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Electrochemiluminescence from Isoniazid Itself and Its Analytical Application

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Abstract

A weak electrochemiluminescence (ECL) of isoniazid in NaOH solution was observed at a platinum wire anode. When cetyltrimethylammonium bromide (CTAB) was present, the weak ECL was enhanced. The stronger ECL mechanism of isoniazid in NaOH–CTAB solution could be described as follows: isoniazid was electrochemically oxidized via one-electron and one-proton transfer to isoniazid hydrazyl radical. Then the formed radical was further chemically oxidized by dissolved oxygen to the excited state isonicotinate that subsequently emitted light. Based on the stronger ECL phenomenon of isoniazid, a flow injection ECL method for the determination of isoniazid was proposed. The ECL intensity was linear with isoniazid concentration in the range of 4.0×10^{-7} to 1.0×10^{-5} mol l^{-1} and the limit of detection ($s/n = 3$) was 1.9×10^{-7} mol l^{-1}. The proposed method was simple and convenient operation, and has been applied to the determination of isoniazid in pharmaceutical preparations and human urine.

Keywords: Electrochemiluminescence; Isoniazid; Flow injection

Introduction

Electrochemiluminescence (ECL), the production of light from electrochemically generated reagents, has been paid considerable attention during the past several decades due to its versatility, simplified optical setup, very low background signal, and good temporal and spatial control [1]. According to reaction mechanisms, the known ECL has been mainly classified into three main types [2-4]. The first type is the annihilation reaction between electro-generated oxidized and reduced forms of the same or different specie such as rubrene/rubrene and 9, 10-diphenylanthracene/ thianthrene systems [5]. The second type is the coupled reaction between electro-oxidized or electro-reduced product of coreactant and luminescence agent such as Ru (2,2′-bipyridine)$_3^{2+}$/tri-*n*-propylamine and norfloxacin/$S_2O_8^{2-}$ systems [6,7]. The third type is the conventional CL reactions initiated by electrochemical means [8]. Typical sample of the third type is luminol ECL reaction. It is based on electrochemical oxidation of luminol to form luminol radical and subsequently chemical oxidation of the formed radical by such oxidants as hydrogen peroxide, superoxide radical and hypobromite to produce CL [9-11]. Besides luminol, indole, tryptophan and rifampicin ECL systems, to best of our knowledge, no other examples based on successive electro- and chemo-oxidation ECL have been reported [12,13].

In this paper, a stronger ECL of isoniazid in NaOH–cetyltrimethylammonium bromide (CTAB) solution was observed at a platinum wire anode. Based on this phenomenon, a flow injection ECL method was proposed for the determination of isoniazid. The method was applied to the determination of isoniazid in pharmaceutical preparations and human urine. The ECL mechanism of isoniazid in NaOH–CTAB solution was also discussed in detail.

Experimental

Reagents and solutions

All reagents used were of analytical reagent-grade. Twice glass distilled water was used throughout the experiments. Isoniazid was of biochemical-reagent-grade and was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The stock standard solution of isoniazid (1.00×10^{-2} mol l^{-1}) was prepared by dissolving 0.137 g isoniazid in 100 ml water. Standard working solutions of isoniazid were prepared daily by appropriate dilution of the stock solution with water. About 0.6 mol l^{-1} NaOH and 6.0×10^{-3} mol l^{-1} cetyltrimethylammonium bromide (CTAB) solutions were routinely prepared. Isoniazid tablet (Xi'an Pharmaceutical Plant, China) was purchased from local hospital.

Apparatus

Apparatus included two setups, ECL research and flow injection ECL detection ones (Figure 1A and 1B). The ECL research setup included a model IFFM-D flow injection CL analyzer (Xi'an Remax Electronic Science Tech. Co. Ltd., Xi'an, and China), a batch ECL cell and a model CHI660 electrochemical working station (CH Instruments, USA). The batch ECL cell utilized a conventional three-electrode system and was placed upon a photomultiplier tube (PMT) of the model IFFM-D flow injection CL analyzer. The working electrode was a flat spiral-coiled platinum wire (1 mm × 30 cm); a Pt flake (7 mm × 7 mm) and Ag/AgCl (saturated KCl solution) were used as the auxiliary electrode and reference electrode, respectively. As a linear scan potential was applied to the working electrode, ECL intensity versus potential curves and cyclic voltammograms were recorded simultaneously on the model IFFM-D flow injection CL analyzer and the model CHI 660 electrochemical workstation. All the potentials were reported according to the Ag/AgCl (saturated KCl) reference electrode.

The flow injection ECL detection setup consisted of the model IFFM-D flow injection CL analyzer, a flow-through electrolysis cell (EFC) and a Model KLT-1 Coulombmeter (Jiangshu Electroanalysis

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Instrument Plant, China). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The EFC was made of a flat spiral-coiled colorless glass tube without the gap between loops (1 mm × 40 cm). Two platinum wires (0.5 mm × 30 cm and 0.5 mm × 2 cm) were inserted into the glass tube from its inlet and outlet, and the end distance of the two platinum wires in the glass tube was about 1 cm. The longer and the shorter platinum wires were used as anode and cathode, respectively. The EFC was placed upon the PMT of the model IFFM-D flow injection CL analyzer. The constant direct current for electrochemical oxidation was performed using the Model KLT-1 Coulombmeter.

Procedure for ECL kinetic profile and ECL spectra

The ECL kinetic profile was recorded using the ECL research setup. 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. The ECL signal was recorded at different wavelength bands when the potential of the platinum wire working electrode was controlled at 0.69 V.

Procedure for flow injection ECL experiment

By keeping the valve in washing position, NaOH and CTAB solutions were continuously pumped into the manifold until a stable baseline was established on the recorder. Then 120 µl of sample solution was injected into the NaOH solution, and at the same time 15 mA of direct current was applied. The NaOH solution was then merged with CTAB solution in the Y-shaped mixing element (Y) before the ECL flow-through electrolysis cell. When the mixed solution flowed into the ECL flow-through electrolysis cell, ECL reaction occurred. The ECL signal produced was recorded. Calibration graphs were constructed by plotting the intensity (peak height) of the ECL signal versus the concentration of isoniazid.

Results and Discussion

Discussion of the possible ECL mechanism

Preliminary experiments showed that a weak ECL of isoniazid in NaOH solution was observed at a platinum wire anode. When cetyltrimethylammonium bromide (CTAB) was present, the weak ECL was enhanced.

A series of experiments were performed to explain the stronger ECL of isoniazid in NaOH–CTAB solution with the research setup. Cyclic voltammetry and corresponding ECL intensity–potential curves were recorded in isoniazid–NaOH– CTAB solution without deoxygenation (Figure 2). In the potential range of 0–0.8 V, isoniazid yielded one irreversible oxidation peak at 0.63 V (Figure 2A). At the same time, a stronger ECL was observed at 0.69 V (Figure 2B). Isoniazid is a hydrazines derivative, which can be oxidized either by direct electro-oxidation or by successive electro- and chemo-oxidation [14-17]. The effect of dissolved oxygen on the stronger ECL was also investigated. After isoniazid–NaOH–CTAB solution was deoxygenated with pure nitrogen gas for 20 min, the oxidation peak of isoniazid at 0.63 V hardly changed, while the stronger ECL intensity at 0.69 V decreased by 85%. These results suggested that the stronger ECL of isoniazid in NaOH–CTAB solution resulted from successive electro- and chemo-oxidation. The oxidation peak at 0.63 V resulted from one-electron and one-proton oxidation at the hydrazine group and led to the formation of isoniazid hydrazyl radical [18-20]. The formed isoniazid hydrazyl radical, an unstable species, can be chemically oxidized by dissolved oxygen to an excited state that subsequently emitted light [21-24].

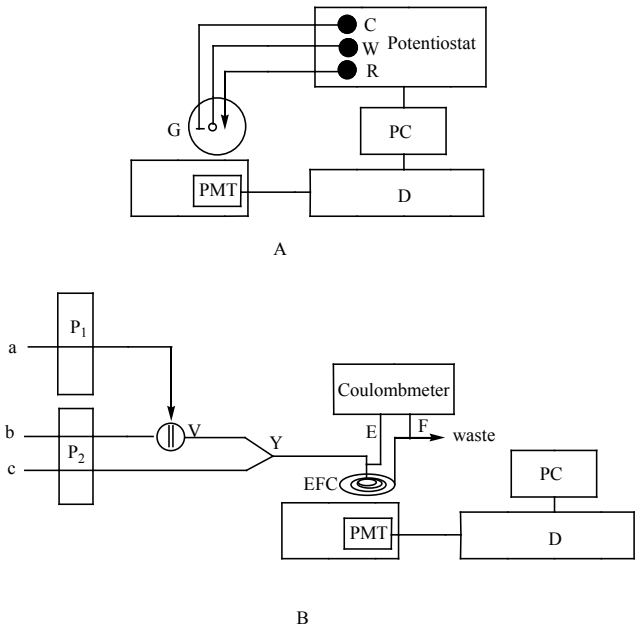


Figure 1: The block diagram of ECL research setup (A) and ECL flow injection ECL detection setup (B). W, working electrode; R, reference electrode; C, counter electrode; G, batch ECL cell; D, CL detector; PC, computer; PMT, photomultiplier tube; P_1 and P_2, peristaltic pumps; V, six-way valve; Y, three-way pipe; EFC, ECL flow-through electrolysis cell; E, anode; F, cathode; a, isoniazid solution; b, NaOH solution; c, CTAB solution.

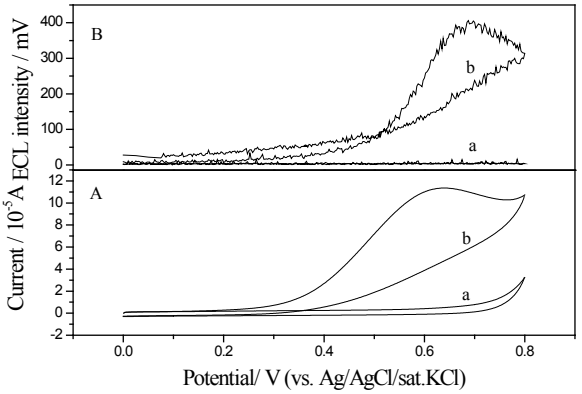


Figure 2: Cyclic voltammograms (A) and ECL intensity–potential curves (B) of 0.6 mol l^{-1} NaOH–6.0 × 10^{-3} mol l^{-1} CTAB solution in the absence (curve a) and the presence of 1.0 × 10^{-5} mol l^{-1} isoniazid (curve b).

For obtaining information about the emitter of the present ECL, ECL dynamic response curves and corresponding spectrum were recorded using 0.69 V potential in the isoniazid–NaOH–CTAB solution without deoxygenation (Figure 3 and 4). The ECL emission spectrum was obtained by using 11 narrow band interference filters (400–680 nm). The maximum ECL emission appeared at 420–460 nm (Figure 4), which was in good agreement with the CL spectrum of isonicotinate (λ_{max} = 430 nm) [25,26]. This result suggested that the emitting species in the present ECL system was excited state isonicotinate.

Based on the results mentioned above, the ECL mechanism of isoniazid in NaOH–CTAB solution was described as follows: Electrochemical oxidation of isoniazid in NaOH–CTAB solution produces isoniazid hydrazyl radical (I). The radical is further oxidized

by dissolved oxygen to yield excited state isonicotinate (II). When the excited state isonicotinate goes back to its ground state, a stronger ECL is emitted. The possible ECL mechanism of isoniazid in NaOH–CTAB solution is written in Scheme 1.

Optimized condition for isoniazid determination

Based on the stronger ECL of isoniazid in NaOH–CTAB solution, a flow injection ECL method for the determination of isoniazid was proposed. The analytical conditions including electrochemical parameters, selection of carrier and surfactant solutions, and flow rate were optimized using 2.0×10^{-6} mol l^{-1} isoniazid.

Effect of direct current on the ECL

The ECL of isoniazid can be produced by using either potential– or current–controlled electrolysis. The current–controlled electrolysis was selected as it was simple in set-up. The effect of direct current on the ECL intensity of isoniazid was examined over the range of 2–50 mA. The ECL intensity increased sharply as the direct current raised from 2 to 15 mA. With the direct current increasing from 15 to 50 mA, the ECL intensity increased slowly. Therefore, 15 mA of direct current was used throughout the experiments.

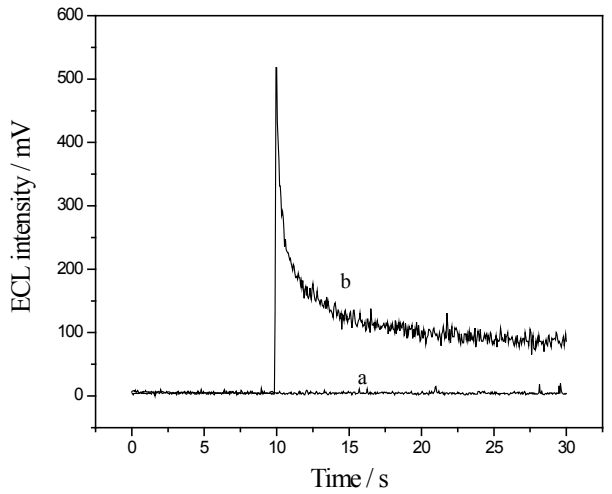


Figure 3: ECL dynamic response curves of 0.6 mol l^{-1} NaOH–6.0×10^{-3} mol l^{-1} CTAB in the absence of (curve a) and the presence of 1.0×10^{-5} mol l^{-1} isoniazid (curve b).

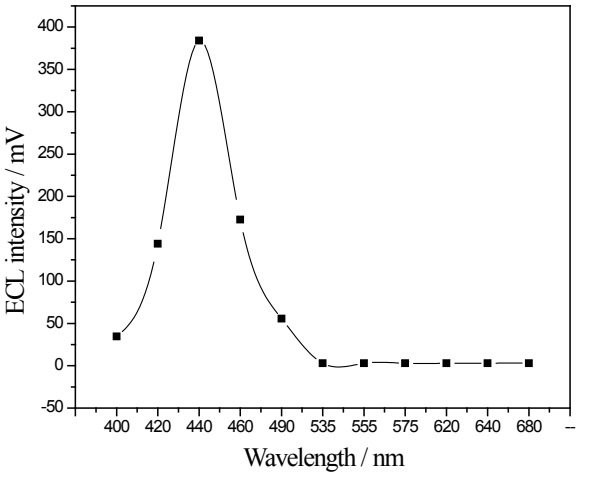
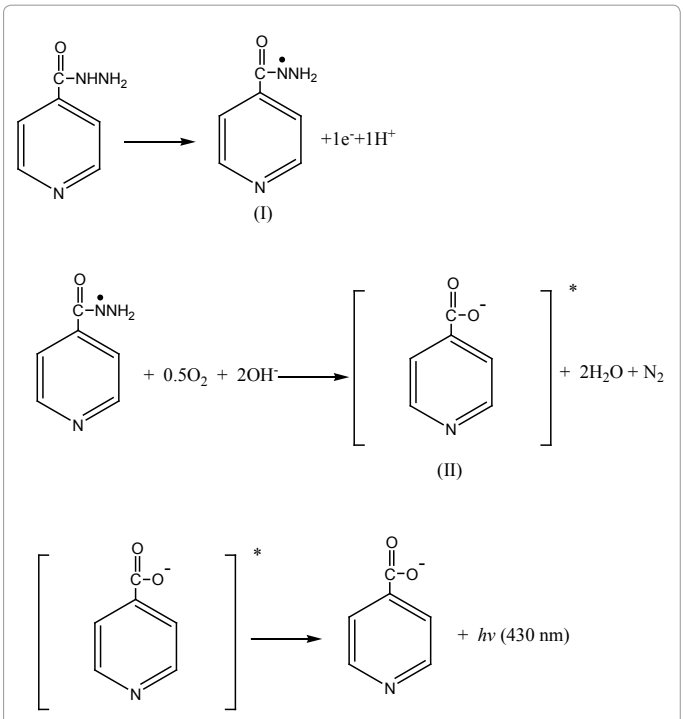


Figure 4: ECL spectrum of 1.0×10^{-5} mol l^{-1} isoniazid in 0.6 mol l^{-1} NaOH–6.0×10^{-3} mol l^{-1} CTAB solution.



Scheme 1: The ECL mechanism of isoniazid in NaOH–CTAB solution.

Selection of carrier solution

Different carrier solutions such as Na_2CO_3, Na_3PO_4, $NH_3{\cdot}H_2O$, NaAc and NaOH solutions were examined in the present ECL system. Experiment showed that NaOH solution gave the maximum ECL signal, and also the best reproducibility for monitoring isoniazid. The effect of NaOH concentration was tested in the range of 0.01–0.80 mol l^{-1}. When NaOH concentration was higher than 0.6 mol l^{-1}, the ECL intensity reached maximum and kept constant. Accordingly, 0.6 mol l l^{-1} NaOH solution was used.

Selection of surfactant

The ECL intensity of isoniazid in the presence of three kinds of surfactants was examined. The surfactants examined included two neutral surfactants (Tween 80 and Tween 20), three cationic surfactants (tetramethyl ammonium chloride, tetrabutyl ammonium chloride and CTAB) and one anionic surfactant (sodium dodecyl sulfate). It was found that all the surfactants enhanced the ECL of isoniazid to some extent. This enhancement may be attributed to the increase of electrode surface hydrophobicity [27,28], which prolongs the lifespan of electro-generated isoniazid hydrazyl radical and facilitates more isoniazid hydrazyl radical to be oxidized [29,30]. Experiment also showed that CTAB gave the maximum ECL signal, and the best reproducibility for monitoring isoniazid. So CTAB was selected. The effect of CTAB concentration on the ECL intensity was examined in the range of 0 to 1.0×10^{-2} mol l^{-1}. As shown in Figure 5, the ECL intensity reached its maximum value at 6.0×10^{-3} mol l^{-1} CTAB. Thus 6.0×10^{-3} mol l^{-1} CTAB was used.

Effect of flow rate

Pump P_2 was used to deliver both NaOH and CTAB solutions.

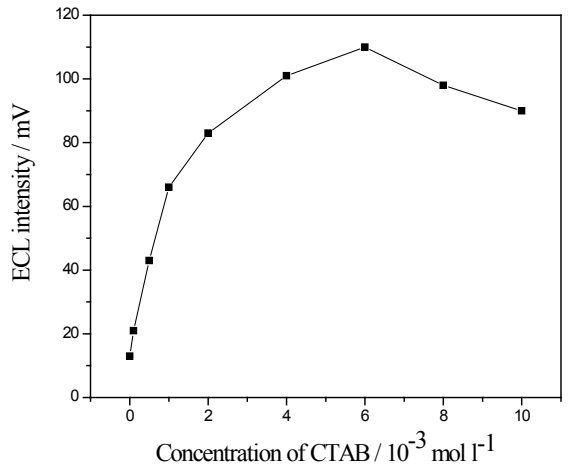


Figure 5: Effect of CTAB concentration on the ECL intensity in 2.0 × 10^{-6} mol l^{-1} isoniazid–0.6 mol l^{-1} NaOH solution.

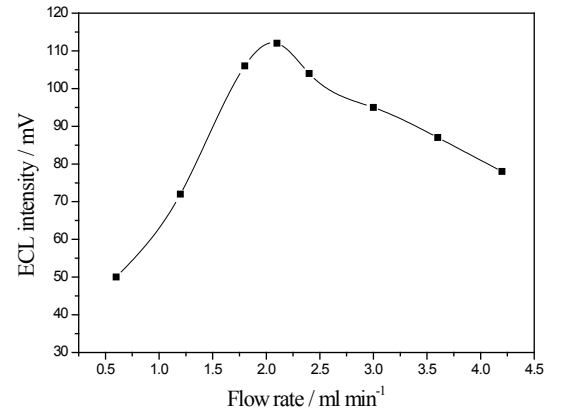


Figure 6: Effect of flow rate on the ECL intensity in 2.0×10^{-6} mol l^{-1} isoniazid–0.6 mol l^{-1} NaOH–6.0 × 10^{-3} mol l^{-1} CTAB solution.

The effect of its flow rate on the ECL intensity was investigated in the range of 0.3–4.4 ml min^{-1}. As shown in Figure 6, the ECL intensity was increased sharply with the increase of the flow rate in the range of 0.3–2.1 ml min^{-1}. When the flow rate was 2.1 ml min^{-1}, the ECL intensity reached maximum. When the flow rate was higher than 2.1 ml min^{-1}, the ECL intensity decreased gradually. It may be that higher flow rate results in a decrease of electrolysis efficiency [31]. Thus, 2.1 ml min^{-1} was used.

Analytical characteristics

Under the selected experimental conditions, the ECL intensity was linear with isoniazid concentration in the range of 4.0 × 10^{-7}–1.0 × 10^{-5} mol l^{-1}. The detection limit was 1.9 × 10^{-7} mol l^{-1} (s/n = 3) and the relative standard deviation for 2.0 × 10^{-6} mol l^{-1} isoniazid (n = 9) was 1.9%. The linear regression equation was $I = 12.16 + 4.98 \times 10^{7} C$ (where I is ECL intensity and C is isoniazid concentration, units are mV and mol l^{-1}, respectively) with a correlation coefficient of 0.9998 (n = 9). The sample measurement frequency was calculated to be about 20 samples per hour.

Analytical application

In order to assess the proposed method to the analysis of isoniazid in pharmaceutical dosage forms and human urine samples, the interferences of commonly used excipients and additives, co-existing ions were examined. The tolerance limit was taken as the maximum concentration of the interfering substances which caused an approximately ± 5% relative error for the determination of isoniazid. The tolerable ratio of the interfering substances to 2.0 × 10^{-6} mol l^{-1} isoniazid was 500 for L-glutamic acid, uric acid, K^{+}, Br^{-}, CO_3^{2-}, SO_4^{2-}, NO_3^{-}, PO_4^{3-}, Ni^{2+} and Cu^{2+}; 200 for L-threonine, L-tyrosine, folic acid, Al^{3+} and Pb^{2+}; 100 for starch, dextrin, L-histidine, L-lysine, L-cystine, isonicotinic acid, acetylhydrazine, Ca^{2+}, Mn^{2+}, Co^{2+} and Mg^{2+}; 20 for thiamine hydrochloride, riboflavin, polyethylene glycol 4000, Fe^{2+} and Fe^{3+}; 10 for sucrose and glucose; 5 for ascorbic acid. From above results, it can be concluded that the proposed method has good selectivity for isoniazid determination, and the normal components presented in pharmaceuticals and human urines do not interfere with its determination [32].

It was reported that around 90% of orally administrated isoniazid was assimilated and around 10% of the excreted isoniazid remains unmetabolized [33]. Isoniazid was metabolized mainly through acetamidation and partly through hydrolysis. Its main metabolites included N-acetylisoniazid, isonicotinic acid and acetylhydrazine [34]. As showed in the inferences study results mentioned above, the tolerable concentration ratios for both isonicotinic acid and acetylhydrazine are about 100. As the contents of isonicotinic acid and acetylhydrazine in a patient's urine sample are very low [35], no interference with the determination of isoniazid can be expected.

The proposed method was applied to the determination of isoniazid in tablet and spiked urine samples. Ten tablets of isoniazid were weighed and pulverized. An accurately weighed amount of the powder was dissolved in water. After filtering, aliquots of the filtrate were appropriately diluted with water before measurement. The results, shown in Table 1, agreed well with those obtained by Pharmacopoeia method [36]. Moreover, recovery studies were also carried out in samples to which known amounts of isoniazid were added. Each recovery was calculated by comparing the results obtained before and after the addition. As shown in Table 1, the recoveries were between 96% and 104% (n = 5).

Urine samples were collected from three healthy individuals (from the Hospital of Xi'an University of Science and Technology). Added 0.70 or 3.00 ml standard isoniazid solution (1.0 × 10^{-5} mol l^{-1}) to 1 ml of each urine sample, respectively, and then diluted the obtained solution

Sample number	Labeled (mg)	Proposed method (mg)	Official method (mg)	Added (×10^{-6} mol l^{-1})	Found (×10^{-6} mol l^{-1})	Average recovery (%, n = 5)	R.S.D (%, n = 5)
1	100	99	98	2.00	2.06.	103	2.3
				5.00	4.81.	96	2.2
2	100	97	96	2.00	1.93	97	2.6
				5.00	5.21	104	2.5

Table 1: Results for the determination of isoniazid in tablets.

UrTable 2ine Sample	added ($\times 10^{-6}$ mol l^{-1})	Found ($\times 10^{-6}$ mol l^{-1})	Average recovery (%, n = 5)	R.S.D. (%, n = 5)
1	0.70	0.72	103	2.2
	3.00	2.90	97	1.7
2	0.70	0.74	106	1.8
	3.00	3.13	104	2.9
3	0.70	0.69	99	2.6
	3.00	3.22	107	1.9

Table 2: Results for the determination of isoniazid in spiked urine samples.

to 10 ml with water. The isoniazid content in the spiked urine sample was determined directly by the proposed method. The recoveries obtained were showed in Table 2. The recoveries were between 97% and 107% with average recovery 103% (n = 5).

Conclusions

A flow injection ECL method for the determination of isoniazid was proposed, which was based on successive electrochemical and chemical oxidation of isoniazid itself. As compared with the known ECL method, based on analyte as coreactant in the luminescence agent–coreactant ECL reaction [37], or based on the enhancement or inhibition of analyte on ECL reaction of the luminescence agent [38-40], the proposed ECL method was easily realized by successive electrochemical and chemical oxidation of organic analyte itself and did not require any luminescence agent. As a number ECL phenomena based on successive electrochemical and chemical oxidation of analytes themselves may occur naturally, this type of ECL method may have potential application in analytical field.

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