Voltammetric Determination of Droxidopa in the Presence of Tryptophan Using a Nanostructured Base Electrochemical Sensor

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ABSTRACT

A novel carbon paste electrode modified with Cu-TiO₂ nanocomposite, 2-(ferrocenylethynyl)fluoren-9-one (2FF) and ionic liquid (IL) (2FF/Cu-TiO₂/IL/CPE) was fabricated and employed to study the electrocatalytic oxidation of droxidopa, using cyclic voltammetry (CV), chronoamperometry (CHA) and differential pulse voltammetry (DPV) as diagnostic techniques. It has been found that the oxidation of droxidopa at the surface of modified electrode occurs at a potential of about 295 mV less positive than that of an unmodified CPE. DPV exhibits a linear dynamic range from 5.0×10^{-8} to 4.0×10^{-4} M and a detection limit of 30.0 nM for droxidopa. Finally this modified electrode was used for simultaneous determination of droxidopa and tryptophan. Also the 2FF/Cu-TiO₂/IL/CPE shows excellent ability to determination of droxidopa and tryptophan in real samples.

Keywords : Droxidopa, Tryptophan, Ionic liquid, Carbon paste electrode, Cu-TiO₂ nanocomposite, 2-(ferrocenylethynyl)fluoren-9-one

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1. Introduction

Droxidopa (L-threo-3,4-dihydroxyphenylserine) (Scheme 1) is an orally administered synthetic precursor amino acid converted both peripherally and centrally into norepinephrine by the ubiquitous enzyme, dopa-decarboxylase [1]. Droxidopa is the ideal agent to treat orthostatic hypotension due to its direct conversion to norepinephrine, bypassing the rate-limiting hydroxylation step of the normal formation of norepinephrine from dopamine [2]. It is clinically used in Japan mainly for the treatment of

neurogenic orthostatic hypotension (nOH), frozen gait, or dizziness associated with Parkinson disease (PD) since 1989. In February 2014, droxidopa received accelerated Food and Drug Administration (FDA) approval for the treatment of symptomatic nOH [3]. Although droxidopa is widely used in clinical practice, the report method concerning the quantitation of droxidopa in human plasma is scanty [3-5]. The most widely used method is a high performance liquid chromatography (HPLC), but this method needs a complex alumina extraction process and consumed large volume of biological samples [6].

Tryptophan (TRP) (Scheme 1) is an essential amino acid with various physiological roles [7]. Tryptophan is commonly synthesized in plants and microorganisms from shikimic acid [8]. The

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Scheme 1. Chemical structure of (A) droxidopa and (B) Tryptophan.

improper metabolite of tryptophan leads to abnormal levels of melatonin and serotonin. The brain's serotonin level depends on the tryptophan level in our body. The improper metabolite of tryptophan causes hallucination, delusions, depression, and Alzheimer's and Parkinson's diseases [9-12].

Therefore, simultaneous determination of droxidopa and tryptophan is very important in blood and urine sample in Parkinson's disease. Hence, a simple, fast, selective, sensitive and accurate method for the determination of these drugs is very important. Electroanalytical methods have been more attractive in recent years because they are fast, simple, power efficient, inexpensive, accurate and highly sensitive [13-18]. Also, efforts have been made to modify the electrode surfaces for the purpose of lowering the overpotential, improving the sensitivity with high current density for effective enrichment of the desired substance and/or restraining the effect of interferences [19-25].

Among many modifiers, transition metals oxides often have semiconductor properties, such properties can improve by decreasing the size of the crystals. So it is clear that nanoparticles of these oxides exhibit better function. Nano metal hydroxides/oxides have outstanding properties such as high surface area and enhanced chemical/electrochemical activities. In between them, TiO_2 has exceptional properties, such as high conductivity, high inertness, stability, non-toxicity, bio-compatibility, low cost, etc [26]. TiO_2 may be used with different shapes such as nanoparticles, nanotubes, and nanoneedles in the electrochemical sensors and biosensors. The catalytic activity of TiO_2 is improved if Pt, Pd, Au or Ag are used as dopants, but these metals are rare and expensive. Addition of copper can, in some cases, improve the catalytic activity, but the mechanism responsible for the improvement is not yet explained. Novel Cu-modified TiO_2 nanostructured have promising catalytic properties, they can be cheaper and more efficient [27-29].

Ionic liquids (ILs), which are compounds that consist only of ions, are liquids at around room temperature.

Scheme 2. Chemical structure of 2-(ferrocenylethynyl)fluoren-9-one.

They have great potential as the green reaction media due to the advantages such as no measurable vapor pressure, good thermal and chemical stability, high conductivity, and low toxicity [30]. Very recently, ionic liquids have been proposed to be very interesting and efficient pasting binder in place of non conductive organic binders for the preparation of carbon ionic liquid electrodes (CILEs). These types of electrodes show some advantages over traditional carbon paste electrodes (CPEs) such as high conductivity, provision of fast electron transfer, and antifouling properties [31,32].

Also Ferrocene (Fc) and ferrocene-based derivatives have been investigated due to their use as color pigments and as high burning rate catalysts. Therefore they are widely used as modified materials in analytical chemistry, particularly as electron-transfer mediators in electrochemical biosensors [33].

To the best of our knowledge, most previously published electrochemical studies have dealt with individual determination of droxidopa or tryptophan utilizing carbon paste electrodes or other kinds of modified electrode. Therefore, in continuation of our recently studies concerning the preparation of modified electrodes, in the present work, we describe the preparation of a new carbon paste electrode composed of Cu- TiO_2 nanocomposite, 2-(ferrocenylethynyl)fluoren-9-one (2FF) (Scheme 2) and ionic liquid (IL) and investigate its performance for the electrocatalytic determination of droxidopa in aqueous solutions. We also evaluate the analytical performance of the modified electrode for quantification of droxidopa in the presence of tryptophan.

2. Experimental

2.1 Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat

(PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at $25 \pm 1^\circ\text{C}$. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and 2FF/Cu-TiO₂/IL/CPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. Droxidopa, tryptophan and all of the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

2.2 Synthesis of Cu-TiO₂ nanocomposite

TiO₂ and Cu doped TiO₂ nano powder was prepared by the controlled sol-gel method with titanium(IV) n-butoxide Ti(OCH₂CH₂CH₂CH₃)₄ as raw materials. All the chemicals were purchased from Merck (Darmstadt, Germany) and were used without any further purification. Deionized water obtained with a Milli-Q purification system (Millipore, Bedford, MA, USA), and filtered through 0.45 µm Millipore solvent filter, was used throughout. The dopant starting material was metallic copper sulfate. In a typical process, 5 mL of Ti(OBu)₄ was dissolved in 15 mL of absolute ethanol in a dry atmosphere and ultrasonically dispersed to produce a mixture (solution A). Meanwhile, 5 mL of water and 1 mL of HNO₃ (65%) were added to another 20 mL of absolute ethanol in turn to form an alcohol/acid/water solution (solution B). After the two resulting solutions were stirred, respectively, the solution A was slowly added drop wise to the solution B under vigorously stirring to carry out a hydrolysis. Subsequently, the roughly stirring was conducted so that the temperature was raised from room temperature to 80°C at the end of the reaction. The gel was dried in the air for about 24 h at 85°C and subsequently the resulting material was powdered and then calcined in an electric muffle furnace at 450°C for 2 h to obtain crystalline powders of TiO₂. Cu doped TiO₂ nanoparticles were synthesized using almost the same method. The molar amount of transition metal ion dopant (Cu²⁺) was calculated in order to substitute 1% of titanium ions in TiO₂ and was solubilized in an appropriate amount of ethanol/nitric acid/water solution prior to the

Fig. 1. SEM image of synthesized Cu-TiO₂ nanocomposite.

hydrolysis. The remaining procedures were the same as described above. After hydrolysis, the greenish transparent sol was obtained. A typical SEM image of the synthesized Cu-TiO₂ nanocomposite is shown in Fig. 1.

2.3 Synthesis of 2-(ferrocenylethynyl)fluoren-9-one

PdCl₂(PPh₃)₂ (29 mg) and CuI (7.6 mg) were added to a stirred solution of aryl halides (1.0 mmol, 0.26 g) and ethynylferrocene (1.2 mmol, 0.25 g) in Et₃N (5 mL) and DMF (5 mL) and the resulting mixture was stirred at reflux temperature for 3 h. The progress of the reaction was monitored by TLC. After addition of 200 mL of H₂O and stirring for 30 min, the resulting crude product was filtered. The resulting raw material was dissolved in CH₂Cl₂, then washed with diluted HCl and saturated NaCl solution. After drying with MgSO₄ and solvents removal, the crude products was purified by column chromatography on silica gel using hexane-CH₂Cl₂ as eluent to afford the pure product in 87% yield.

Red solid; mp: 147-148°C; IR: ν (cm⁻¹) = 750, 1602, 1712 (C=O), 2201 (C≡C); ¹H NMR (500 MHz, CDCl₃): δ (ppm) = 4.20-4.30 (pseudo s, 7H), 4.54 (s, 2H), 7.30-7.76 (m, 7H); ¹³C NMR (125 MHz, CDCl₃): δ (ppm) = 64.62, 69.04, 70.00, 71.48, 85.03, 90.51, 120.20, 120.45, 124.36, 124.88, 127.02, 129.12, 134.16, 134.27, 134.81, 137.19, 142.94, 144.07, 193.11; Anal. Found: C, 76.98; H, 3.96. Calc. for C₂₅H₁₆FeO: C, 77.34; H, 4.15%.

2.4 Preparation of the electrode

The Cu/TiO₂-IL-2FF/CPEs were prepared by hand mixing 0.04 g of Cu/TiO₂ nanocomposite, 0.01 g of 2FF with 0.95 g graphite powder and 0.2 mL ionic

liquid with a mortar and pestle. Then, ~ 0.7 mL of paraffin oil was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, unmodified CPE in the absence of Cu/TiO₂ nanocomposite, 2FF and ionic liquid were also prepared in the same way.

2.5 Procedure of real samples preparation

Urine samples were stored in a refrigerator immediately after collection. Ten millilitres of the samples were centrifuged for 15 min at 2,000 rpm. The supernatant was filtered out by using a 0.45 μm filter. Next, different volumes of the solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine samples were spiked with different amounts of droxidopa and tyrosine. The droxidopa and Tryptophan contents were analysed by the proposed method by using the standard addition method.

The serum sample was centrifuged, and after filtering, diluted with PBS (pH 7.0) without any further treatment. The diluted serum sample was spiked with different amounts of droxidopa and Tryptophan. The droxidopa and tyrosine contents were analysed by the proposed method by using the standard addition method.

3. Results and Discussion

3.1 Electrochemical properties of 2FF/Cu-TiO₂/IL/CPE

2FF/Cu-TiO₂/IL/CPE was prepared and its electrochemical properties were studied in a PBS (pH 7.0) using CV. It should be noted that one of the advantages of 2FF as an electrode modifier is its insolubility in aqueous media. Experimental results showed reproducible and well-defined CVS. Anodic and cathodic peak potentials were 0.35 and 0.25 V vs. Ag/AgCl/KCl (3.0 M) respectively. The observed peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 100 mV, was greater than the value of 59/n mV which is expected for a reversible system [34], suggesting that the redox couple of 2FF in 2FF/Cu-TiO₂/IL/CPE has a quasi-reversible behavior in

aqueous medium.

In addition, the long-term stability of the 2FF/Cu-TiO₂/IL/CPE was tested over a three-week period. When CVs were recorded after the modified electrode was stored in atmosphere at an ambient temperature, the peak potential for droxidopa oxidation was unchanged and the current signals showed less than 2.6% decrease relative to the initial response. The antifouling properties of the modified electrode toward droxidopa and its oxidation products were investigated by recording the CVs of the modified electrode before and after use in the presence of droxidopa. CVs were recorded in the presence of droxidopa after having cycled the potential 15 times at a scan rate of 10 mV s⁻¹. The peak potentials were unchanged and the currents decreased by less than 2.3%. Therefore, at the surface of 2FF/Cu-TiO₂/IL/CPE, not only the sensitivity increases, but the fouling effect of the analyte and its oxidation product also decreases.

3.2 Electrocatalytic oxidation of droxidopa at a 2FF/Cu-TiO₂/IL/CPE

The electrochemical behavior of droxidopa is dependent on the pH value of the aqueous solution,

Fig. 2. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0); (b) unmodified CPE in 0.1 M PBS (pH 7.0) containing 0.3 mM droxidopa; (c) 2FF/Cu-TiO₂/IL/CPE in 0.1 M PBS (pH 7.0); (d) Cu-TiO₂/CPE in 0.1 M PBS (pH 7.0) containing 0.3 mM droxidopa; (e) 2FF/IL/CPE in 0.1 M PBS (pH 7.0) containing 0.3 mM droxidopa and (f) 2FF/Cu-TiO₂/IL/CPE in 0.1 M PBS (pH 7.0) containing 0.3 mM droxidopa. In all cases the scan rate was 10 mV s⁻¹.

whereas the electrochemical properties of Fc/Fc^+ redox couple are independent on pH. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of droxidopa. Thus the electrochemical behavior of droxidopa was studied in 0.1 M PBS in different pH values (2.0-9.0) at the surface of 2FF/Cu-TiO₂/IL/CPE by voltammetry. It was found that the electrocatalytic oxidation of droxidopa at the surface of 2FF/Cu-TiO₂/IL/CPE was more favored under neutral conditions than in acidic or basic medium. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the cyclic voltammograms of 2FF/Cu-TiO₂/IL/CPE. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of droxidopa oxidation at the surface of 2FF/Cu-TiO₂/IL/CPE.

Fig. 2 depicts the CV responses for the electrochemical oxidation of 300.0 μM droxidopa at an unmodified CPE (curve b), Cu-TiO₂/CPE (curve d), 2FF/IL/CPE (curve e) and 2FF/Cu-TiO₂/IL/CPE

(curve f). As it is seen, while the anodic peak potential for droxidopa oxidation at the Cu-TiO₂/CPE, and unmodified CPE are 600 and 645 mV, respectively, the corresponding potential at 2FF/Cu-TiO₂/IL/CPE and 2FF/IL/CPE is about 350 mV. These results indicate that the peak potential for droxidopa oxidation at the 2FF/Cu-TiO₂/IL/CPE and 2FF/IL/CPE electrodes shift by ~ 250 and 295 mV toward negative values compared to Cu-TiO₂/CPE and unmodified CPE, respectively. However, 2FF/Cu-TiO₂/IL/CPE shows much higher anodic peak current for the oxidation of droxidopa compared to 2FF/IL/CPE, indicating that the combination of Cu-TiO₂ nanocomposite and the mediator (2FF) has significantly improved the performance of the electrode toward droxidopa oxidation. In fact, in the absence of droxidopa 2FF/Cu-TiO₂/IL/CPE exhibited a well-behaved redox reaction (Fig. 2, curve c) in 0.1 M PBS (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of 300.0 μM droxidopa (curve f), which can be related to the strong electro-

Scheme 3. Electrocatalytic oxidation of droxidopa and electrochemical oxidation of tryptophan at 2FF/Cu-TiO₂/IL/CPE.

Fig. 3. LSVs for oxidation of 200.0 μM droxidopa at the surface of 2FF/Cu-TiO₂/IL/CPE at different scan rates of 5, 10, 25, 50, 75, 100, 300 and 500 mV s^{-1} . Insets: Variation of (A) anodic peak current versus the square root of scan rate. (B) Variation of the scan rate normalized current ($I_p/v^{1/2}$) with scan rate.

catalytic effect of the 2FF/Cu-TiO₂/IL/CPE towards this compound [34]. According to these results, we suggest an EC' catalytic mechanism [34] for the electrochemical oxidation of droxidopa at 2FF/Cu-TiO₂/IL/CPE, as shown in Scheme 3. It has been proposed that in a catalytic reaction (C), droxidopa is oxidized by the oxidized form of 2FF which is produced during an electrochemical reaction (E) at the electrode surface.

The effect of potential scan rate on the electrocatalytic oxidation of droxidopa at the 2FF/Cu-TiO₂/IL/CPE was investigated by linear sweep voltammetry (LSV) (Fig. 3). As can be observed in Fig. 3, the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) vs. the square root of scan rate ($v^{1/2}$) was found to be linear in the range of 5–500 mV s^{-1} , suggesting that, at sufficient over potential, the process is diffusion rather than surface controlled (Fig. 3A). A plot of the scan rate-normalized current ($I_p/v^{1/2}$) vs. scan rate (Fig. 3B) exhibits the characteristic shape typi-

Fig. 4. LSV (at 5 mV s^{-1}) of 2FF/Cu-TiO₂/IL/CPE in 0.1 M PBS (pH 7.0) containing 200.0 μM droxidopa. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.

cal of an EC' process [34].

Fig. 4 shows the Tafel plot for the sharp rising part of the voltammogram at the scan rate of 5 mV s^{-1} . If deprotonation of droxidopa is a sufficiently fast step, the Tafel plot can be used to estimate the number of electrons involved in the rate determining step. A Tafel slope of 0.096 V was obtained which agrees well with the involvement of one electron in the rate determining step of the electrode process [34], assuming a charge transfer coefficient, α of 0.38.

3.3 Chronoamperometric measurements

Chronoamperometric measurements of droxidopa at 2FF/Cu-TiO₂/IL/CPE were carried out by setting the working electrode potential at 0.4 V for the various concentrations of droxidopa in PBS (pH 7.0) (Fig. 5). For an electroactive material (droxidopa in this case) with a diffusion coefficient of D , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [34]. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of droxidopa (Fig. 5A). The slopes of the resulting straight lines were then plotted vs. droxidopa concentration (Fig. 5B). From the resulting slope and Cottrell equation the mean value of the D

Fig. 5. Chronoamperograms obtained at 2FF/Cu-TiO₂/IL/CPE in 0.1 M PBS (pH 7.0) for different concentrations of droxidopa. The numbers 1-5 correspond to 0.1, 0.5, 1.0, 1.5 and 2.0 mM of droxidopa. Insets: (A) Plots of I vs. $t^{1/2}$ obtained from chronoamperograms 2-5. (B) Plot of the slope of the straight lines against droxidopa concentrations.

Fig. 6. DPVs of 2FF/Cu-TiO₂/IL/CPE in 0.1 M (pH 7.0) containing different concentrations of droxidopa. Numbers 1-12 correspond to 0.05, 0.3, 1.0, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0, 200.0, 300.0 and 400.0 μM of droxidopa. Inset: Plot of the electrocatalytic peak current as a function of droxidopa concentration in the range of 0.05–400.0 μM .

was found to be $1.5 \times 10^{-6} \text{ cm}^2/\text{s}$.

3.4 Calibration plot and limit of detection

The electrocatalytic peak current of droxidopa oxidation at the surface of the modified electrode can be used for determination of droxidopa in solution. Therefore, DPV experiments were performed using modified electrode in PBS (pH 7.0) containing various concentration of droxidopa (Fig. 6). The mediated oxidation peak currents of droxidopa at the surface of a modified electrode were proportional to the concentration of the droxidopa within the ranges 5.0×10^{-8} to 4.0×10^{-4} M with slope of $0.0548 \mu\text{A } \mu\text{M}^{-1}$ in the DPV. The detection limit (3σ) was 30.0 nM.

3.5 Simultaneous determination of droxidopa and tryptophan

To our knowledge, no paper has used the modified Cu-TiO₂ nanocomposite electrode and specially modified Cu-TiO₂ nanocomposite carbon paste electrode for simultaneous determination of droxidopa and tryptophan and this is the first report for simultaneous determination of droxidopa and tryptophan using modified Cu-TiO₂ nanocomposite carbon paste

Fig. 7. DPVs of 2FF/Cu-TiO₂/IL/CPE in 0.1 M PBS (pH 7.0) containing different concentrations of droxidopa and tryptophan in μM , from inner to outer: 10.0+10.0, 75.0+75.0, 120.0+175.0, 170.0+300.0, 250.0+400.0, 300.0+500.0, 325.0+750.0, and 400.0+1000.0 respectively. Insets (A) plot of I_p vs. droxidopa concentration and (B) plot of I_p vs. tryptophan concentrations.

electrode. The electrochemical determination of droxidopa using bare electrodes suffers from interference by tryptophan, because the oxidation potential for tryptophan is fairly close to that of droxidopa.

Determination of two compounds was performed by simultaneously changing the concentrations of droxidopa and tryptophan, and recording the DPVs (Fig. 7). The voltammetric results showed well-defined anodic peaks at potentials of 315 and 715 mV, corresponding to the oxidation of droxidopa and tryptophan, respectively, indicating that simultaneous determination of these compounds is feasible at the 2FF/Cu-TiO₂/IL/CPE as shown in Fig. 7.

The sensitivity of the modified electrode towards the oxidation of droxidopa was found to be 0.055 $\mu\text{A } \mu\text{M}^{-1}$. This is very close to the value obtained in the absence of tryptophan (0.0548 $\mu\text{A } \mu\text{M}^{-1}$, see Section 3.4), indicating that the oxidation processes of these compounds at the 2FF/Cu-TiO₂/IL/CPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

3.6 Interference studies

The influence of various substances as compounds potentially interfering with the determination of droxidopa was studied under optimum conditions. The potentially interfering substances were chosen from the group of substances commonly found with droxidopa in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maxi-

mum concentration of the interfering substance that caused an error of less than $\pm 5\%$ in the determination of droxidopa. According to the results, L-lysine, glucose, NADH, acetaminophen, uric acid, L-asparagine, L-serine, L-threonine, L-proline, histidine, glycine, tryptophan, phenylalanine, lactose, saccharose, fructose, benzoic acid, methanol, ethanol, urea, Mg^{2+} , Al^{3+} , NH_4^+ , F^- , SO_4^{2-} and S^{2-} did not show interference in the determination of droxidopa.

3.7 Determination of droxidopa and tryptophan in human blood serum and urine samples

In order to evaluate the analytical applicability of the proposed method, also this method was applied to the determination of droxidopa and tryptophan in human blood serum and urine samples. The samples were found to be free from droxidopa and tryptophan. Therefore different amounts of droxidopa and tryptophan were spiked to the sample and analyzed by the proposed method. The results for determination of the two species in real samples are given in Table 1. Satisfactory recovery of the experimental results was found for droxidopa and tryptophan. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

4. Conclusions

The construction of a chemically modified carbon paste electrode by the incorporation of Cu-TiO₂ nanocomposite, 2FF and ionic liquid as modifying

Table 1. Determination of droxidopa and tryptophan in human blood serum and urine samples. All the concentrations are in μM (n=5).

Sample	Spiked		Found		Recovery (%)		R.S.D. (%)	
	Droxidopa	Tryptophan	Droxidopa	Tryptophan	Droxidopa	Tryptophan	Droxidopa	Tryptophan
Human blood serum	0	0	-	-	-	-	-	-
	7.5	10.0	7.4	10.3	98.7	103.0	3.3	1.9
	12.5	20.0	12.6	19.5	100.8	97.5	2.9	3.5
	17.5	30.0	17.4	30.3	99.4	101.0	1.8	2.2
	22.5	40.0	23.3	39.8	103.6	99.5	2.3	2.9
Urine	0	0	-	-	-	-	-	-
	10.0	12.0	10.1	11.7	101.0	97.5	1.9	2.5
	15.0	17.0	14.8	17.5	98.7	102.9	2.4	1.7
	20.0	22.0	20.6	21.8	103.0	99.1	3.5	2.2
	25.0	27.0	24.8	27.9	99.2	103.3	2.2	3.1

species is reported in this work. The electrochemical behavior of the droxidopa was studied using CV. The results showed that the oxidation of droxidopa is catalyzed at pH 7.0, with the peak potential of droxidopa shifted by 295 mV to a less positive potential at the surface of the modified electrode. A potential difference of 400 mV between droxidopa and tryptophan was detected, which was large enough to determine droxidopa and tryptophan individually and simultaneously. Finally, the modified electrode was used for the determination of droxidopa and tryptophan in some real samples.

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