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Electrochemiluminescence determination of pipemidic acid using sulfite as energy transfer mediator

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Abstract—An electrochemiluminescence (ECL) based on energy transfer from electro-generated triplet sulfur dioxide to pipemidic acid (PPA) was studied. A weak ECL from triplet sulfur dioxide $^3SO_2^*$ was observed when sulfite was electrochemically oxidized in sulfuric acid solution on a Pt electrode. When PPA was present, the weak ECL was enhanced. The enhanced ECL was attributed to energy transfer from $^3SO_2^*$ to PPA. Based on the enhanced ECL, a flow-injection (FIA) ECL method for the determination of PPA was proposed. The proposed method allowed the measurement of PPA over the range of 1.0×10^{-7} to 2.0×10^{-5} mol l^{-1}. The detection limit was 3.9×10^{-8} mol l^{-1}, and the relative standard deviation for 1.0×10^{-6} mol l^{-1} PPA ($n = 9$) was 1.3%. This method was evaluated by the analysis of PPA in pharmaceutical preparations and urine samples.

Energy transfer electrochemiluminescence. Chemiluminescence (CL) has been used widely in different research fields for its low detection limit and easy operation procedure. Usually, CL can be achieved by direct reactions or by energy-transfer mechanisms. Many energy-transfer CL systems have been widely used for the determination of some fluorophore compounds.[1–4] Among them, SO_3^{2-}-based energy transfer CL is a typical one. In this case, sulfite ion as energy-transfer mediator receives chemical energy via chemical oxidation by such oxidants as cerium (IV), permanganate, hydrogen peroxide, hypobromite, O_2, manganese (III), and manganese (IV), producing excited state sulfur dioxide (SO_2^*).[5–8] Because these oxidants possessed different oxidation ability, the excited state sulfur dioxide (SO_2^*) produced by chemical oxidation should possess different energy.[9] Only suitable fluorophore as sensitizer could just accept the energy from the SO_2^* in different energy level, which led to the enhanced CL. Thus, the sensitized CL intensity mainly depended on the chemical and spectroscopy characters of the oxidant.[10] However, the enhanced CL often suffered from an overlapping between the CL band of sensitizer and the absorption band of the used oxidants itself.[11] Moreover, homogeneous CL reaction between oxidant and energy-transfer mediator was complicated, and side reaction often occurred. Because oxidation ability of the used oxidants was invariable under certain chemical conditions, the selection of appropriate oxidant was limited in CL analysis.

Compared with energy transfer CL, energy transfer electrochemiluminescence (ECL) has many additional advantages. First, the ECL reaction is easily regulated and manipulated by employing electrochemical manner, which introduces a degree of electrochemically high selectivity into the methodology.[12] Second, electrochemical oxidation ability is continuously variable and controllable, which allows the efficient and selective production of an excited state species under certain chemical conditions. Third, oxidation of mediator by electrochemical manner effectively avoids side reaction that often takes place in homogeneous CL reaction. Up to now, however, most of ECL studies have focused on electron transfer ECL reaction such as annihilation, luminol and coreactant systems.[13] Only a few ECL studies are concerned with the energy-transfer mechanism.[14–17] Therefore, it is of significance to study this kind of ECL and to enlarge its application in analysis field.

In this paper, a weak ECL was observed when sulfite in sulfuric acid solution was electrochemically oxidized at 1.34 V (vs. Ag/AgCl/sat. KCl) on a platinum electrode.

Keywords: Pipemidic acid; Electrochemiluminescence; Triplet sulfur dioxide.
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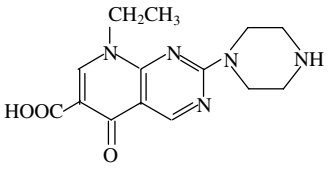
With the presence of pipemidic acid (PPA) (Scheme 1), the weak ECL was enhanced. Both the weak and the enhanced ECL mechanisms were discussed. Moreover, based on the enhanced ECL, a rapid and sensitive flow-injection (FIA) ECL method for the determination of PPA was proposed and evaluated by the analysis of PPA in pharmaceutical preparations and urine samples.

Reagents and apparatus. All reagents were of analytical reagent-grade. Twice glass-distilled water was used throughout the experiments. PPA was of biochemical-reagent grade and purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A stock standard solution of PPA (1.00×10^{-3} mol l^{-1}) was prepared in 0.01 mol l^{-1} sodium hydroxide solution. The standard working solutions were prepared by diluting the stock solution with water before they were used. Sodium sulfite solution (Xi'an Chemical Reagent Plant, Xi'an, China) (8.0×10^{-3} mol l^{-1}) was prepared daily. PPA tablet (Xi'an Pharmaceutical Plant, China) was purchased from local hospital. Urine samples were collected from three healthy individuals (from the Hospital of Northwest University, Xi'an).

All the ECL intensity was recorded by a Model IFFM-D-FI-CL analyzer (Xi'an Remax Electronic Science-Tech Co. Ltd., China). It consisted of two peristaltic pumps, a six-way injection valve, a Y-shaped mixing element (Y), and a photomultiplier tube (PMT). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The ECL signal was collected with the PMT. Cyclic voltammograms were recorded on a model CHI660 electrochemical workstation (CH Instruments, USA). A commercial cylindroid glass cell was used as batch ECL cell and employed with a three-electrode system consisting of a platinum coil (30 cm × 0.5 mm i.d.) as working electrode, a platinum wire as auxiliary electrode, and an Ag/AgCl/satd KCl as reference electrode. The ECL flow-through electrolysis cell, a flat spiral-coiled colorless glass tube (40 cm × 1.0 mm i.d.) with two-electrode system, was placed in front of the PMT and was paralleled with the flat of the window of PMT. Two platinum wires (30 cm × 0.5 mm i.d. and 2 cm × 0.5 mm i.d.), directly inserted from the inlet and the outlet of the glass tube into the ECL flow-through electrolysis cell and kept the end distance between the two platinum electrodes about 1 cm, were used as working and auxiliary electrodes, and were connected with anode port and cathode port of a galvanostat (Model KLT-1 coulombmeter) (Jiangshu Electroanalysis Instrument Plant, China). The constant direct current for electrolysis was supplied by the galvanostat too. The fluorescence spectra were monitored using Model RF-540 fluorescence spectrometer (Shimadzu, Japan).



Scheme 1. Structural formula of pipemidic acid.

Procedures for ECL profiles versus potential, cyclic voltammograms and ECL spectra. The batch ECL cell was placed in front of the PMT. When the potential of the work electrode was scanned from 0 to 1.5 V at scan rate 50 mV s^{-1}, ECL profiles versus potential and corresponding cyclic voltammograms were recorded simultaneously.

The ECL spectra were achieved with a set of 11 narrow band interference filters (400–680 nm). The filters were set between the batch ECL cell and the PMT. When the potential was stepped from 0 to 1.34 V, the ECL signal produced was recorded at different wavelength bands.

Procedure for flow injection ECL experiment. By keeping the valve in washing position, sulfuric acid solution and sodium sulfite solution were continuously pumped into the manifold until the baseline was established on the recorder. Then, 120 μl of sample (or standard) solution was injected into the sulfuric acid solution, and at the same time 15 mA direct current was applied. The solution was then merged with sodium sulfite solution in the Y-shaped mixing element (Y) before the ECL flow-through electrolysis cell. The ECL signal produced was recorded. Calibration graphs were constructed by plotting the ECL intensity (peak height of the ECL signal) versus the concentration of PPA (Fig. 1).

Characteristics of ECL. Experiment showed that a weak ECL signal appeared when the potential was stepped from 0 to 1.34 V (vs. Ag/AgCl/satd KCl) in sodium sulfite–sulfuric acid solution (Fig. 2b), while no ECL signal was observed in PPA–sulfuric acid solution (Fig. 2a). When PPA was added into the sodium sulfite–sulfuric acid solution mentioned above, the weak ECL was enhanced (Fig. 2c). The enhancement in ECL intensity increased with the increase of PPA concentration. In order to understand both the weak and the enhanced ECL mechanisms, ECL profiles versus potential, and cyclic voltammograms, the fluorescence and ECL spectra were examined.

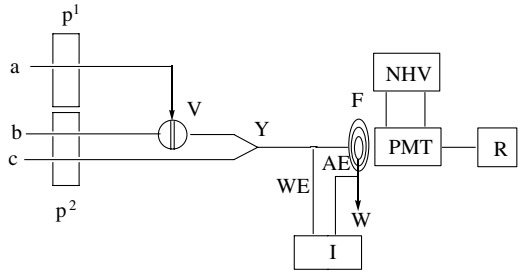


Figure 1. Schematic diagram of the flow injection ECL manifold used for the determination of PPA: P^1 and P^2, peristaltic pump; Y, three-way pipe; V, six-way valve; F, ECL flow-through electrolysis cell; W, waste; WE, working electrode; AE, auxiliary electrode; NHV, negative high voltage; PMT, photomultiplier tube; R, computer; I, galvanostat; a, sample stream; b, sulfuric acid carrier stream; c, sodium sulfite stream.

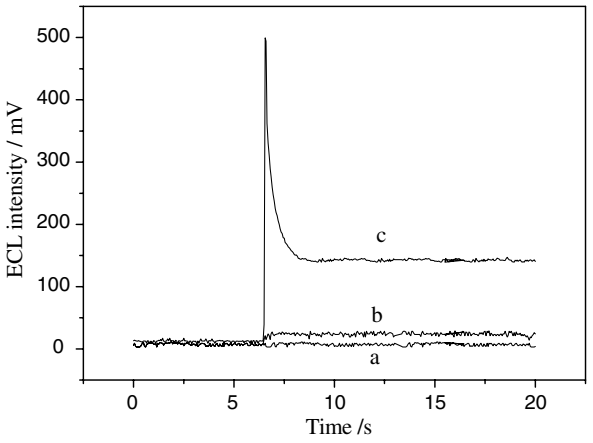


Figure 2. ECL emission intensity versus time profile of 2.0×10^{-6} mol l^{-1} PPA -0.10 mol l^{-1} sulfuric acid solution (a) and 8.0×10^{-3} mol l^{-1} sodium sulfite-0.10 mol l^{-1} sulfuric acid solution in the absence (b) and the presence (c) of 2.0×10^{-6} mol l^{-1} PPA.

As shown in Figure 3, in sulfuric acid solution, one irreversible oxidation peak appeared at 1.15 V (vs. Ag/AgCl/sat. KCl) (Fig. 3A-a), which was due to the formation of PtO_2 on the surface of Pt electrode.[18] When sulfite was added in the sulfuric acid solution, another two new irreversible oxidation peaks were observed at 0.65 and 1.34 V (Fig. 3A-b), respectively. The oxidation peaks of 0.65 and 1.34 V corresponded to electro-oxidation of sulfite absorbed at Pt electrode and diffused from bulk solution, respectively.[19] Both absorbed sulfite and bulk sulfite were electrochemically oxidized to form the same hydrogen sulfite radical (HSO_3·),[15,20–22] and the formed radical subsequently recombined to form dithionate ($S_2O_6^{2-}$).[23,24] Since the formed $S_2O_6^{2-}$ was unstable in the diffusion layer of the Pt electrode, it rapidly decomposed to triplet sulfur dioxide ($^3SO_2^*$) and sulfate.[24] When $^3SO_2^*$ fell back to its ground, a weak ECL peak at 1.34 V appeared (Fig. 3B-b). Nevertheless, the formed $S_2O_6^{2-}$ absorbed at Pt electrode surface was stable due to the formation of a surface complex consisting of $S_2O_6^{2-}$ and SO_2.[23,25] As a result, no ECL signal appeared at 0.65 V. When PPA was added in the sulfite-sulfuric acid solution, the weak ECL at 1.34 V was enhanced (Fig. 3B-c). Moreover, no change of both oxidation peak current and peak potential of sulfite was observed in the presence of PPA (Fig. 3A-c), indicating that PPA itself did not participate in electrode reaction. Obviously, the molecular structure of PPA was not destroyed in the enhanced ECL reaction.

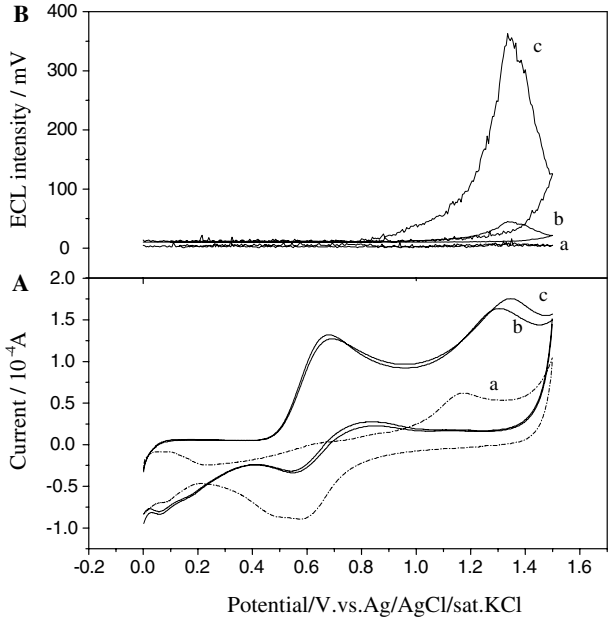


Figure 3. The cyclic voltammograms (A) and ECL profiles versus potential (B) of 0.10 mol l^{-1} sulfuric acid solution (a), 8.0×10^{-3} mol l^{-1} sodium sulfite-0.10 mol l^{-1}sulfuric acid solution in the absence (b) and the presence (c) of 2.0×10^{-6} mol l^{-1} PPA. The scan rate was 50 mV s^{-1}.

The fluorescence and ECL spectra were also examined. Only one fluorescence peak (λ_{max} = 450 nm) in the range of 400–700 nm was observed in PPA–sulfuric acid solution. The fluorescence peak was from PPA itself.[26] After sodium sulfite was added into the PPA–sulfuric acid solution, the fluorescence spectrum and the peak intensity (λ_{max} = 450 nm) hardly changed. The results showed that no direct chemical reaction occurred between sulfite and PPA. Moreover, when the potential was stepped from 0 to 1.5 V in the PPA–sulfite–sulfuric acid solution, the fluorescence spectrum and the peak intensity (λ_{max} = 450 nm) were still unchanged. The results further confirmed that the molecular structure of PPA was not destroyed. Furthermore, the ECL spectra of sulfite–sulfuric acid in the absence and the presence of PPA were recorded in the range of 400–680 nm. When PPA was absent, no obvious ECL spectrum was observed (Fig. 4a), which was because the ECL from $^3SO_2^*$ was too weak to penetrate the interference filters. The ECL spectrum in the presence of PPA showed one band, 420–490 nm (Fig. 4b). The band coincided with the fluorescence spectrum of PPA (λ_{max} = 450 nm) obtained by this work in PPA–sulfuric acid solution and the CL spectrum of PPA in Ce(IV)–sulfite and acidic hydrogen

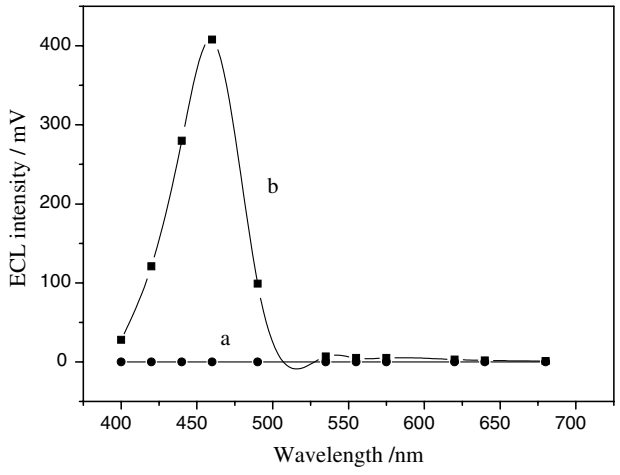


Figure 4. ECL spectra of 8.0×10^{-3} mol l^{-1} sodium sulfite-0.10 mol l^{-1} sulfuric acid solution in the absence (a) and the presence (b) of 6.0×10^{-6} PPA.

peroxide–nitrite CL systems.[27,28] Thus, the emitting species in the enhanced ECL was excited state PPA (PPA*).

It was reported that PPA could easily receive energy and had high fluorescence quantum efficiency in acid solution.[26] The energy from excited state sulfur dioxide (SO_2^*) was easily transferred to a fluorophore intentionally added to the sulfite-based weak CL system, which was responsible for the enhanced CL.[29,30] Under the condition of this work, the electro-oxidation of acidic sulfite at Pt electrode produced weak ECL. The weak ECL was from triplet sulfur dioxide ($^3SO_2^*$). When PPA was present in the sulfite–sulfuric acid solution, the energy from $^3SO_2^*$ was transferred to PPA, forming PPA*. When PPA* fell back to its ground state, the enhanced ECL occurred. Because PPA* had higher quantum efficiency than $^3SO_2^*$, stronger light was emitted. The enhanced ECL mechanism was suggested as follows:

$$HSO_3^- \xrightarrow{-e^-} HSO_3^\bullet$$
$$2HSO_3^\bullet \rightarrow S_2O_6^{2-} + 2H^+$$
$$S_2O_6^{2-} \rightarrow SO_4^{2-} + {}^3SO_2^*$$
$$^3SO_2^* + PPA \rightarrow PPA^* + SO_2$$
$$PPA^* \rightarrow PPA + h\nu\,(450\ nm)$$

Optimization of experimental variables. The enhanced ECL can be used to determine PPA. A FIA ECL method was proposed. The analytical conditions were optimized.

The current-controlled electrolysis is used as it is thought to be simple in instrument. The effect of direct current on the ECL intensity in the presence of 2.0×10^{-6} mol l^{-1} PPA was investigated when the flow rate was fixed at 2.1 ml min^{-1}. As shown in Figure 5, the ECL intensity increased dramatically with the increase of direct current from 1 to 15 mA. Above 15 mA, the ECL intensity remained at a higher level, however, it decreased slightly with the increase of direct current. The reason may be that when the higher direct current was applied, the oxidation reaction of water took place and the intermediate products of water oxidation might react with the electrogenerated hydrogen sulfite radical.[24] Therefore, 15 mA direct current was used.

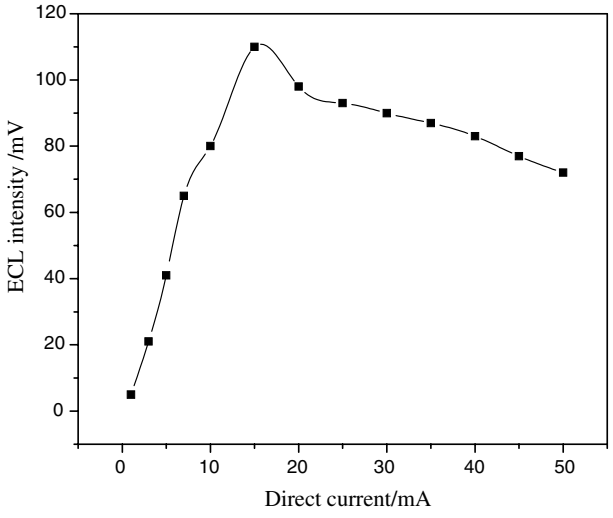


Figure 5. Effect of direct current on the ECL intensity. Sodium sulfite, 8.0×10^{-3} mol l^{-1}; sulfuric acid, 0.10; PPA, 2.0×10^{-6} mol l^{-1}.

Many different carrier streams such as H_2SO_4, HCl, H_3PO_4, NaOH, Na_2CO_3, $NaHCO_3$, Na_3PO_4, Na_2HPO_4, and $NH_3\cdot H_2O$ were used. Experiment showed that H_2SO_4 gave the maximum ECL signal, and also the best reproducibility for the ECL intensity. So H_2SO_4 solution was selected as carrier stream.

The effect of sulfuric acid concentration on the ECL intensity was examined over the range of 1.0×10^{-3} to 1.0 mol l^{-1}. The maximum ECL intensity was obtained when sulfuric acid concentration was 0.10 mol l^{-1}. The raising of sulfuric acid concentration over 0.10 mol l^{-1} caused a decrease of ECL intensity. This was presumably due to the fact that the luminescent form of PPA was gradually destroyed by acidolysis when the pH value was less than 1.[24] Therefore, 0.10 mol l^{-1} sulfuric acid was used.

The effect of sodium sulfite concentration on the ECL intensity was examined. The ECL intensity rose sharply as the sodium sulfite concentration increased from 1.0×10^{-4} to 8.0×10^{-3} mol l^{-1}. When the sodium sulfite concentration increased from 8.0×10^{-3} to 2.0×10^{-2} mol l^{-1}, the ECL intensity decreased slightly. Therefore, 8.0×10^{-3} mol l^{-1} sodium sulfite was used.

Pump P^2 was used to deliver the carrier (sulfuric acid) and sodium sulfite solutions. Sodium sulfite solution and sulfuric acid carrier solution that contained sample were first mixed at the Y-shaped mixing element (Y), and then the mixed solution was delivered by pump P^2 to the ECL flow-through electrolysis cell placed in front of the PMT. The flow rate affected the amount of electrogenerated hydrogen sulfite radical. The effect of the flow rate on the ECL intensity was examined in the range of 0.7–4.2 ml min^{-1}. The ECL signal reached its maximum value at 2.1 ml min^{-1}. The raising of flow rate over 2.1 ml min^{-1} caused the decrease of ECL intensity. This was due to the fact that higher flow rate led to a decrease of electrolysis efficiency.[31] Thus, 2.1 ml min^{-1} flow rate was used.

Performance of the proposed method for PPA measurements. Under the selected experimental conditions, the ECL intensity was linear with PPA in the range of 1.0×10^{-7} to 2.0×10^{-5} mol l^{-1}. The detection limit was 3.9×10^{-8} mol l^{-1} ($s/n = 3$) and the relative standard deviation for 1.0×10^{-6} mol l^{-1} PPA ($n = 9$) was 1.3%. The linear regression equation was $I = 9.05 + 5.07\times10^7$C (where I is ECL intensity and C is PPA concentration, units are mV and mol l^{-1}, respectively) with a correlation coefficient of 0.9995 ($n = 13$). The sample measurement frequency was calculated to be about 20 samples h^{-1}.

Table 1. The tolerable concentration ratios of some interfering species to 2.0×10^{-6} mol l^{-1} PPA

Substance	Tolerable concentration ratio
Cation	
K^+, Na^+, Ca^{2+}, NH_4^+	1000
Mg^{2+}, Al^{3+}, Zn^{2+}	500
Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}	100
Anion	
Cl^-, SO_4^{2-} , PO_4^{3-}, NO_3^-	1000
Vitamin	
Thiamine hydrochloride (vitamin B_1)	200
Riboflavin (vitamin B_2), folic acid (vitamin B_c)	100
Ascorbic acid (vitamin C)	50
Amino acid	
L-Valine, L-serine, L-arginine,	500
L-Glutamic acid, L-tyrosine	200
L-Threonine, L-lysine	100
L-Histidine, L-cystine	50
Others	
Urea, starch	500
Glucose, sucrose	200
Oxalic acid, uric acid	100

Interferences study. In order to assess the proposed method to the analysis of PPA in pharmaceutical dosage forms and urine samples, the interference of commonly used excipients and additives, co-existing ions or the other compounds was examined. The solutions for this purpose contained 2.0×10^{-6} mol l^{-1} PPA and increasing amounts of interfering species. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately ±5% relative error for the determination of PPA. The results of interference tests are listed in Table 1. The tolerated amounts of interfering species were far in excess of their normal occurrence in urine samples.[32]

Determination of PPA in tablet and urine samples. The proposed method was applied to the determination of PPA in tablet. Ten tablets of PPA were weighed and pulverized. An accurately weighted amount of the powder was dissolved in 0.01 mol l^{-1} sodium hydroxide. After filtering, aliquots of the filtrate were further diluted with water until the final PPA concentration was within the working range. The determination was performed according to procedure for flow injection ECL experiment. The results, shown in Table 2, agreed well with those obtained by UV method (Pharmacopoeia method).[33] Moreover, recovery studies were also carried out on each sample solution to which the known amounts of PPA standard solution were added. Each recovery was calculated by comparing the results obtained before and after the addition. As shown in Table 2, the recoveries were between 97% and 105%.

Table 3. Results for the determination of PPA in spiked urine samples

Sample no.	Added ($\times 10^{-6}$ mol l^{-1})	Found[a] ($\times 10^{-6}$ mol l^{-1})	Recovery (%)
1	0.40	0.41 ± 0.01	103
	2.00	1.95 ± 0.03	98
2	0.40	0.42 ± 0.02	105
	2.00	2.02 ± 0.02	101
3	0.40	0.39 ± 0.01	98
	2.00	1.97 ± 0.02	99

[a] Mean value ± SD ($n = 5$).

Because all of PPA was unchanged during metabolism,[34] and its content in patient urine was between 3.3 and 330 μmol l^{-1},[35] the proposed method could be applied to the determination of PPA in urine sample. The PPA content in the spiked urine sample was determined directly by the proposed method. No PPA was found. Then, recovery tests were carried out by standard addition method. The recoveries obtained are shown in Table 3. The recoveries were between 98% and 105%.

In summary, the electro-generated excited state species can be easily realized by electrochemical manner, which allows the efficient and selective measurements. The ECL based on energy transfer from $^3SO_2^*$ to PPA, which was taken as an example, was more sensitive and convenient than sulfite-based and hydrogen peroxide-nitrite energy transfer CL.[27–29] In addition, the ECL based on energy transfer mechanism could occur greatly and has potential application for analytical purpose.

Acknowledgment

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Table 2. Results for the determination of PPA in tablets

Sample no.	Label value (mg)	Amount (mg)		Added ($\times 10^{-6}$ mol l^{-1})	Found[c] ($\times 10^{-6}$ mol l^{-1})	Recovery (%)
		Proposed method[a]	Official method[b]			
1	250	253 ± 3	251 ± 2	0.40	0.42 ± 0.02	105
				2.00	2.02 ± 0.04	101
				4.00	4.08 ± 0.03	102
2	250	246 ± 2	246 ± 2	0.40	0.39 ± 0.01	98
				2.00	2.03 ± 0.03	102
				4.00	3.88 ± 0.05	97

[a,b,c] Mean value ± SD ($n = 5$).

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