mol L^{-1} phosphate buffer of pH 7.5, no obvious redox peaks were observed. When 1.0×10^{-3} mol L^{-1} $Ru(bpy)_3^{2+}$ was added into phosphate buffer, we could see that a pair of reversible redox peaks appeared (**curve b**). After 5.0×10^{-4} mol L^{-1} CPZ (**curve c**) and PMZ (**curve d**) was added into **b**, individually, it could be seen that both CPZ and PMZ augment the peak current of $Ru(bpy)_3^{2+}$. This indicated that two substances could react with $Ru(bpy)_3^{2+}$ at the electrode surface. In addition, we also observed that the peak current of CPZ was larger than that of PMZ.

Under the same experiment conditions, we also investigated the corresponding signals of ECL (Fig. 2B). The ECL behaviour was similar to that of cyclic voltammograms. It can be seen that ECL signals of $Ru(bpy)_3^{2+}$ (**curve b**) were enhanced greatly due to the presence of CPZ (**curve c**) and PMZ (**curve d**). The ECL intensity produced by CPZ with $Ru(bpy)_3^{3+}$ (**curve c**) was higher than that of PMZ with $Ru(bpy)_3^{3+}$ (**curve d**), which agreed with the results of the cyclic voltammograms. The higher concentration of drugs was studied, higher intensity of ECL can be obtained in a certain range. This phenomenon illustrated that the oxidation of CPZ and PMZ is an important factor in this ECL emission system. The maximum ECL peak intensity occurred at 1.15 V, corresponding to the oxidation potential[38] of $Ru(bpy)_3^{2+}$.

Optimization of detection conditions

Effect of applied potential on ECL: The effect of the applied potential on the ECL intensity of CPZ and PMZ were studied as shown in Fig. 3.

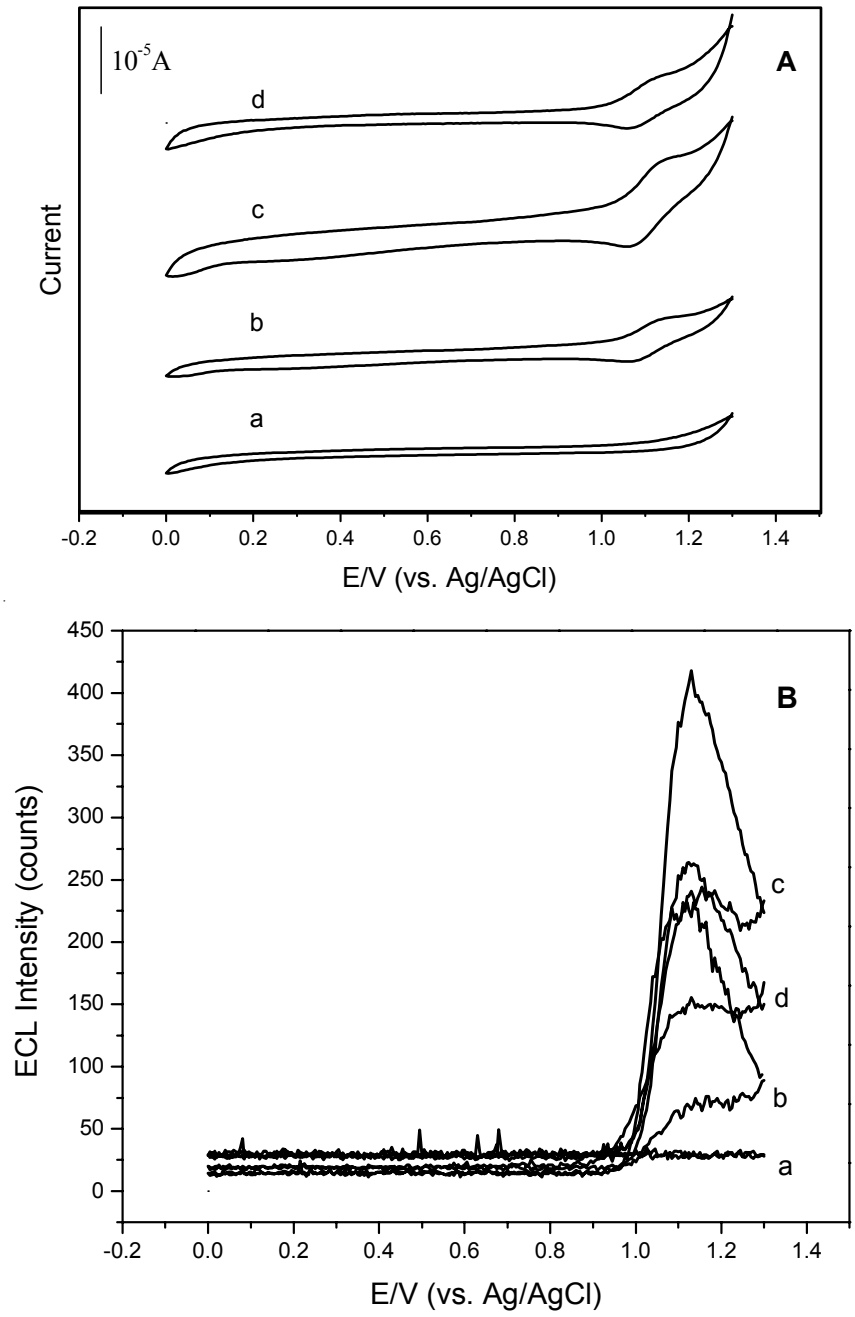


Fig. 2. Cyclic voltammograms (A) and the corresponding ECL intensity-potential curve (B) of 0.1 mol L^{-1} phosphate buffer of pH 7.5 (a), a + 1.0×10^{-3} mol L^{-1} $Ru(bpy)_3^{2+}$ (b), 5.0×10^{-4} mol L^{-1} CPZ (c) and PMZ (d) was spiked into b at a Pt electrode (500 μm-diameter), individually. Scan rate: 100 mV s^{-1}

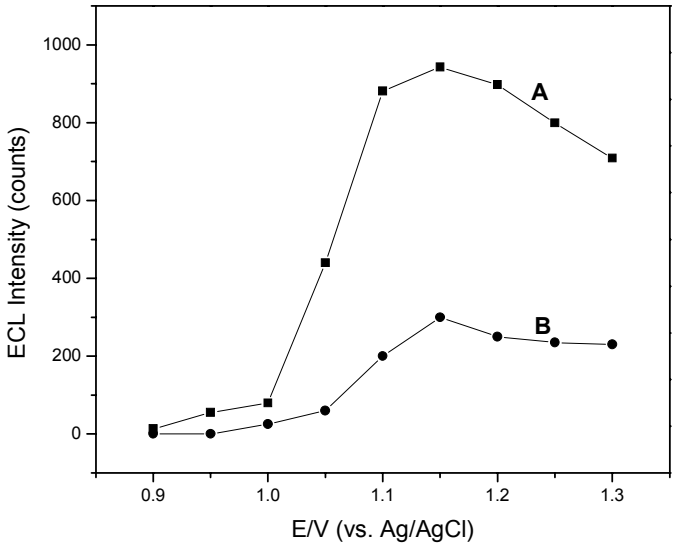


Fig. 3. Effects of applied potential on the ECL intensity of 5.0×10^{-6} mol L^{-1} CPZ (A) and 2.5×10^{-5} mol L^{-1} PMZ (B) at a 500 μm diameter Pt electrode. CE-ECL conditions: separation capillary, 45 cm length (25 μm i.d.); sample injection, 3 s at 10 kV; separation voltage, 15 kV; running buffer, 3.0×10^{-2} mol L^{-1} sodium phosphate of pH 6.0; 5.0×10^{-2} mol L^{-1} of sodium phosphate buffer (pH 7.5) containing 5.0×10^{-3} mol L^{-1} of $Ru(bpy)_3^{2+}$ in ECL detection cell

The ECL intensity for CPZ (**curve A**) was relatively low when the applied potential varied from 0.9 to 1.0 V because $Ru(bpy)_3^{2+}$ was not oxidized on the working electrode surface. However, when the applied potential was higher than 1.0 V, the ECL intensity of CPZ increased rapidly with the applied potential stepped from 1.0 to 1.15 V and reached a maximum at 1.15 V. Subsequently, the ECL signal decreased gradually with higher applied potential. For PMZ (curve B), the effect of applied potential on the ECL intensity presented similar behaviour to that of CPZ. But as shown in Fig. 3, the ECL intensity produced by PMZ with $Ru(bpy)_3^{2+}$ was lower than that of CPZ with $Ru(bpy)_3^{2+}$ which agreed with the results of the cyclic voltammograms and ECL behaviour. We know that CPZ is more sensitive than PMZ in CE-ECL detection using $Ru(bpy)_3^{2+}$ reagent. The ECL intensity of CPZ and PMZ reached a maximum together and a lower background noise was obtained at 1.15 V. So a 1.15 V (*vs.*Ag|AgCl) was selected as the ECL optimum applied potential. Under the optimized conditions, a stable ECL signal was observed.

Influence of parameters in the detection cell: $Ru(bpy)_3^{2+}$ is a luminescence reagent. The concentration of $Ru(bpy)_3^{2+}$ added in the detection cell is one of the most important detection parameters[9]. The concentration of

$Ru(bpy)_3^{2+}$ has influence on background ECL and ECL of $Ru(bpy)_3^{2+}$/trialkylamine system. The ECL intensity of CPZ and PMZ as well as background noise have enhanced with increase of the concentration of $Ru(bpy)_3^{2+}$. But considering the consumption of larger amount of expensive reagent ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), 5.0×10^{-3} mol L^{-1} $Ru(bpy)_3^{2+}$ was used in present experiment. At the same time, a low background noise and a higher signal/noise (S/N) value were obtained at this concentration.

The ECL emission of $Ru(bpy)_3^{2+}$/trialkylamine system is pH dependent. Knight and Greenway[39] studied the effect of pH on the ECL intensity and found that the ECL intensity was smaller at lower pH because of the protonation of amine. At high pH value, some hydroxide ions were easily oxidized to produce oxygen. Oxygen has a quenching impact on formation of the excited state[40] of $Ru(bpy)_3^{2+}$*. At the same time, in alkaline media $Ru(bpy)_3^{2+}$ emits light by reacting with OH-species. Thus it is disadvantage to form the excited state of $Ru(bpy)_3^{2+}$* at the electrode surface at very low and very high pH value. The ECL intensity achieved maximum in weakly alkali aqueous solution[41]. We investigated the ECL intensity of CPZ and PMZ in the pH range of 6.0-9.0 (Fig. 4). Results showed that the ECL intensity increased with the pH value and the highest ECL intensity was both obtained at the pH value of 7.5 and then decreased slightly. Thus, the optimized pH value in the detection cell was set at 7.5 in this study.

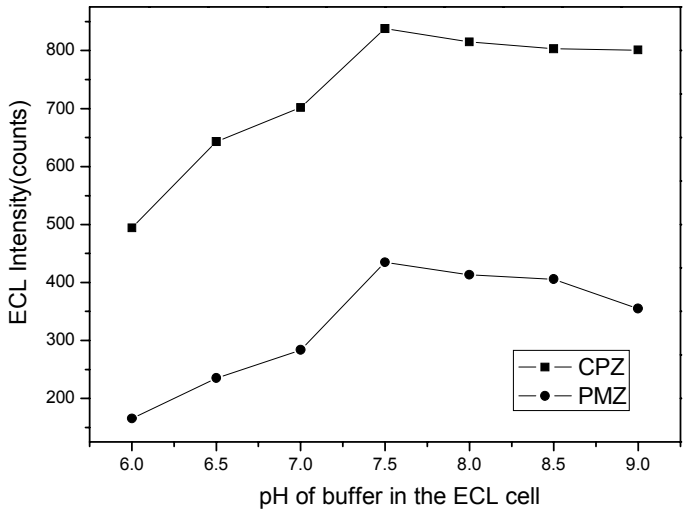


Fig. 4. Effects of the buffer pH in the detection cell on the ECL intensity of CPZ and PMZ. CE-ECL conditions: detection potential, 1.15 V *vs.* Ag/AgCl; other conditions were the same as in Fig. 3

The ionic strength of background electrolyte also affected the ECL intensity. We studied the effect of the buffer concentration in the detection cell at pH 7.5. As shown in Fig. 5, the higher ECL intensity was obtained at the concentration of 5.0×10^{-2} mol L^{-1}. In fact, in phosphate system, log k' becomes lower than 3 when the concentration is lower than 10^{-3} mol L^{-1} (where k' is kinetic rate constant). Thus, emission must wait the ammonium proton lost, which is produced slower than the fast potential pulse applied[42]. On the other hand, the quantity of $Ru(bpy)_3^{2+}$ ions on the working electrode surface would be reduced at high concentrations, because other ions in the detection cell might affect the activity of $Ru(bpy)_3^{2+}$. Furthermore, reproducibility became worse when the buffer concentration in the detection cell was too high. Therefore, on the basis of these factors, 5.0×10^{-2} mol L^{-1} phosphate electrolyte was chosen for this CE-ECL method.

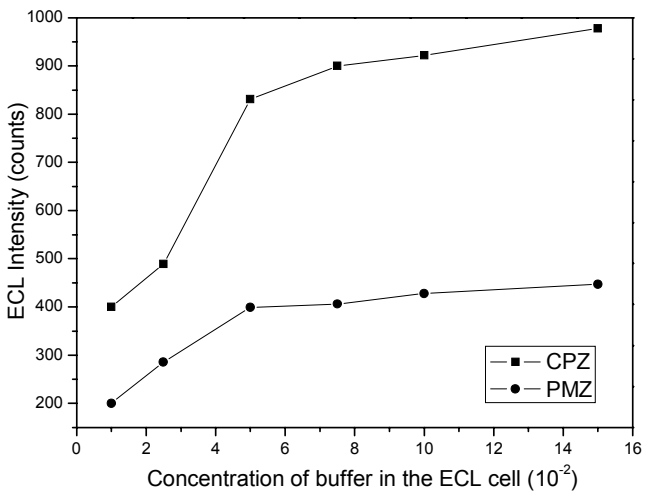


Fig. 5. Effects of the buffer concentration in the detection cell on the ECL intensity of CPZ and PMZ. Conditions were the same as in Fig. 4

Separation of CPZ and PMZ: CPZ and PMZ have similar chemical structures and molecular weights, which leads to the similar physical and chemical properties. Thus it is difficult to determine the drugs individually from mixture.

The pH value of the running buffer influences both the net charge of the analytes and the electroosmotic flow (EOF) inside the capillary, which, in turn, results in different migration times for analytes. Thus, the resolution within CPZ and PMZ is strongly influenced by the running buffer pH value. We investigated carefully the effects of the pH value of the running buffer on the CE-ECL system, especially, the migration time, resolution and the ECL intensity.

The buffer concentration was fixed at 3.0×10^{-2} mol L^{-1} in order to obtain a low separation current, avoiding the Joule heating effect. Fig. 6 showed the effect of the pH value of the running buffer on resolution (R_S) when injecting the sample mixture for 3 s. Resolution (R_S) can be calculated by the equation as follows:

$$Rs = 1.18 (t_2 - t_1) / (W_{0.5(1)} + W_{0.5(2)})$$

where t_1, t_2 are the migration time of CPZ and PMZ ($t_2 > t_1$) and $W_{0.5(1)}$, $W_{0.5(2)}$ are the peak width at half height.

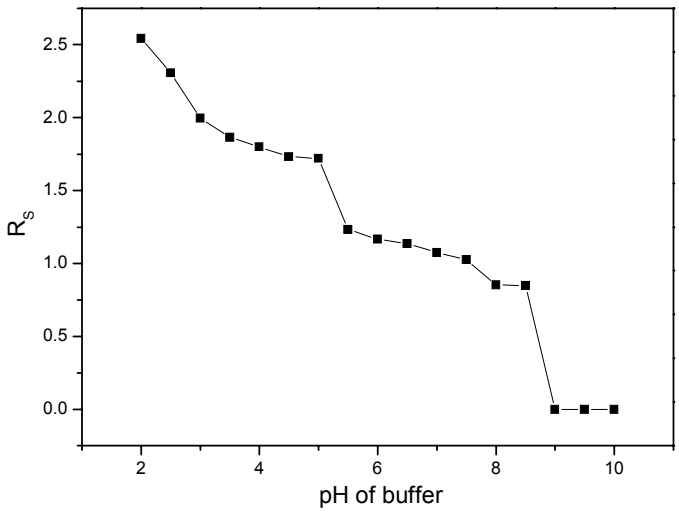


Fig. 6. Effect of pH value of the running buffer on resolution (R_S) conditions were the same as in Fig. 4

Peaks of CPZ and PMZ could be separated from pH 2.0 to 5.0. Resolution (R_S) decreased with the increasing pH from 2.0 to 10.0, especially at higher pH (> 5.0). Even two peaks overlapped at pH 9.0-10.0. This phenomenon was mainly the result of effect of the pH value of the running buffer on electroosmotic flow (EOF) as well as the migration time in CE[41,43]. EOF increased with the increasing pH when a fused-silica capillary was used. High EOF prolonged migration time and enhanced band broadening. Hence too high pH would decrease resolution dramatically. The results were in accordance with those of studies on the existence of maximum resolution between 2.5-4.0 in the separation of phenothiazines[32].

The pH value of the running buffer is an important parameter influencing the ECL intensity, so the impact of the running buffer pH on the ECL intensity of CPZ and PMZ was also evaluated. Fig. 7 showed the variations of the ECL intensity of CPZ and PMZ as a function of buffer pH in the range of 2.0-10.0 with a pH value increments of 0.5. For CPZ, when pH

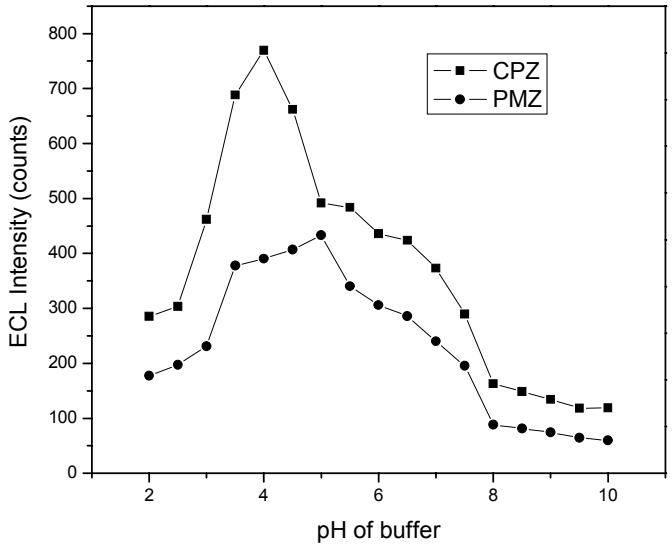


Fig. 7. Effect of pH value of the running buffer on the ECL intensity of CPZ and PMZ. Conditions were the same as in Fig. 4

changed from 2.0 to 4.0, the ECL intensity increased and reached a maximum value at 4.0. After pH value was higher than 4.0, the ECL intensity decreased gradually. For PMZ, maximum ECL intensity was observed at pH 5.0 and its ECL intensity decreased when pH value was greater than 5.0. this was similar to the trend of CPZ. It was supposed that this trend was due to the corresponding change of the state of the protonated CPZ and PMZ when the pH varied. The sulfur and nitrogen atoms in the tricyclic structure and the aliphatic side chain of CPZ and PMZ were readily oxidized to deprotonate and formed high reducing intermediate under acidic conditions[15]. Their protonated degree was minimum with the increase of pH. A critical step involved in the mechanism of $Ru(bpy)_3^{2+}$/trialkylamine system is the oxidation and subsequent deprotonation of amine to form reducing free radical intermediate. At pH values above 9, a precipitation of PMZ would happen[34], resulting in a decrease of ECL intensity. In addition, the ECL intensity decreased in the pH range of 8.0-10.0 because of the reduced availability of $Ru(bpy)_3^{3+}$. Hydroxide ions was assumed to be at considerable concentration level at high pH value[40], which competed with $Ru(bpy)_3^{3+}$. When pH was above 8.0, the ECL baseline became unstable. We found that the ECL intensity reached maximum values at pH 4.0 and 5.0 for CPZ and PMZ, respectively. Moreover, the ECL intensity for CPZ decreased 36 % when pH varied between 4.0 and 5.0. At the same time, only 10 % variation for PMZ was obtained.

Therefore, in view of favourable resolution and the ECL intensity of two compounds, pH 4.0 was chosen as the optimal running buffer pH value for the detection of CPZ and PMZ. Typical separation electropherogram of 2.5×10^{-6} mol L^{-1} CPZ and 2.5×10^{-5} mol L^{-1} PMZ standard mixture solution was shown in Fig. 8a, where the migration sequence was CPZ and PMZ according to the ratio of their charge to mass. We observed that the peak shape of CPZ and PMZ was very sharp. This might be due to the fact that the oxidation of drugs was very rapid and two electrons were lost rapidly at the electrode surface and hence they showed sharp peak. Through the CE-ECL method, CPZ and PMZ were separated without any additives.

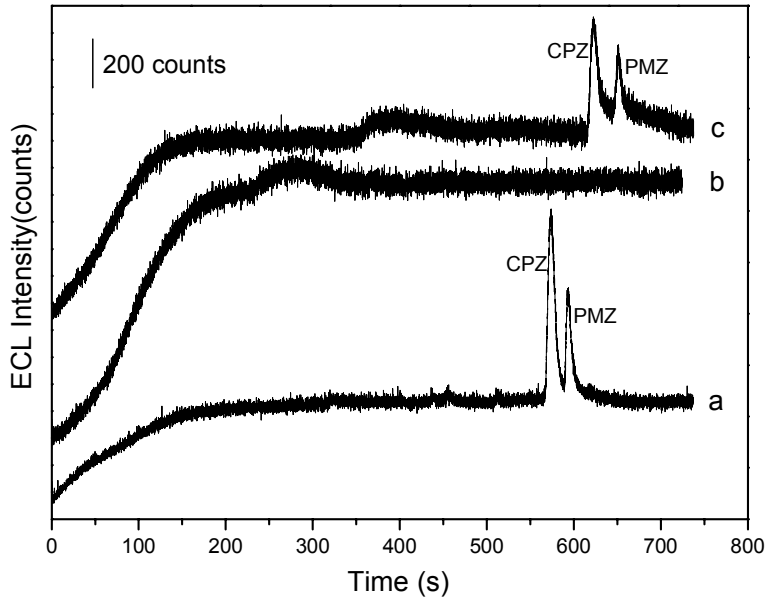


Fig. 8. Typical electropherograms of 2.5×10^{-6} mol L^{-1} CPZ and 2.5×10^{-5} mol L^{-1} PMZ standard mixture solution (a); 1:9 diluted blank human urine sample (b); 2.5×10^{-6} mol L^{-1} CPZ and 2.5×10^{-5} mol L^{-1} PMZ standard mixture solution was spiked to b (c). CE-ECL conditions: separation capillary, 45 cm length (25 μm i.d.); sample injection, 3 s at 10 kV; separation voltage, 15 kV; detection potential, 1.15 V *vs.* Ag/AgCl; 3.0×10^{-2} mol L^{-1} phosphate buffer at pH 4.0; 5.0×10^{-3} mol L^{-1} of $Ru(bpy)_3^{2+}$ and 5.0×10^{-2} mol L^{-1} of phosphate buffer at pH 7.5 in the detection reservoir

Detection limit, linearity and reproducibility: The optimized CE-ECL conditions were as follows: running buffer, 3.0×10^{-2} mol L^{-1} sodium phosphate of pH 4.0; detection potential, 1.15 V; 5.0×10^{-2} mol L^{-1} of sodium phosphate buffer (pH 7.5) containing 5.0×10^{-2} mol L^{-1} of $Ru(bpy)_3^{2+}$ in ECL detection cell. Under the optimized conditions, the different concentrations of CPZ

and PMZ were individually measured. Linear curves of the ECL intensity as a function of concentration were established. Calibration for CPZ was linear with a regression curve of Y = 21.52 + 192.55X (r = 0.9998) over the concentration range of 2.0×10^{-7} to 1.0×10^{-5} mol L^{-1}. In the concentration range of 1.7×10^{-5} to 1.0×10^{-4} mol L^{-1} the dependency of ECL intensity on the concentrations of CPZ was also linear: Y = 2514.50 + 18.4X (r = 0.9912). A linear range of PMZ from 2.0×10^{-6} to 5.0×10^{-5} mol L^{-1} was obtained with a correlation coefficient of 0.9969. The results were identical with the results determined by other method[22,24,34] and were clearly shown in Table-1.

TABLE-1
LINEAR RANGE AND THE DETECTION LIMITS OF CPZ AND PMZ

Analytes	Linear range (mol L^{-1})	Regression equation	Correlation coefficiency	Detection limit (S/N = 3) (mol L^{-1})
CPZ	2.0×10^{-7} to 1.0×10^{-5}	Y = 21.52 + 192.55X	0.9998	1.0×10^{-8}
	1.7×10^{-5} to 1.0×10^{-4}	Y = 2514.50 + 18.4X	0.9912	
PMZ	2.0×10^{-6} to 5.0×10^{-5}	Y = 39.79 + 9.24X	0.9969	1.0×10^{-6}

(Y= the ECL intensity, X = the concentration of the measured solution, μ mol L^{-1})

The detection limits (S/N = 3) were 1.0×10^{-8} mol L^{-1} and 1.0×10^{-6} mol L^{-1} for CPZ and PMZ, respectively. Compared with the results in literatures, the detection limits of CPZ and PMZ were lower than that obtained by HPLC-UV method (88 ng mL^{-1}, correspond to 2.5×10^{-1} mol L^{-1} for CPZ)[26], CE with diode-array detector (3.3 μg mL^{-1}, correspond to 1.0×10^{-5} mol L^{-1} for PMZ)[34], CE-scan voltammetry method (1.0×10^{-6} mol L^{-1} for CPZ and 5.0×10^{-6} mol L^{-1} for PMZ) and FIA-ECL method (0.24 μg mL^{-1}, correspond to 6.75×10^{-7} mol L^{-1} for CPZ)[35]. The detection limits were comparable to the results obtained by GC-MS method (5 μg L^{-1}, correspond to 1.4×10^{-8} mol L^{-1} for CPZ)[20], but GC-MS method needs an extra derivatization step.

Inter-day and intra-day detection reproducibility was also studied. 8 Consecutive injections of 1.0×10^{-6} mol L^{-1} CPZ and 1.0×10^{-5} mol L^{-1} PMZ, respectively, were assayed on the same day (Table-2). The relative standard deviations (RSD) of the ECL intensity and the migration time were 2.54 and 1.93 % for CPZ, 2.86 % and 1.79 % for PMZ, respectively. Between runs, the electrode and the capillary were treated as depicted in 2.2 and 2.3 sections. Through the treatment process, the reproducibility was remarkably improved.

As for the intra-day detection reproducibility, they were assessed on 8 different days under the same operating conditions and the results obtained were presented in Table-2. The RSD for the ECL intensity (≤ 4.55 %) and

TABLE-2
STUDY OF THE SYSTEM PRECISION OF THE PROPOSED METHOD

Analyte	I_{ECL}	t_m
Intraday RSD (%) (n = 8)		
CPZ (1×10^{-6} mol L^{-1})	2.54	1.93
PMZ (1×10^{-5} mol L^{-1})	2.86	1.79
Interday RSD (%) (n = 8)		
CPZ (1×10^{-6} mol L^{-1})	2.99	2.10
PMZ (1×10^{-5} mol L^{-1})	4.55	1.77

(I_{ECL} = the ECL intensity, t_m = the migration time).

the migration time (≤ 2.10 %) proved that CE-ECL was a precise method. The high reproducibility indicated that the CE-ECL method was accurate for the detection of CPZ and PMZ.

Applications: In order to examine the application to practical analysis, the CE-ECL method was applied to the determination of CPZ and PMZ in tablets, injections and human urine samples.

Applications on pharmaceutical preparations: CPZ injection 1 was dissolved (with a nominal CPZ content of 25 mg mL^{-1}) appropriately to 1.0×10^{-6} mol L^{-1}. This sample was directly injected into the capillary, six determinations were chosen to calculate the CPZ content and the average content of CPZ in CPZ injection 1 is 25.0 ± 0.5 mg mL^{-1}. Then CPZ standard solution was spiked into CPZ injection 1. The increase of the ECL signal was proportional to the amount spiked with CPZ standard solution by employing the standard addition method, the average recovery was in the range of 97.2-108.3 % when 1.0×10^{-6} mol L^{-1} CPZ standard solution was spiked into CPZ injection 1 (n = 6). Similarly, we also analyzed and calculated the content and the recoveries of CPZ injection 2, PMZ injection 1, PMZ injection 2, PMZ tablets 1 and PMZ tablets 2, respectively. The mean recoveries were comparable to those obtained using spectrophotometric determination and voltammetric determination[17,22] for each of the studied drugs. The results were listed in Table-3. According to the recovery results, good accuracy was obtained and usually presented in dosage form. The compounds such as starch, glucose, lactose or povidone did not affect the results obtained. This indicated that the CE-ECL method is valid for the assay of CPZ and PMZ in pharmaceutical preparation. Furthermore, the precise of proposed method was also validated in the determination of CPZ and PMZ injection and PMZ tablets. These results proved that the CE-ECL was a high reproducible method.

Detection of CPZ and PMZ in urine: The proposed CE-ECL technique was employed to detect CPZ and PMZ in human urine samples. The blank urine samples from a volunteer were collected in the laboratory and

TABLE-3
ANALYSIS OF CPZ AND PMZ IN PHARMACEUTICAL FORMULATIONS BY THE PROPOSED METHODS (AVERAGE OF SIX DETERMINATIONS)

Pharmaceutical formulations	Nominal amount (mg/mL or piece)	Found amount (mg/mL or piece)	RSD (%)	Added amount (μg/mL)	Found amount (μg/mL)	Recovery (%)
CPZ injection 1	25.0	25.0(±0.5)	2.06	0.36	0.37(±0.02)	97.2-108.3
CPZ injection 2	25.0	25.4(±0.8)	2.97	7.11	7.28(±0.29)	98.6-106.7
PMZ injection 1	25.0	24.6(±1.1)	3.18	1.60	1.59(±0.16)	89.7-109.6
PMZ injection 2	25.0	24.8(±1.0)	3.36	3.21	3.17(±0.13)	95.2-103.2
PMZ tablet 1	12.5	12.9(±0.2)	1.14	3.21	3.47(±0.07)	106.1-110.5
PMZ tablet 2	12.5	12.7(±0.3)	1.70	6.42	6.52(±0.13)	99.9-103.9

diluted 10-fold with doubly distilled water, then filtered with 0.22 μm membrane. The filtrate was injected into the CE-ECL system and analyzed. The content of CPZ and PMZ in urine was obtained by three replicate injections of diluted urine sample. Fig. 8a showed the typical electropherograms of the CPZ and PMZ standard mixture solution. The electro-phoregram of blank urine was shown in Fig. 8b. 2.5×10^{-6} mol L^{-1} CPZ and 2.5×10^{-5} mol L^{-1} PMZ was spiked into the urine sample. Two peaks appeared in the electrophoregram as shown in Fig. 8c. Their ECL intensity was lower than that of the CPZ and PMZ standard mixture solution and their migration in urine sample was also slower than that of corresponding standard mixture solution and background noise enhanced. These phenomena could be explained by that the ion strength was higher in urine samples than that in standard sample. With CE-ECL method, it was unnecessary to do the extraction procedure and it was simple, rapid, sensitive and convenient.

Conclusion

In this work, the end-column electrochemiluminescence detection using $Ru(bpy)_3^{2+}$ by capillary electrophoresis has been used for the determination of CPZ and PMZ. Simultaneously, CPZ and PMZ could be separated without any additives. The method had good sensitivity, selectivity and short analysis time. The CE-ECL method was utilized to analyze CPZ and PMZ in practical samples and no interference was found from samples matrix. The method could be applied for the determination of CPZ and PMZ in pharmaceutical formulations and human urine samples.

ACKNOWLEDGEMENTS

Financial supports from the National Natural Science Foundation of China, the State Key Laboratory of Electroanalytical Chemistry of Changchun Institute of Applied Chemistry and Foundation of National Excellent PhD Thesis are gratefully acknowledged.

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(*Received*: 28 September 2007; *Accepted*: 8 February 2008) AJC-6319