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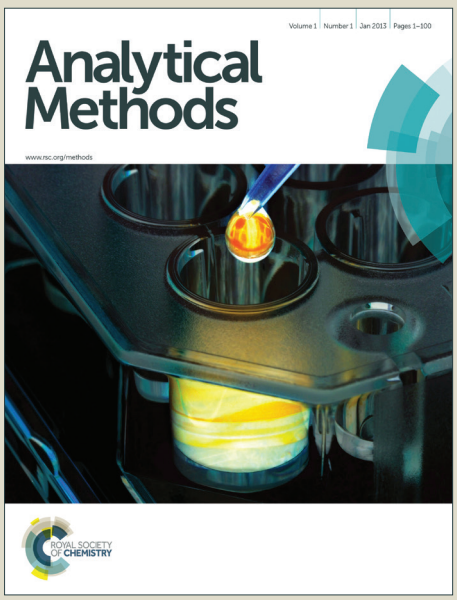

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Enhanced solid-state electrochemiluminescence of $Ru(bpy)_3^{2+}$ with nano-CeO_2 modified carbon paste electrode and its application in tramadol determination

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A sensitized solid-state electrochemiluminescence (ECL) was successfully used for determination of tramadol. The ECL signal was produced by a sensitive $Ru(bpy)_3^{2+}$-tramadol reactions. A modified carbon paste electrode (CPE) was applied as a working electrode. Incorporation of cerium oxide nanoparticles in the carbon paste led to the fabrication of a novel ECL sensor. CeO_2 showed attractive sensitizing effect on $Ru(bpy)_3^{2+}$ -tramadol electrochemiluminescence system. Under the optimal conditions for $Ru(bpy)_3^{2+}$ - tramadol system, a linear relationship between the ECL intensity and concentration of tramadol in the range of 1.0×10^{-10} to 2.5×10^{-8} mol/L was obtained with detection limit of 9.0×10^{-11} mol/L and relative standard deviation of 2.9% (S/N=3). The method is sensitive, selective, and simple and has been successfully applied to the analysis of tramadol in human serum, urine and pharmaceutical formulation.

1. Introduction

Tramadol (TMD) ((±)cis-2-[(dimethylamino) methyl]-1-(3methoxyphenyl) cyclohexanol hydrochloride) is an opioid, synthetic analog of codeine and is not currently classified as a controlled substance [1-3]. Tramadol has been used since 1977 like other narcotics applied for the treatment of acute or chronic pain [4]. In Iran, 350 million tramadol tablets (100 mg) were sold in 2006–2007 and recently, it has become one of the most widely dispensed analgesics in Iran's essential drugs list [5,6]. TMD abuse and its probable risks such as dependence or addiction to TMD have increased due to its effectiveness and low incidence of side effects. It binds to opioid receptors in the brain and restrains reuptake of norepinephrine and serotonin [7]. It can be administered orally, rectally, intravenously, or intramuscularly [6]. The racemic TMD is rapidly

absorbed after oral administration and approximately 10–30% of its dose is excreted in urine as unmetabolized drug [8]. TMD therapeutic concentration is within the range of 100–800 g/L [9]. After a single oral administration of 100 mg TMD, its concentration can be detected instantly in plasma. TMD elimination is slow and characterized by an elimination half-life of 6 h [10,11]. Several analytical methods have been reported for the determination of TMD and its metabolites such as HPLC methods with UV [12,13], electrochemical [14], fluorescence [15,16], or mass spectrometry (MS) detection [17,18], GC with flame ionization detection (FID) [19], nitrogen phosphorus [20] and MS detection [21, 22], Capillary electrophoresis [23]. However these methods suffer from disadvantages including long analysis time, high costs and requirement for sample pretreatment which is time consuming, making them unsuitable for routine analysis. To overcome these defects, development of a simple, inexpensive, sensitive and accurate analytical method for determination of TMD would be of considerable value.

Electrochemiluminescence (ECL) based on tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) was first reported by Tokel and Bard [24]. After that, ($Ru(bpy)_3^{2+}$) ECL as a sensitive detection method has received considerable attention in chemical analysis because of its excellent stability and high efficiency in aqueous phase [25-33]. Moreover, since ($Ru(bpy)_3^{2+}$) is regenerated during the ECL process, a reagentless ECL sensor can be constructed by immobilizing the ($Ru(bpy)_3^{2+}$) on an electrode surface [34]. Compared with the solution-phase ECL procedure, the immobilization of the ($Ru(bpy)_3^{2+}$) reduces the consumption of expensive reagent and simplifies experimental design. The Carbon paste electrodes have been widely applied in electrochemical research due to its simplicity of fabrication, low cost, low background current, easily renewable surface [35]. Nowadays, their usages continue in the developments of new materials based electrode capable to change the electrode surface with better analytical performance, including graphene, metal

nanoparticles, and carbon nanotubes. Metal nanoparticles have some distinct advantages such as higher mass transport, lower influence of the solution resistance, low detection limit, and better signal-to-noise ratio over the conventional macroelectrodes [36]. In the present study, we report a selective, rapid and sensitive ECL method to determine TMD by employing a nano-composite CeO_2 NPs -$Ru(bpy)_3^{2+}$- carbon paste electrode.

2 Experimental

2.1 Reagents and chemicals

All chemicals were of analytical reagent grade and used without further purification. Tramadol was obtained from local pharmaceutical factory (Iran hormon) as a gift sample. Tris (2.2′-bipyridyl) ruthenium(II) ($Ru(bpy)_3^{2+}$) chloride hexahydrate was obtained from Sigma and used without further purification. Carbon graphite powder and paraffin oil were from Fluka. All of other reagents were analytical reagent grade. Nafion perfluorinated ion-exchange (5% solution in 90% light alcohol) was obtained from Fluka (Buchs, Switzerland).

2.2 Apparatus

Cyclic voltammetry (CV) was performed using a PalmSens PC potentiostat–galvanostat (Netherlands) with a conventional three-electrode set-up in which a CeO_2 NPs -$Ru(bpy)_3^2$+-CPE, an Ag|AgCl|KCl sat'ed electrode, and a platinum wire served as the working, reference and auxiliary electrodes, respectively. Electrochemical and ECL measurements were carried out in a 4 mL quartz cell. The working electrode was mounted in an equatorial position in a quartz cell, where its surface was exactly in front of the window of a Fl-win lab photomultiplier LS 50 (Perkin-Elmer) and fixed on houlder (see Fig.S1). The detector and ECL cell were enclosed in a

light tight black box. The size and morphology of nanoparticles were measured by scanning electron microscopy (SEM) using a KYKY-EM 3200 Digital Scanning Electron Microscope (China).

2.3 Sensor preparation

General procedure for the preparation of the carbon paste electrode was as follows: prior to use, the graphite powder was treated at 800°C for 60 s in a furnace and then cooled to room temperature in a desiccator in the presence of activated silica gel. 200µL of 2.5×10^{-2} mol/l $Ru(bpy)_3^{2+}$ was dispersed in 500 µL 5wt% nafion solution to obtain $Ru(bpy)_3^{2+}$ - nafion solution. Graphite powder (90mg) and appropriate amount of CeO_2 nanoparticles was homogenized and was mixed thoroughly with 200 µL $Ru(bpy)_3^{2+}$ - nafion solution. This solution was dried at room temperature for 30 min. The modified carbon powder and 50µL paraffin oil were then mixed together by hand-mixing in order to obtain uniformly wetted paste. A portion of modified carbon paste was placed into the end of pyrex tube (5 mm i.d). Electrical contact to the paste was established by inserting copper wire down the tubes and into the back of the mixture. Finally modified CPE was smoothed at fine piece of paper (see Fig.S2).

2.4 Synthesis of cerium oxide nanoparticles

Aqueous cerium nitrate solution was prepared by dissolving cerium oxide powder in an appropriate amount of nitric acid with the aid of ultrasound radiation. This solution was diluted by ethanol and added to polyvinyl alcohol 20 wt% water solution under vigorous stirring in a glass beaker. This mixed solution was heated up to 90°C and stirred for 2 h. By condensation of the hydroxyl network (by giving off water) gelation was achieved and a dense porous gel was

obtained. The gel was dried in drying oven at 110°C and the dried gel was calcinated at 400°C for selected time periods to obtain ceria NPs [37,38].

3 Result and discussion

3.1 Characterization of the NPs

The morphology of the CeO_2 nanoparticles and incorporation of CeO_2 nanoparticles in carbon paste was studied by SEM, as shown in Fig. 1(A,B). As it can be observed, the nanoparticles are spherical in shape and have uniform surface with narrow size distribution.

Fig. 1

3.2 Electrochemical and ECL behaviors CeO_2 NPs -$Ru(bpy)_3^{2}$+-CPE

The catalytic effect of metal and metal oxide NPs such as Pt-NPs [39], Au-NPs [40], Ag-NPs [41], Pd-NPs [42] and ZnO-NPs [43] on chemiluminescence (CL) and ECL reaction has been shown in previous studies. In order to investigate the electrocatalytic effect of the CeO_2 NPs decorated carbon paste on $Ru(bpy)_3^{2+}$ oxidation and possible ECL reaction, a CV study was performed in the potential range between 0.0 and 1.4 V *vs.* Ag|AgCl|KClsat at a scan rate of 100 mV s^{-1} and the output CV and ECL signals were recorded. Fig. 2A presents the cyclic voltammograms of CPE, CeO_2 NPs -$Ru(bpy)_3^{2}$+-CPE in pH 8.5 phosphate buffer at 100 mVs^{-1}. As shown in Fig. 2A (c), the larger charging currents at the CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE is attributed to the increase of the electrode surface area due to the presence of CeO_2 NPs. Beside the effect of increasing the surface area, CeO_2-nanostructured-modified carbon paste electrode can enhance the signal due to

the electrocatalytic activity. The redox behavior of Ce^{4+}/Ce^{3+} in the CeO_2 and Ce_2O_3 can significantly electrocatalysis the oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$.

CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE exhibited a couple of redox peaks at +1.153 V and +0.81 V. A similar enhancement effect was observed in their corresponding ECL signals (Fig.2B). The intensity of the ECL signal of $Ru(bpy)_3^{2+}$ increased 2 fold by modification of the CPE.

Fig. 2.

3.3 Electrocatalytic response of immobilized $Ru(bpy)_3^{2+}$ to oxidation of TMD

TMD contains tertiary amino groups that can be oxidized at an enough positive potential. Fig. 3 (a) shows the cyclic voltammogram of TMD at CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE in 0.1 M pH 8.5 phosphate buffer. One oxidation peak of TMD could be observed at about +0.81 V at 100 mV s^{-1}. The oxidation peak potential was more negative than +1.153 V of immobilized $Ru(bpy)_3^{2+}$ [Fig. 3 (b)]. Thus, it was possible that the oxidation state of $Ru(bpy)_3^{2+}$ oxidized TMD to produce an electrocatalytic response. Fig. 3(c) shows the change of electrochemical response of CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE upon addition of 10^{-8} mol/L TMD into the pH 8.5 phosphate buffer. The anodic peak current increased. It showed an electrocatalytic action of immobilized $Ru(bpy)_3^{2+}$ to the oxidation of TMD. The electrocatalytic process resulted in the formation of an emitting species to produce ECL signal. The ECL spectrum of this system showed an emission peak at nearly 611nm(Fig.4), close to the emission peaks at 610 or 620nm for $Ru(bpy)_3^{2+}$ dissolved in aqueous solution [44], indicating a similar emission process to that reported in the literature [45,46]. In

order to optimize the performance of the proposed ECL sensor, CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE towards tramadol detection, effect of pH, loadings of CeO_2 NPs on the electrode surface, concentration of $Ru(bpy)_3^{2+}$ and scan rate on the intensity of ECL signal was investigated.

Fig. 3.

Fig. 4.

3.4 Effect of pH

The relationship between the ECL intensity and pH value was investigated over a pH range of 6.5 to 11.5. Fig. 5 shows the ECL intensity increased considerably with the rising of pH from 6.5 to 8.5. Contrasting, if the pH value of the buffer solution had been more than 8.5, the ECL intensity of tramadol would have decreased. Thus, phosphate buffer solution of pH 8.5 was chosen for further ECL determination, because the most satisfactory response was found at pH 8.5.

Fig. 5.

3.5 Effect of $Ru(bpy)_3^{2+}$ concentration

Effect of the $Ru(bpy)_3^{2+}$ concentration of in the carbon paste electrode on the intensity of the ECL signal was investigated. The intensity of the ECL signal in the presence of 0.01 μM tramadol increased linearly with an increase in the concentration of $Ru(bpy)_3^{2+}$ from 1×10^{-2} mol/L to 2.5×10^{-2} mol/L in sensor preparation. Further increase in the concentration of $Ru(bpy)_3^{2+}$ (up to 2.5×10^{-2} mol/L) did not cause any further enhancement on the intensity of the ECL signal. However, the background ECL signal of $Ru(bpy)_3^{2+}$ increased with an increase in the

concentration of $Ru(bpy)_3^{2+}$ from 1.0×10^{-2} mol/L to 2.5×10^{-2} mol/L, continuously. Therefore, 2.5×10^{-2} mol/L was selected as the optimum concentration of $Ru(bpy)_3^{2+}$ in CeO_2 NPs - $Ru(bpy)_3^{2+}$- CPE for determining tramadol.

3.6 Effect of scan rate

The effect of scan rate (v) on ECL and CV signals of $Ru(bpy)_3^{2+}$ was investigated in the presence of 0.01 μM tramadol. The results showed with increasing v the intensity of the ECL signal of $Ru(bpy)_3^{2+}$ passed over a maximum at about $100mVs^{-1}$. At the same time, the cyclic voltammograms of CeO_2 NPs -$Ru(bpy)_3^{2}$+-CPE at scan rates between 50 and 350 mVs^{-1} showed (Fig. 6) that the anodic peak current increased linearly with $v^{1/2}$ revealing a diffusion-controlled redox process. A scan rate of $100mVs^{-1}$ was selected for further experiments since maximum ECL sensitivity is achieved for that scan rate.

Fig. 6.

3.7 Interference studies

The influence of some common foreign species on the determination of TMD was studied under the optimum experimental conditions stated above. The tolerable limit of a foreign species was taken as a relative error not greater then ±5% in the ECL signal of TMD. No interference has been found when including up to 1000-fold (Mg^{2+}, K^+, Cl^-)500-fold glucose, 300-fold (L-methionine, Citric Acid, uric acid, Dopamine), 100-fold ascorbic acid.

3.8 Analytical performance

Under the optimum conditions given above, the response to TMD was linear in the range of 1.0×10^{-10} to 2.5×10^{-8} mol/L, with a detection limit of 9.0×10^{-11} mol/L (S/N=3). Fig. 7A shows typical calibration traces recorded for TMD using the proposed ECL sensor. The relative standard deviation was 2.9% for the determination 1.0×10^{-8} mol/L TMD (n=7).

Fig. 7.

The reproducibility of the modified electrode was studied. When the sensor was immersed in a phosphate buffer containing 2.0 $\times10^{-8}$ M TMD, there was no detectable change for the ECL intensity under then repetitive cyclic potential scans, suggesting good reproducibility of the ECL determination of TMD (Fig.8). Also, the stability of the modified electrode was tested by means of a repetitive measurement ECL response. After two months, no evident decrease in the ECL response was observed and the sensor could still maintain 92% of the original response. The results suggested that the modified electrode has a good stability.

Fig. 8.

A comparison between this work and other previous reported methods for TMD determination is listed in Table 1. In comparison with these methods, the proposed sensor has a wider linear range. The detection limit of the proposed sensors is 9.0×10^{-11} mol L^{-1} which is lower than spectrophotometric and fluorescence methods. In term of the linear range and detection limit, it can be seen that the proposed sensor displays even more sensitivity than most of the reported methods [4,13, 14, 16, 47].

Table 1

3.9 Mechanism of enhancement of ECL by TMD

The ECL signal of CeO_2 NPs -$Ru(bpy)_3^{2+}$-CPE in phosphate buffer without the presence of TMD showed only weak background ECL emission, indicating that the ECL emission of the system presumably arose from the energetic electron-transfer reaction between electrogenerated $Ru(bpy)_3^{3+}$ and the reducing intermediate, the deprotonated form of oxidized TMD ion free radical, to produce the excited state $Ru(bpy)_3^{2+*}$, an emitting species. The electrochemical mechanism for the response was presumably analogous to that of the TPA–$Ru(bpy)_3^{2+}$ system[48].

$$Ru(bpy)_3^{2+} - e \rightarrow Ru(bpy)_3^{3+}$$

$$Tramadol - e \rightarrow Tramadol^{\cdot +}$$

$$Tramadol^{\cdot +} \rightarrow Tramadol^{\cdot} + H^{+}$$

$$Tramadol^{\cdot} + Ru(bpy)_3^{3+} \rightarrow Tramadol\ fragments + Ru(bpy)_3^{2+*}$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\nu \quad (\lambda_{max}=610nm)$$

3.9 Analytical applications

The applicability of a proposed sensor for the determination of tramadol in human serum, urine and drug samples was examined. ECL intensity were obtained by spiking prepared real solutions with appropriate samples and using CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE at optimum conditions as described earlier. Concentrations were measured by applying the calibration plot. The results are

shown in Table 2. The recoveries indicate that both the accuracy and repeatability of proposed sensor are very satisfactory. As experimental results, it is very clear that this method has great potential for the determination of trace amounts of this compound in biological samples and pharmaceutical preparations.

Table 2

4 Conclusion

Immobilization of $Ru(bpy)_3^{2+}$ and CeO_2 NPs in the carbon paste is successfully achieved for preparation of a novel ECL sensor. The immobilized $Ru(bpy)_3^{2+}$ shows a diffusion electrode process and has an electrocatalytic action to the oxidation of tramadol, which results in the formation of an emitting species to produce ECL signal for sensitive determination of tramadol. The method presented has a good reproducibility and is sensitive enough for detection of tramadol in real samples.

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Figures Legend

Fig. 1. SEM image of CeO_2 NPs (A), incorporation of CeO_2 NPs in carbon paste (B)

Fig. 2. (A) cyclic voltammograms CPE (a), CPE + $Ru(bpy)_3^{2+}$ (b), CeO_2 NPs -$Ru(bpy)_3^{2+}$-CPE(c) and (B) ECL responses of CPE + $Ru(bpy)_3^{2+}$ (a), CeO_2 NPs -$Ru(bpy)_3^{2+}$-CPE (b) supporting electrolyte buffer solution (0.1M and pH 7.4); potential scan rate, 100 mVs^{-1}

Fig. 3. Cyclic voltammograms of Nafion-CPE in 0.1 M pH 8.5 phosphate buffer in the absence (a) and presence (b) of 10 ng TMD ,(c) CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE + 10 ng TMD in 0.1 M pH 8.5

Fig. 4. ECL spectrum of CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE in 0.1 M pH 8.5 phosphate buffer containing 40 μM TMD, Inset, Visual ECL of CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE .

Fig. 5. Effect of pH on the ECL Intensity

Fig. 6. Cyclic voltammograms of CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE in).1 M pH 8.5 phosphate buffer at 50, 100, 150, 200, 250 , 300 and 350 mVs^{-1}. Inset: plots of peak currents *vs*. scan rate.

Fig. 7. (A) ECL responses of nanoCe_2O -$Ru(bpy)_3^{2+}$- CPE at in the presence of 0.1n M, 5 nM, 10 nM, 15 nM, 20 nM, and 25 nM of TMD, Inset shows linear relationship between the ECL intensity and the concentration of TMD.

Fig.8. Stability of ECL signal from modified carbon paste electrode in 0.1 M pH 8.5 phosphate buffer solution, containing 2.0×10^{-9} M TMD under ten continuous cycles of CV scan.

Table 1

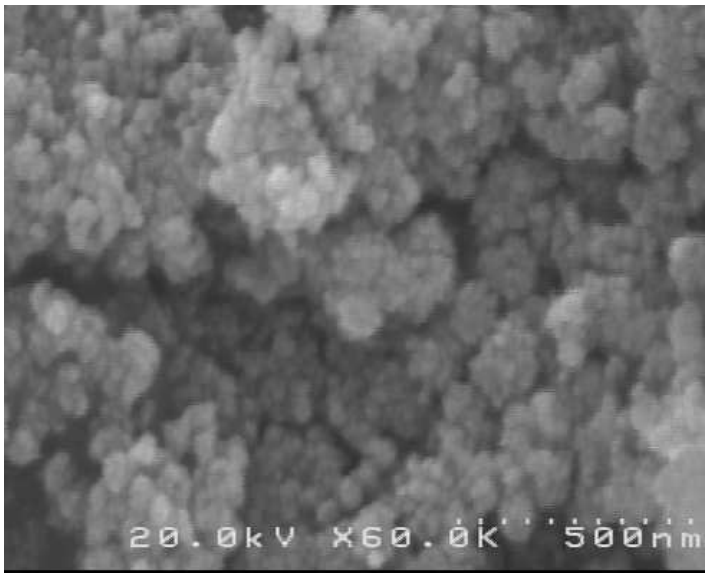
Comparison of the characteristics of the proposed sensor with those of the previously reported for tramadol.

method	Detection Limit (M)	Dynamic Range (M)	Ref.
HPLC-UV detector	3.8×10^{-8}	7.6×10^{-6}-3.8×10^{-8}	13
HPLC-Electrochemical detector	3.8×10^{-8}	7.6×10^{-6}-3.8×10^{-8}	14
HPLC- Fluorescence detector	9.5×10^{-9}	7.6×10^{-9}-1.9×10^{-6}	16
Potentiometry	1.8×10^{-6}	9.2×10^{-6}-1.0×10^{-1}	43
Molecularly imprinted solid-phase extraction	1.1×10^{-8}	-	4
Electrochemiluminescence	9.0×10^{-11}	2.5×10^{-8}-1.0×10^{-10}	This work

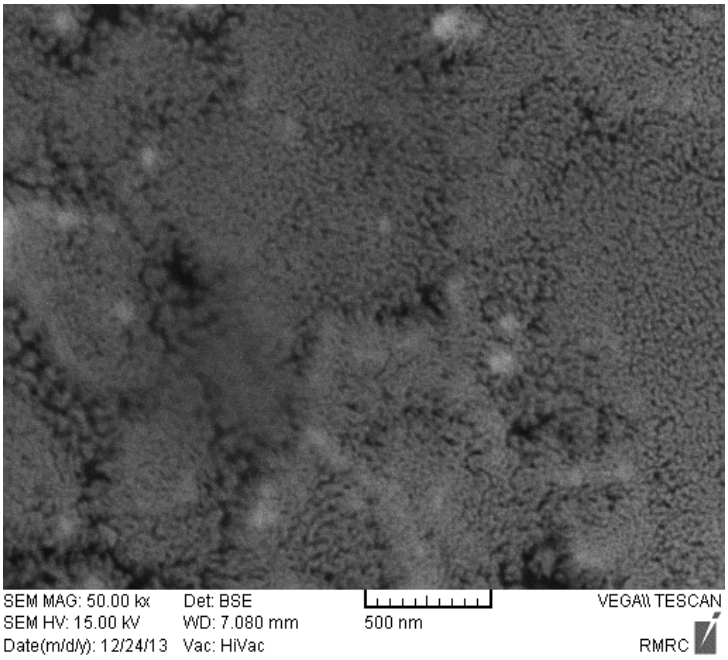
Table 2.

ECL determination results and recoveries of TMD samples using a CeO_2 NPs -$Ru(bpy)_3^{2+}$- CPE modified

Sample	Added ($molL^{-1}$)	Found [a] ($molL^{-1}$)	Recovery(%)
Human Serum	4.0×10^{-9}	$4.1\ (\pm0.2)\times10^{-9}$	102.5
	6.0×10^{-9}	$6.1(\pm\ 0.2)\times10^{-9}$	101.6
Urine	3.5×10^{-9}	$3.4\pm(\ 0.1)\times10^{-9}$	97.1
	5.3×10^{-9}	$5.5(\pm\ 0.2)\times10^{-9}$	103.7
Tablet	7.0×10^{-9}	$6.9\pm(\ 0.1)\times10^{-9}$	98.5
	5.3×10^{-9}	$5.5(\pm\ 0.2)\times10^{-9}$	103.7



A



B

Fig. 1

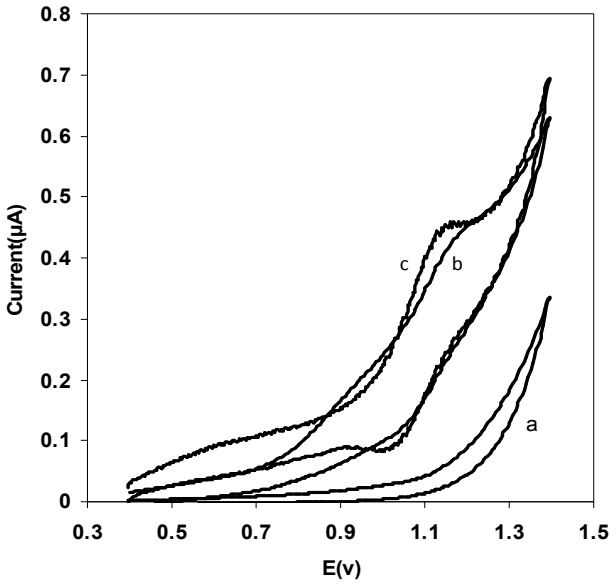


Fig. 2. (A)

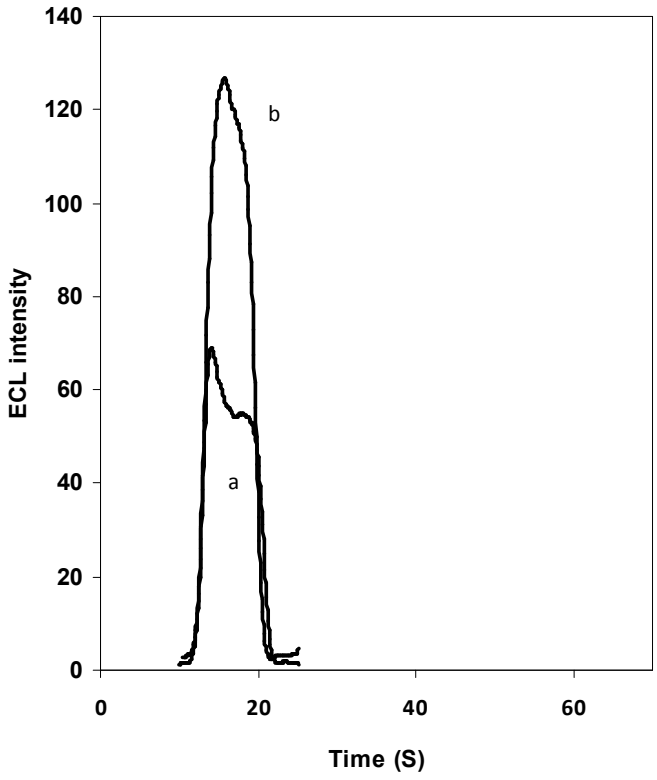


Fig. 2. (B)

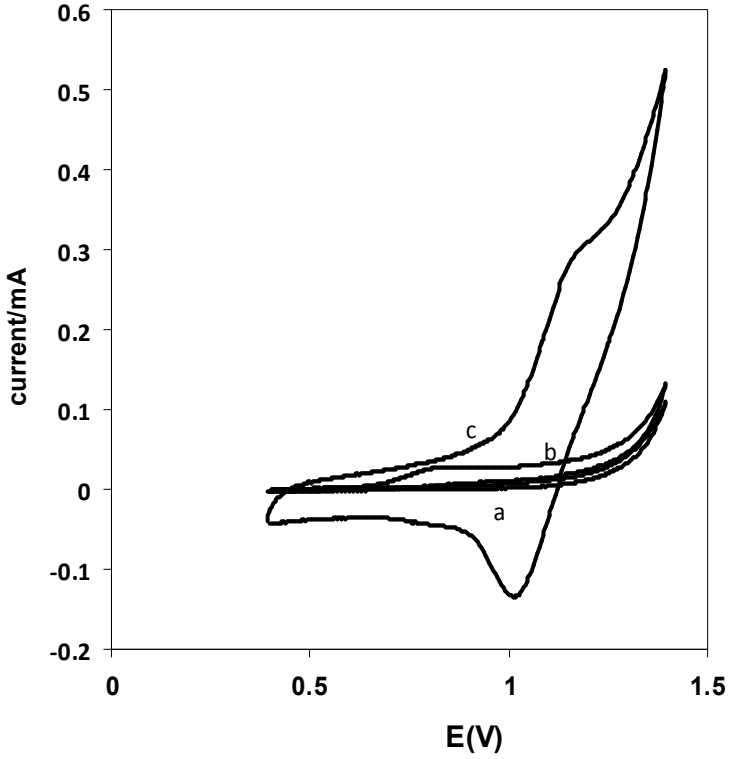


Fig. 3.

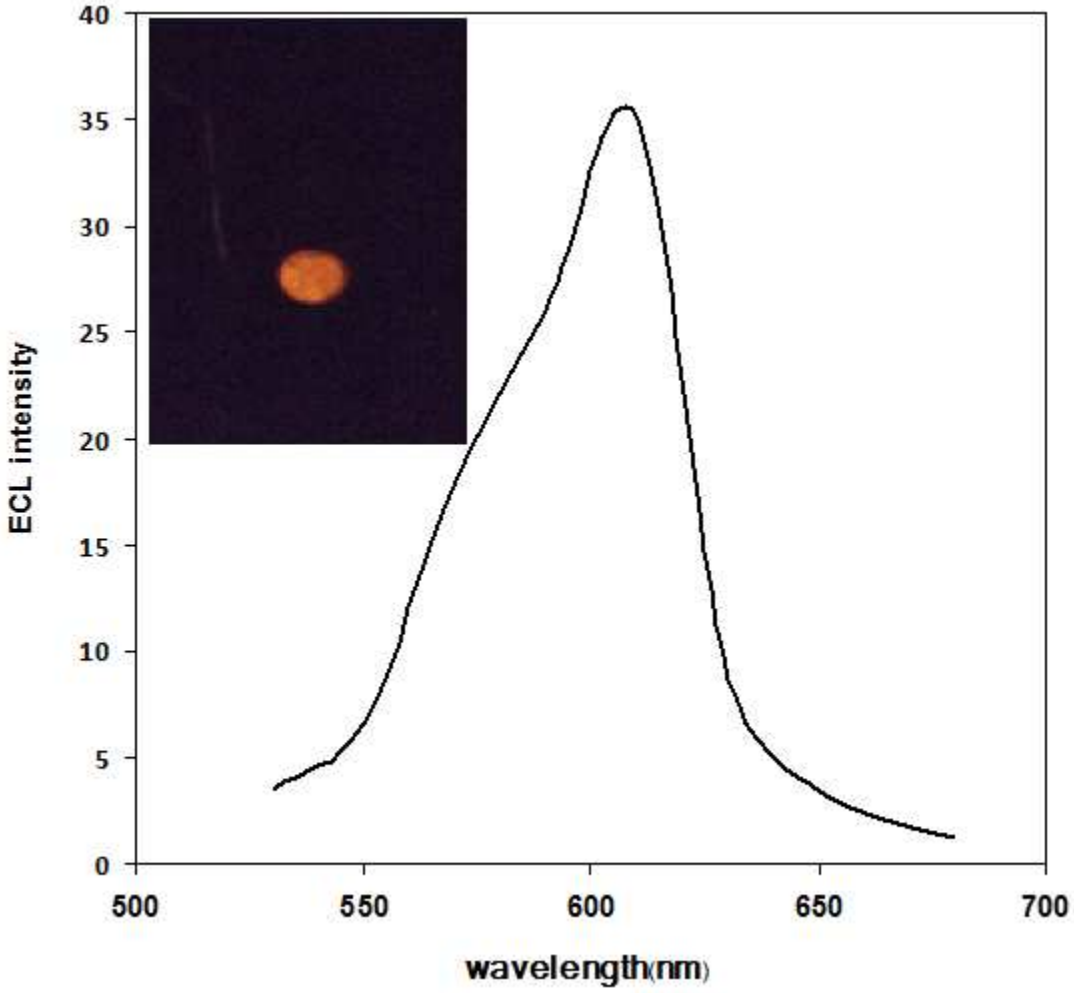


Fig. 4.

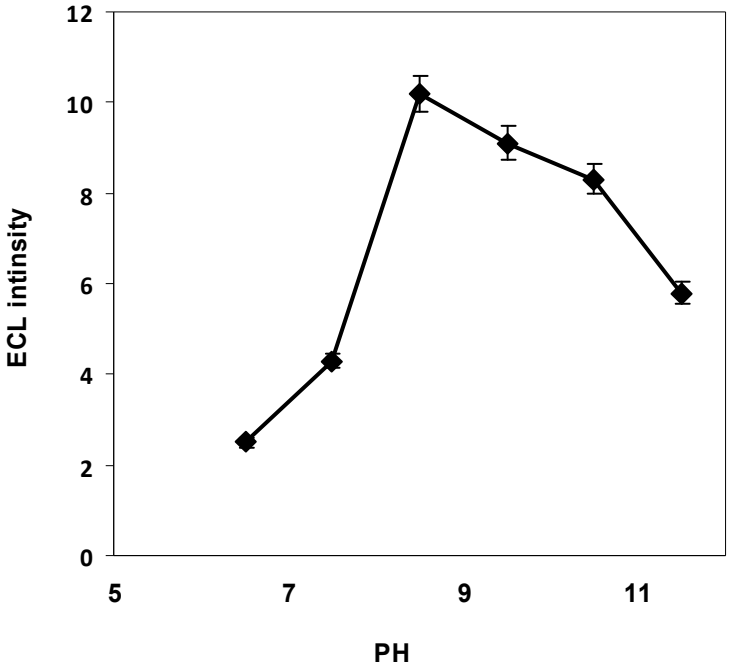


Fig. 5.

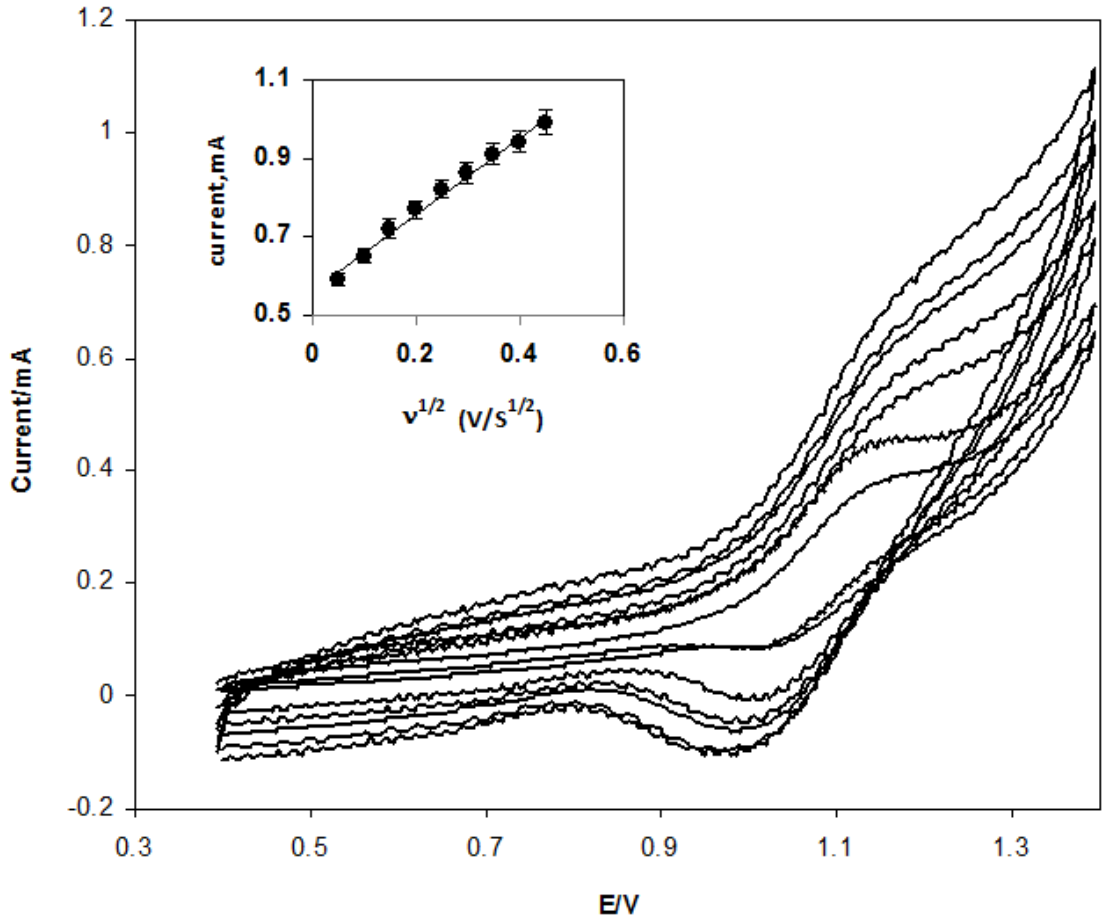


Fig. 6.

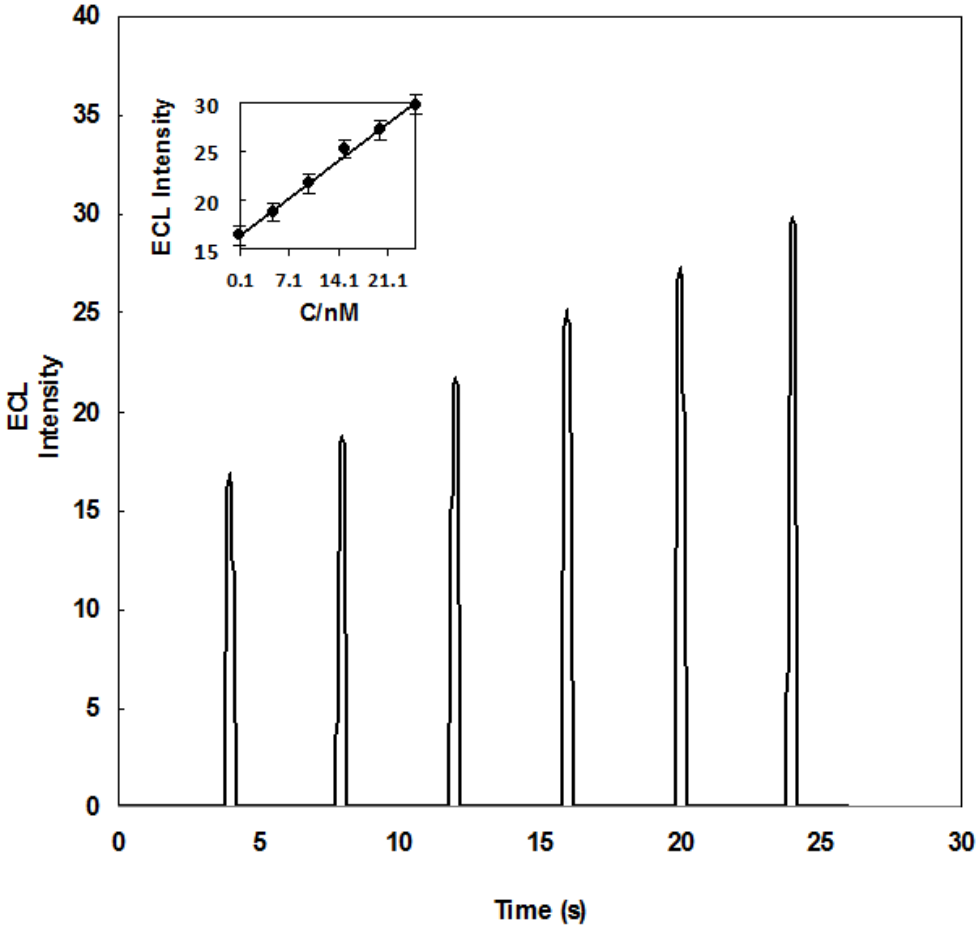


Fig. 7.

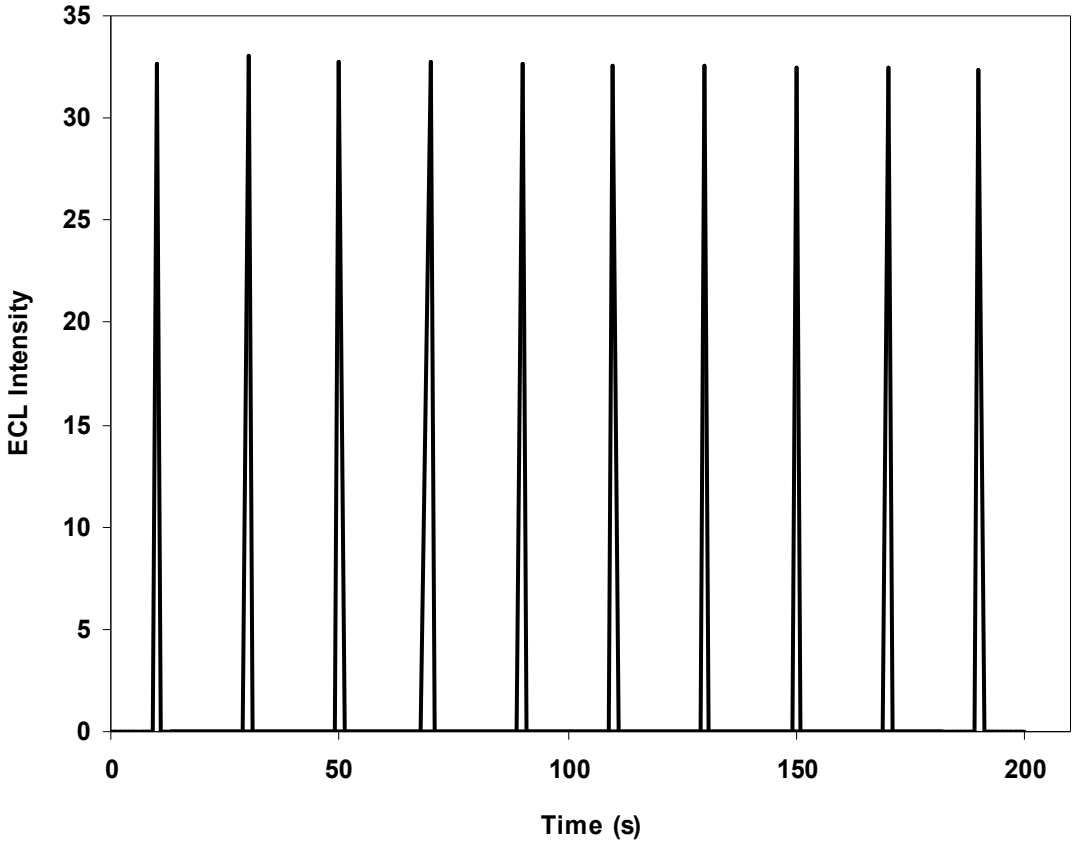


Fig.8