Anal. Bioanal. Electrochem., Vol. 10, No. 7, 2018, 873-889

Analytical & Bioanalytical Electrochemistry

2018 by CEE
www.abechem.com

Full Paper

Synergic Effect of Cu/TiO_2 Nanocomposite, 2-(ferrocenylethynyl)fluoren-9-one and Room Temperature Ionic Liquid for the Fabrication of Highly Sensitive Voltammetric Sensor for Levodopa Determination in the Presence of Tyrosine

Sedighe Akbari,[1,*] Somayeh Tajik,[2,*] Hadi Beitollahi,[3] Shohreh Jahani,[2] Hadi Hassani Nadiki,[4] Pouria Biparva[5] and Rahman Hosseinzadeh[6]

[1]*Islamic Azad University, Shahrbabak Branch, Shahrbabak, Iran*

[2]*NanoBioElectrochemistry Center, Bam University of Medical Sciences, Bam, Iran*

[3]*Environment Department, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran*

[4]*Islamic Azad University, Kerman Branch, Kerman, Iran*

[5]*Department of Basic Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran*

[6]*Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran*

*Corresponding Author, Tel.: +98 3426226613; Fax: +98 3426226617

E-Mails: s.akbari1389@yahoo.com; Tajik_s1365@yahoo.com

Received: 18 October 2017 / Received in revised form: 11 November 2017 / Accepted: 23 November 2017 / Published online: 31 July 2018

Abstract- Levodopa, one of the prescribed medications in Parkinson disease, is electrochemically determined here. A Cu/TiO_2 nanocomposite/room temperature ionic liquid/2-(ferrocenylethynyl)fluoren-9-one modified carbon paste electrode (Cu/TiO_2-IL-2FF/CPE) was used for the electrochemical determination of levodopa. The electro-oxidation mechanism of levodopa at Cu/TiO_2-IL-2FF/CPE was also studied. The obtained data showed that the electro-oxidation of levodopa at the Cu/TiO_2-IL-2FF/CPE is greatly facilitated, which is attributed to high electrical conductivity of Cu/TiO_2 nanocomposite, room temperature

ionic liquid and 2-(ferrocenylethynyl)fluoren-9-one. The Cu/TiO_2-IL-2FF/CPE showed a good electron mediating behavior followed by well separated oxidation signals of levodopa and tyrosine. Differential pulse voltammetric peak current showed a linear relationship corresponds to the concentrations of levodopa in a linear range of 0.03 to 700.0 μM with detection limit of 12.0 nM. The Cu/TiO_2-IL-2FF/CPE shows excellent ability to determination of levodopa and tyrosine in real samples.

Keywords – Levodopa, Tyrosine, Cu/TiO_2 nanocomposite, Room temperature ionic liquid, 2-(ferrocenylethynyl)fluoren-9-one, Carbon paste electrode, Voltammetry

1. INTRODUCTION

Parkinson's disease is a late-onset, progressive motor disease marked by selective degeneration of dopaminergic neurons of the substantia nigra and formation of fibrillar cytoplasmic inclusions [1,2]. Levodopa, which is able to cross the blood–brain barrier and decarboxylated to dopamine by dopa decarboxylase, is most commonly suggested for symptomatic treatment of Parkinson's disease. However, chronic levodopa treatment may lead to the development of various motor and non-motor complications, such as wearing-off, levodopa-induced dyskinesia (LID) and dopamine dysregulation syndrome (DDS), which limits the usefulness of levodopa. DDS is one of the most severe non-motor fluctuations induced by levodopa. Patients with DDS increase their levodopa doses beyond those required for motor control, resulting in a pattern of compulsive drug taking [3-9].

Amino acids are important enantiomers compounds, they also are the evaluating indicator for many diseases. Many studies also explored the potential relationship between amino acids enantiomers and several diseases, such as alzheimer's disease and schizophrenia [8,9]. Tyrosine belong to enantiomers of aromatic amino acid. Hence, tyrosine can help eliminate anxiety, irritability, and headache in the treatment. Now research has shown depression patients would have been associated with deficiencies of tyrosine [10,11].

In the human body, levodopa is synthesized from amino acid tyrosine by tyrosine hydroxylase [12]. Therefore, simultaneous determination of levodopa and tyrosine is very important in blood and urine sample in Parkinson's disease. Up to now, some analytical methods have been used for the analysis of levodopa, such as high performance chromatography, spectrophotometry, chemiluminecence [13-15]. Nevertheless, each technique has often suffered from diverse disadvantages with regard to cost and selectivity, the use of organic solvents, complex sample preparation procedures and long analysis time. Therefore, it is still highly desirable to develop a simple, rapid and sensitive method for routinely quantifying analytes in complex matrices, especially biological fluids [16-20].

However, electrochemical methods can be superior methods of neurotransmitters determination due to the miniaturization of technology and ability of online analysis. Due to this fact, many researchers focused on designing new electrochemical sensors to measure these compounds more selective and sensitive [21-35]. Due to the possibility of modifying

conventional electrodes with building blocks with specific properties, chemically modified electrodes (CME) received great attention during the last decades. The use of CMEs for drug analysis led to an improved analytical response in terms of detection limit, specificity and anti-fouling property [36-50].

In the past decades, the investigations on the direct electrochemistry and electrochemical applications of metal nanoparticles have aroused considerable interest in analytical chemistry and bioinorganic chemistry [51-56]. Various studies proved that TiO_2 is the most efficient and environmentally benign catalyst. Improved catalytic properties have been found for TiO_2 nanostructured used in environmental applications [57]. The catalytic activity 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 [58-61].

Room-temperature ionic liquids (RTILs) are ion compounds that remain in the liquid state at room temperature. RTILs possess unique properties such as high thermal stability and viscosity, good conductivity, and solubility, which make them novel solvent (electrolyte) systems with great potential in electrochemical applications [62]. The direct electron-transfer reaction between redox analytes and RTIL-based nanocomposite electrodes has received considerable attention in recent years. However, not all RTILs are suitable for biocatalysis [63]. Park and Kazlauska [64] reported that enzymes are usually active in RTILs that contain BF_4^-, PF_6^-, and Ntf_2^- anions.

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 [65].

In this study, we describe application of Cu/TiO_2 nanocomposite, room temperature ionic liquid and 2-(ferrocenylethynyl)fluoren-9-one (2FF) modified carbon paste electrodes (Cu/TiO_2-IL-2FF/CPE) for voltammetric determination of levodopa. The proposed sensor showed good electrocatalytic effect on levodopa. Cu/TiO_2-IL-2FF/CPE shows advantages in terms of selectivity, reproducibility and sensitivity. Eventually, this new constructed electrochemical sensor was used for determination of levodopa in the presence of tyrosine.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software.

A conventional three electrodes cell was used at 25±1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the Cu/TiO_2-IL-2FF/CPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was employed for pH measurements.

All solutions were freshly prepared with double distilled water. Levodopa, tyrosine and all other reagents were of analytical grade and were obtained from Merck chemical company (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-TiO_2 nanocomposite

TiO_2 and Cu doped TiO_2 nano powder was prepared by the controlled sol-gel method with titanium(IV) n-butoxide $Ti(OCH_2CH_2CH_2CH_3)_4$ as raw materials. All the chemicals were purchaced 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, 5mL of water and 1 mL of HNO_3 (65%) were added to another 20 mL of absolute ethanol in turn to form an alchol/acid/water solution (solution B).

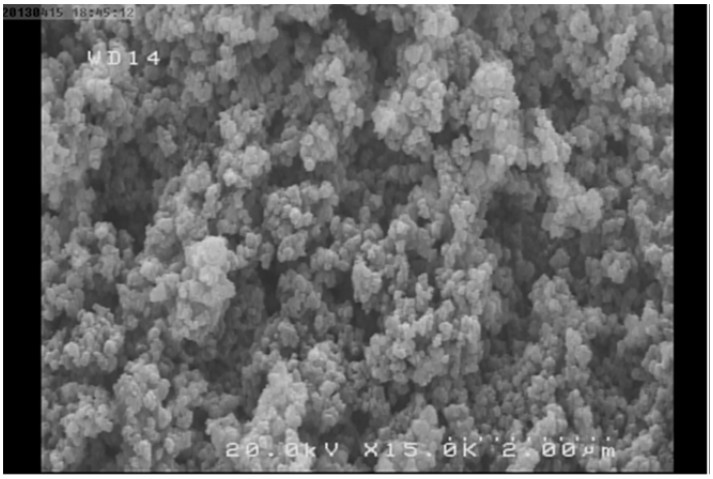


Fig. 1. SEM image of synthesized Cu-TiO_2 nanocomposite

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 ºC and subsequently the resulting material was powdered and then calcined in an electric muffle furnace at 450 ºC for 2 h to obtain crystalline powders of TiO_2. Cu doped TiO_2 nanoparticles were synthesized using almost the same method. The molar amount of transition metal ion dopant (Cu^{2+}) was calculated in order to substitute 1% of titanium ions in TiO_2 and was solubilized in an appropriate amount of ethanol/nitric acid/water solution prior to the 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-TiO_2 nanocomposite is shown in Fig. 1.

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

$PdCl_2(PPh_3)_2$ (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 Et_3N (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 H_2O and stirring for 30 min, the resulting crude product was filtered. The resulting raw material was dissolved in CH_2Cl_2, then washed with diluted HCl and saturated NaCl solution. After drying with $MgSO_4$ and solvents removal, the crude products was purified by column chromatography on silica gel using hexane-CH_2Cl_2 as eluent to afford the pure product in 87% yield.

Red solid; mp: 147-148 °C; IR: υ (cm^{-1})=750, 1602, 1712 (C=O), 2201 (C=C); ^{1}H NMR (500 MHz, CDCl 3): d(ppm)=4.20-4.30 (pseudo s, 7H), 4.54 (s, 2H), 7.30-7.76 (m, 7H); ^{13}C NMR (125 MHz, CDCl 3): d(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 $C_{25}H_{16}FeO$: C, 77.34; H, 4.15%.

2.4. Preparation of the electrode

The Cu/TiO_2-IL-2FF/CPEs were prepared by hand mixing 0.04 g of Cu/TiO_2 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/TiO_2 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 levodopa and tyrosine. The levodopa and tyrosine 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 levodopa and tyrosine. The levodopa and tyrosine contents were analysed by the proposed method by using the standard addition method.

3. RESULTS AND DISCUSSION

3.1. Electrochemical properties of Cu/TiO_2-IL-2FF/CPE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of 2FF in an aqueous media. Therefore, Cu/TiO_2-IL-2FF/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, $\Delta Ep = (E_{pa} - E_{pc})$ of 100 mV, was greater than the value of 59/n mV which is expected for a reversible system, suggesting that the redox couple of 2FF in Cu/TiO_2-IL-2FF/CPE has a quasi-reversible behavior in aqueous medium [66].

In addition, the long-term stability of the Cu/TiO_2-IL-2FF/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 levodopa oxidation was unchanged and the current signals showed less than 2.5% decrease relative to the initial response. The antifouling properties of the modified electrode toward levodopa and its oxidation products were investigated by recording the cyclic voltammograms of the modified electrode before and after use in the presence of levodopa. Cyclic voltammograms were recorded in the presence of levodopa 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 2.7%. Therefore, at the surface of Cu/TiO_2-IL-2FF/CPE, not only the sensitivity increases, but the fouling effect of the analyte and its oxidation product also decreases.

3.2. Electrocatalytic oxidation of levodopa at a Cu/TiO_2-IL-2FF/CPE

The electrochemical behavior of levodopa is dependent on the pH value of the aqueous solution, 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 levodopa. Thus the electrochemical behavior of levodopa was studied in 0.1 M PBS in different pH values (2.0<pH<9.0) at the surface of Cu/TiO_2-IL-2FF/CPE by CV. It was found that the electrocatalytic oxidation of levodopa at the surface of Cu/TiO_2-IL-2FF/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 CVs of Cu/TiO_2-IL-2FF/CPE. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of levodopa oxidation at the surface of Cu/TiO_2-IL-2FF/CPE.

To evaluate the effects of the nature of the working electrodes various studies were conducted and the results in Fig. 2 include the CVs obtained for the electrocatalytic oxidation of a 500.0 μM solution of levodopa using a unmodified CPE (curve b), Cu/TiO_2/CPE (curve d) and IL-2FF/CPE (curve e) as the working electrodes. One can easily see that although the anodic peak potential obtained for the oxidation of levodopa at the surfaces of Cu/TiO_2/CPE, and unmodified CPE are 540 and 650 mV, these values at IL-2FF/CPE and Cu/TiO_2-IL-2FF/CPE are around 350 mV, which indicates that 2FF can be as a good mediator. It is clear that the peak potentials for the oxidation of levodopa at the IL-2FF/CPE and Cu/TiO_2-IL-2FF/CPE showed ~190 and 300 mV shifts toward the negative values as opposed to Cu/TiO_2/CPE and unmodified CPE, respectively. Cu/TiO_2-IL-2FF/CPE, on the other hand, led to a much higher anodic peak current compared to IL-2FF/CPE, which can be held to argue that the mixture of Cu/TiO_2 nanocomposite and 2FF has a much better performance for oxidizing levodopa. Actually, in a 0.1 M PBS (pH=7.0) solution lacking levodopa, the Cu/TiO_2-IL-2FF/CPE produced a well-defined redox reaction (Fig. 2, curve c), while upon reaching a levodopa concentration of 500.0 μM a considerable increase could be observed in the anodic peak current (curve e), most probably due to the strong electrocatalytic effects of the Cu/TiO_2-IL-2FF/CPE in the oxidation of levodopa (Scheme 1).

The effect of potential scan rate on the electrocatalytic oxidation of levodopa at the Cu/TiO_2-IL-2FF/CPE was investigated by linear sweep voltammetry (LSV) (Fig. 3). As can be seen 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 10-300 mV s^{-1}, suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled (Fig. 3) [66].

Fig. 4 illustrates the Tafel plot for the sharp rising part of the LSV at the scan rate of 10 mV s^{-1}. If deprotonation of levodopa 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 0.1648 V was obtained which is consistent well with the involvement of one electron in the rate determining step of the electrode process [66], assuming a charge transfer coefficient, α of 0.64.

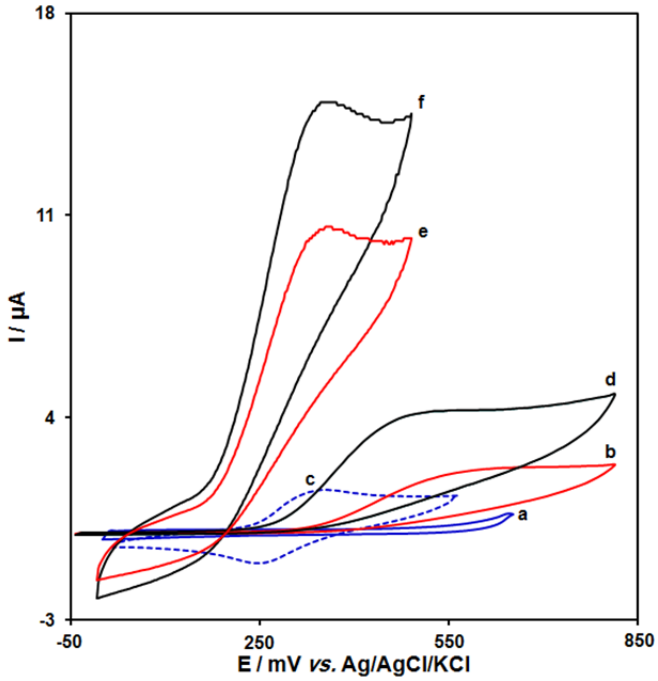
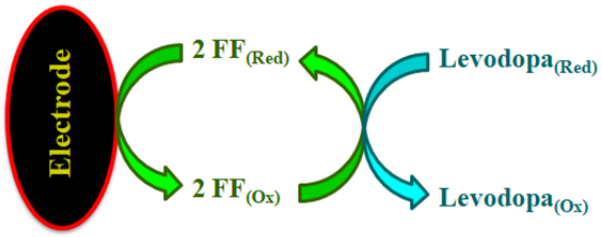


Fig. 2. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0); (b) unmodified CPE in 0.1 M PBS (pH 7.0) containing 0.5 mM levodopa; (c) Cu/TiO_2-IL-2FF/CPE in 0.1 M PBS (pH 7.0); (d) Cu/TiO_2/CPE in 0.1 M PBS (pH 7.0) containing 0.5 mM levodopa, (e) IL-2FF/CPE and (f) Cu/TiO_2-IL-2FF/CPE in 0.1 M PBS (pH 7.0) containing 0.5 mM levodopa



Scheme 1. Electrocatalytic oxidation of levodopa at the Cu/TiO_2-IL-2FF/CPE

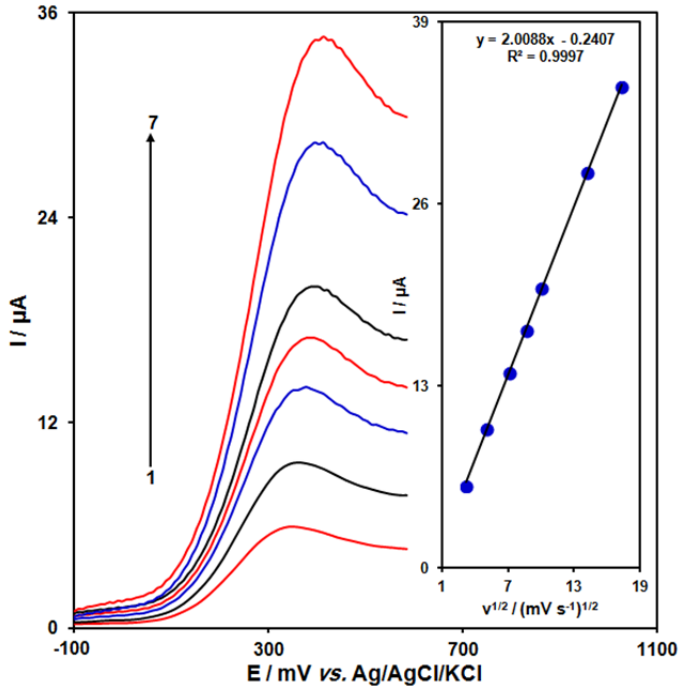


Fig. 3. LSVs for oxidation of 100.0 µM levodopa at the surface of modified electrode at various scan rates; numbers 1-7 correspond to 10, 25, 50, 75, 100, 200 and 300 mV s^{-1}. Inset: Variation of anodic peak current versus the square root of scan rate

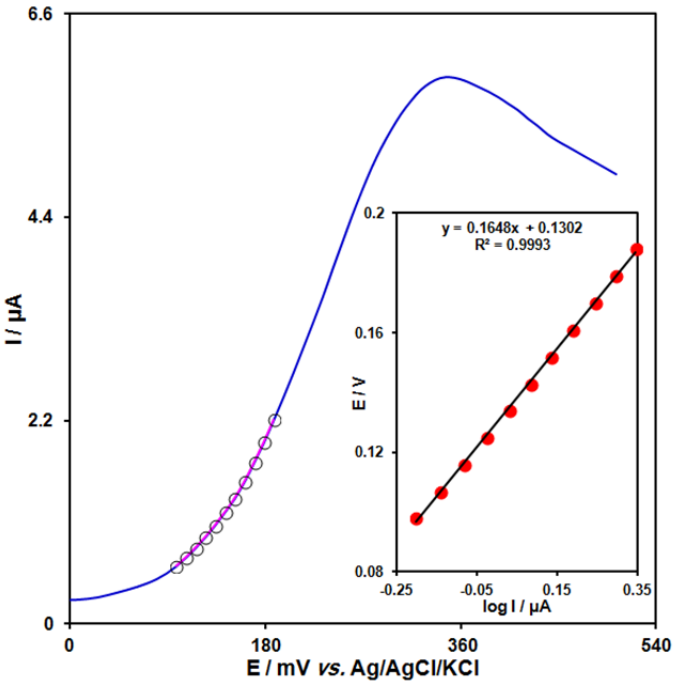


Fig. 4. LSVs (at 10 mV s^{-1}) of electrode in 0.1 M PBS (pH 7.0) containing 100.0 µM levodopa. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV

3.3. Chronoamperometric measurements

Chronoamperometric measurements of levodopa at Cu/TiO_2-IL-2FF/CPE were carried out by setting the working electrode potential at 400 mV for the various concentrations of levodopa in PBS (pH 7.0) (Fig. 5). For an electroactive material (levodopa 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 [66]. Experimental plots of I *vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of levodopa (Fig. 5A). The slopes of the resulting straight lines were then plotted *vs.* levodopa concentration (Fig. 5B). From the resulting slope and Cottrell equation the mean value of the D was found to be 1.7×10^{-6} cm^2/s.

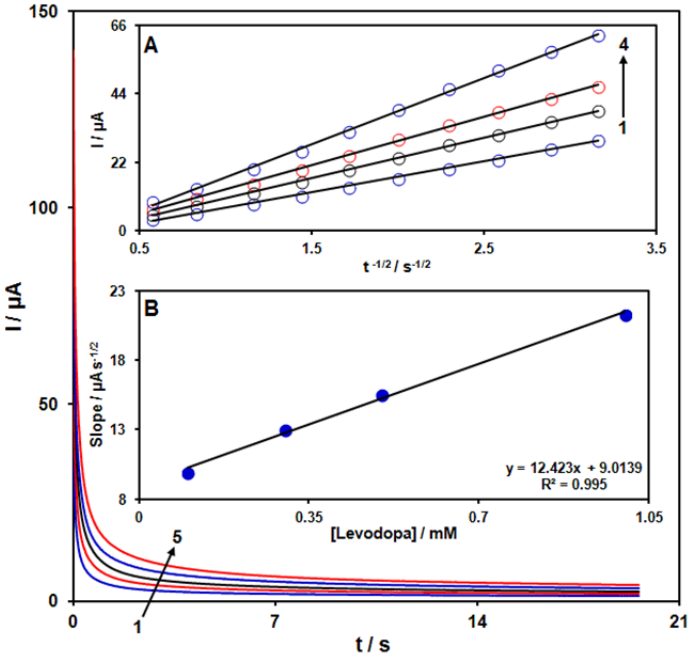


Fig. 5. Chronoamperograms obtained at Cu/TiO_2-IL-2FF/CPE in 0.1 M PBS (pH 7.0) for different concentrations of levodopa. The numbers 1–5 correspond to 0.1, 0.3, 0.5 and 1.0 mM of levodopa. Insets: (A) Cottrell's plot for the data from the chronoamperogram; (B) Plot of the slope of the straight lines against levodopa concentration

3.4. Calibration plot and limit of detection

DPV method was used to determine the concentration of levodopa (Fig. 6) (Initial potential=0.14 V, End potential=0.46 V, Step potential=0.002 V, Modulation Amplitude=0.02505 V). The plot of peak current *vs.* levodopa concentration consisted of linear segment with slope of 0.0236 µA μM^{-1} in the concentration ranges of 0.03 to 700.0 µM. The detection limit (3σ) of levodopa was found to be 0.012 µM. These values are

comparable with the results obtained by some other modified electrode for determination of levodopa (See table 1).

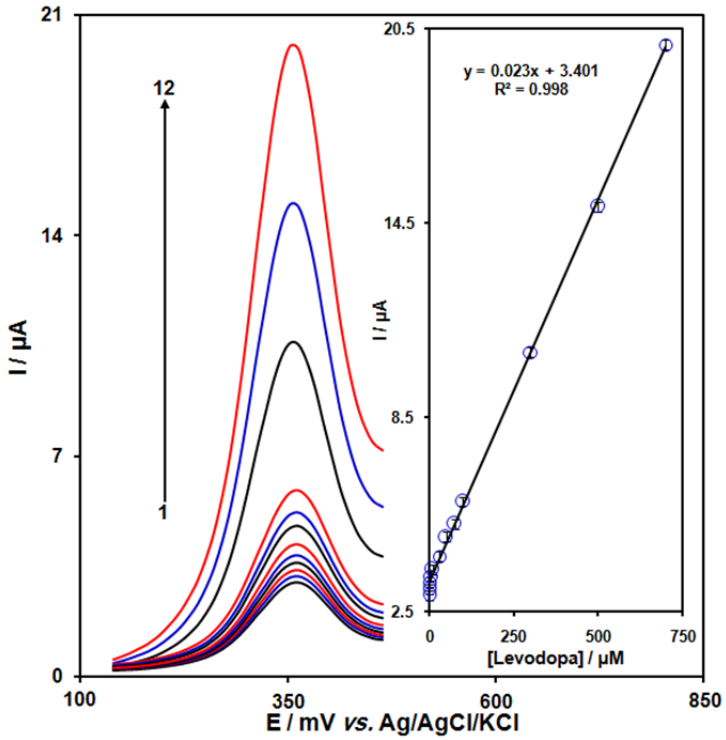


Fig. 6. DPVs of Cu/TiO_2-IL-2FF/CPE in 0.1 M (pH 7.0) containing different concentrations of levodopa. Numbers 1–12 correspond to 0.03, 0.1, 1.0, 5.0, 10.0, 30.0, 50.0, 75.0, 100.0, 300.0 500.0 and 700.0 µM of levodopa. Inset: Plot of the electrocatalytic peak current as a function of levodopa concentration in the range of 0.03–700.0 µM

Table 1. Comparison of some electrochemical methods used in the determination of levodopa

Electrode	**Modifier**	**Linear Range (µM)**	**Detection Limit (µM)**	**Ref.**
Glassy carbon	Fullerene-functionalized carbon nanotubes	0.5-2000.0	0.035	[67]
Gold Screen printed	-	99.0-1200.0	68.0	[68]
Carbon paste	Ferrocene modified carbon nanotubes	2.0-50.0	1.2	[69]
Carbon paste	Trinuclear ruthenium ammine complex incorporated in NaY zeolite	120.0-10000.0	85.0	[70]
Glassy carbon	Carbon nanotubes and chitosan	2.0-220.0	0.6	[71]
Carbon paste	Dysprosium nanowire	0.01-1.0	0.004	[72]
Carbon paste	Cu/TiO_2 nanocomposite, room temperature ionic liquid and 2-(ferrocenylethynyl)fluoren-9-one	0.03-700.0	0.012	This Work

3.5. Simultaneous determination of levodopa and tyrosine

To our knowledge, no paper has used Cu/TiO_2-IL-2FF/CPE for simultaneous determination of levodopa and tyrosine and this is the first report for simultaneous determination of levodopa and tyrosine by using Cu/TiO_2-IL-2FF/CPE. Electrochemical determination of levodopa by using bare electrodes suffers from interference by tyrosine because the oxidation potential of tyrosine is fairly close to that of levodopa. The two compounds were determined by simultaneously changing the concentrations of levodopa and tyrosine, and recording the DPVs (Fig. 7) (Initial potential= 0.03 V, End potential=0.85 V, Step potential=0.002 V, Modulation Amplitude=0.02505 V). The voltammetric results showed well defined anodic peaks at potentials of 315 and 700 mV, corresponding to the oxidation of levodopa and tyrosine, respectively, there by indicating that simultaneous determination of these compounds is feasible at Cu/TiO_2-IL-2FF/CPE, as shown in Fig. 7.

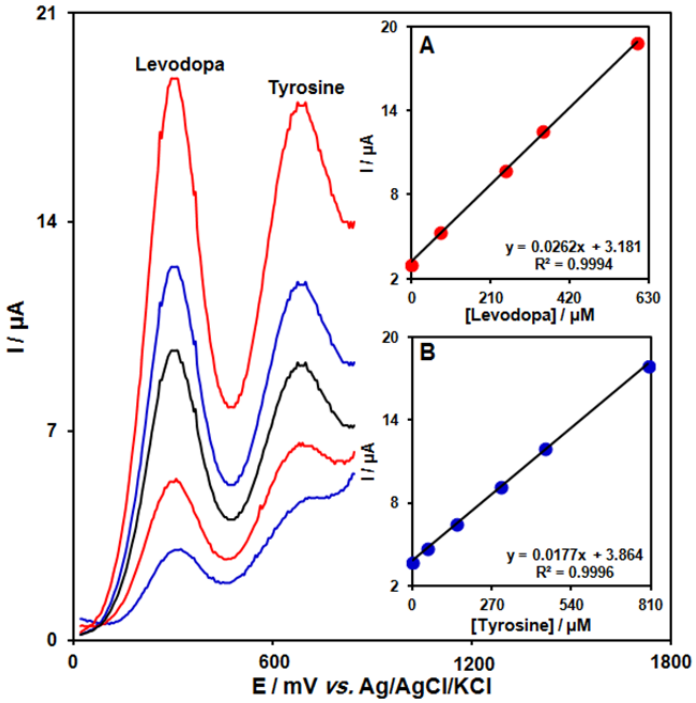


Fig. 7. DPVs of Cu/TiO_2-IL-2FF/CPE in 0.1 M PBS (pH 7.0) containing different concentrations of levodopa+tyrosine in µM, from inner to outer: 0.03+1.0, 75.0+50.0, 250.0+150.0, 350.0+450.0 and 600+800.0 respectively. Insets: (A) plot of Ip *vs*. levodopa concentrations, (B) plot of Ip *vs*. tyrosine concentrations

3.6. Interference studies

The influence of various substances as compounds potentially interfering with the determination of levodopa was studied under optimum conditions. The potentially interfering substances were chosen from the group of substances commonly found with levodopa in

pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5% in the determination of levodopa. According to the results, L-lysine, glucose, NADH, acetaminophen, uric acid, L-asparagine, L-serine, L-cysteine, L-cystine, L-threonine, L-proline, histidine, glycine, tryptophan, phenylalanine, lactose, saccarose, 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 levodopa.

3.7. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of levodopa and tyrosine in human blood serum and urine samples. The results for determination of levodopa in real samples are given in Table 2. Satisfactory recovery of the experimental results was found for levodopa. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 2. Determination of levodopa and tyrosine in human blood serum and urine samples. All the concentrations are in μM (n=5)

Sample	**Spiked**		**Found**		**Recovery (%)**		**R.S.D. (%)**	
	Levodopa	Tyrosine	Levodopa	Tyrosine	Levodopa	Tyrosine	Levodopa	Tyrosine
Human blood serum	0	0	-	-	-	-	-	-
	5.0	7.5	4.9	7.6	98.0	101.3	2.7	2.4
	10.0	12.5	10.3	12.4	103.0	99.2	3.4	1.8
	15.0	17.5	15.2	17.2	101.3	98.3	1.9	2.6
	20.0	22.5	19.8	23.3	99.0	103.5	2.2	3.3
Urine	0	0	-	-	-	-	-	-
	7.5	10.0	7.6	9.7	101.3	97.0	3.2	1.9
	17.5	20.0	17.3	20.7	98.9	103.5	2.8	2.2
	27.5	30.0	28.1	29.8	102.2	99.3	1.7	3.1
	37.5	40.0	37.3	40.2	99.5	100.5	2.1	2.8

4. CONCLUSION

A highly sensitive and good selective electrochemical sensor based on Cu/TiO_2 nanocomposite, room temperature ionic liquid and 2-(ferrocenylethynyl)fluoren-9-one (2FF) was fabricated for analysis of levodopa in the presence of tyrosine. Combination of Cu/TiO_2 nanocomposite, RTIL and 2FF showed unique properties for modification of carbon paste

electrode. The Cu/TiO_2-IL-2FF/CPE successfully resolves the overlapped oxidation peaks of levodopa and tyrosine by about 385 mV. The Cu/TiO_2-IL-2FF/CPE showed good ability for analysis of levodopa and tyrosine in real samples.

REFERENCES

[1] J. Chi, Y. Ling, R. Jenkins, and F. Li, J. Chromatogr. B 1054 (2017) 1.
[2] H. J. Yang, G. Ehm, Y. E. Kim, J. Y. Yun, W. W. Lee, A. Kim, H. J. Kim, and B. Jeon, J. Neurol. Sci. 377 (2017) 6.
[3] C. Monnot, X. Zhang, S. Nikkhou-Aski, P. Damberg, and P. Svenningsson, Exp. Neurol. 292 (2017) 11.
[4] H. Nishijima, T. Ueno, S. Ueno, F. Mori, Y. Miki, and M. Tomiyama, Neur. Res. 115 (2017) 54.
[5] G. Noffs, A. C. Duprat, A. P. Zarzur, R. G. Cury, B. O. Cataldo, and E. Fonoff, J. Voice 31 (2017) 383.e19.
[6] H. Beitollahi, S. Tajik, H. Parvan, H. Soltani, A. Akbari, and M. H. Asadi, Anal. Bioanal. Electrochem. 6 (2014) 54.
[7] H. Y. Yue, H. Zhang, S. Huang, X.Y. Lin, X. Gao, J. Chang, L. H. Yao, and E. J. Guo, Biosens. Bioelectron. 89 (2017) 592.
[8] S. W. Hyun, A. Liu, Z. Liu, E. P. Lillehoj, J. A. Madri, A. B. Reynolds, and S. E. Goldblum, Cell. Signal. 35 (2017) 1.
[9] D. B. Abdjul, H. Yamazaki, W. Maarisit, F. Losung, H. Rotinsulu, D. S. Wewengkang, D. A. Sumilat, and M. Namikoshi, Phytochem. Lett. 20 (2017) 191.
[10] V. Neirinckx, H. Hedman, and S. P. Niclou, Biochim. Biophys. Acta 1868 (2017) 109.
[11] X. Shi, Y. Wang, C. Peng, Z. Zhang, J. Chen, X. Zhou, and H. Jiang, Electrochim. Acta 241 (2017) 386.
[12] L. X. Xie, H. L. Wu, Y. Fang, C. Kang, S. X. Xiang, L. Zhu, X. L. Yin, H. W. Gu, Z. Liu, and R. Q. Yu, Chemometr. Intell. Lab. Sys. 148 (2015) 9.
[13] M. Doležalová, and M. Tkaczykova, J. pharm. Biomed. Anal. 19 (1999) 555.
[14] J. Coello, S. Maspoch, and N. Villegas, Talanta 53 (2000) 627.
[15] W. W. He, X. W. Zhou, and J. Q. Lu, Chin. Chem. Lett. 18 (2007) 91.
[16] M. Mahmoudi Moghaddam, H. Beitollahi, S. Tajik, I. Sheikhshoaie, and P. Biparva, Environ. Monit. Assess. 187 (2015) 407.
[17] J. T. Mehrabad, M. Aghazadeh, M. R. Ganjali, and P. Norouzi, Mater. Lett. 184 (2016) 223.
[18] M. M. Foroughi, H. Beitollahi, S. Tajik, A. Akbari, and R. Hosseinzadeh, Int. J. Electrochem. Sci. 9 (2014) 8407.
[19] A. Kumar,V. K. Vashistha, P. Tevatia, and R. Singh, Anal. Bioanal. Electrochem. 8 (2016) 848.

[20] F. Soofiabadi, A. Amiri, and Sh. Jahani, Anal. Bioanal. Electrochem. 9 (2017) 340.
[21] M. Mahmoudi Moghaddam, H. Beitollahi, S. Tajik, I. Sheikhshoaie, and P. Biparva, Anal. Bioanal. Electrochem. 6 (2014) 634.
[22] G. A. Saleh, H. F. Askal, I. H. Refaat, and F. A. M. Abdel-aal, Anal. Bioanal. Electrochem. 8 (2016) 691.
[23] H. Beitollahi, S. Tajik, S. Z. Mohammadi, and M. Baghayeri, Ionics 20 (2014) 571.
[24] H. Beitollahi, F. Garakani-Nejad, Electroanalysis 28 (2016) 2237.
[25] Q. Q. Ren, J. Wu, W. C. Zhang, C. Wang, X. Qin, G. C. Liu, Z. X. Li, and Y. Yu, Sens. Actuators B 245 (2017) 615.
[26] H. Beitollahi, M. A. Taher, M. Ahmadipour, and R. Hosseinzadeh, Measurement 47 (2014) 770.
[27] W. Gu, J. Bai, B. Dong, X. Zhuang, J. Zhao, C. Zhang, J. Wang, and K. Shih, Chem. Eng. J. 324 (2017) 122.
[28] H. Beitollahi, S. Tajik, M. Malakootian, H. Karimi-Maleh, and R. Hosseinzadeh, Appl. Organomet. Chem. 27 (2013) 444.
[29] C. Cuadrado, L. Ibarra, J. Hurtado, O. Garcia-Beltran, and E. Nagles, Anal. Bioanal. Electrochem. 8 (2016) 910.
[30] H. Beitollahi, S. Ghofrani Ivari, and M. Torkzadeh-Mahani, Mater. Sci. Eng. C, 69 (2016) 128.
[31] T. Alizadeh, M. R. Ganjali, M. Akhoundian, and P. Norouzi, Microchim. Acta 183 (2016) 1123.
[32] Sh. Jahani, and H. Beitollahi, Anal. Bioanal. Electrochem. 8 (2016) 158.
[33] H. Karimi-Maleh, M. Keyvanfard, K. Alizad, M. Fouladgar, H. Beitollahi, A. Mokhtari, and F. Gholami-Orimi, Int. J. Electrochem. Sci. 6 (2011) 6141.
[34] M. P. Deepak, M. P. Rajeeva and G. P. Mamatha, Anal. Bioanal. Electrochem. 8 (2016) 931.
[35] M.R. Akhgar, H. Beitollahi, M. Salari, H. Karimi-Maleh, and H. Zamani, Anal. Methods 4 (2012) 259.
[36] P. Norouzi, H. Haji-Hashemi, B. Larijani, M. Aghazadeh, E. Pourbasheer, and M. R. Ganjali, Curr. Anal. Chem. 13 (2017) 70.
[37] S. Palanisamy, C. Karuppiah, S. M. Chen, C. Y. Yang, and P. Periakaruppan, Anal. Methods 3 (2011) 911.
[38] H. Beitollahi, J. B. Raoof, H. Karimi-Maleh, and R. Hosseinzadeh, J. Solid State Electrochem. 16 (2012) 1701.
[39] P. Norouzi, V. K. Gupta, B. Larijani, S. Rasoolipour, F. Faridbod, and M. R. Ganjali, Talanta 131 (2015) 577.
[40] E. Molaakbari, A. Mostafavi, and H. Beitollahi, Sens. Actuators B 208 (2015) 195.

[41] S. Tajik, M. A. Taher, Sh. Jahani, and M. Shanesaz, Anal. Bioanal. Electrochem. 8 (2016) 899.
[42] H. Beitollahi, S. Nekooei, and M. Torkzadeh-Mahani, Talanta 188 (2018) 701.
[43] M. Mazloum-Ardakani, H. Beitollahi, B. Ganjipour, and H. Naeimi, Int. J. Electrochem. Sci. 5 (2010) 531.
[44] M. R. Ganjali, F. Garkani Nejad, H. Beitollahi, Sh. Jahani, M. Rezapour, and B. Larijani, Int. J. Electrochem. Sci. 12 (2017) 3231.
[45] H. Mahmoudi Moghaddam, H. Beitollahi, S. Tajik, and H. Soltani, Electroanalysis 27 (2015) 2620.
[46] S. Azhari, P. Sathishkumar, R. Ahamad, F. Ahmad, and A. R. Mohd Yusoff, Anal. Methods 8 (2016) 5712.
[47] H. Beitollahi, H. Karimi-Maleh, and H. Khabazzadeh, Anal. Chem. 80 (2008) 9848.
[48] H. Mahmoudi Moghaddam, H. Beitollahi, S. Tajik, Sh. Jahani, H. Khabazzadeh, and R. Alizadeh, Russ. J. Electrochem. 53 (2017) 452.
[49] H. Beitollahi, S. Ghofrani Ivari, M. Torkzadeh-Mahani, Biosens. Bioelectron. 110 (2018) 97.
[50] N. Y. Sreedhar, and K. Sunil, Anal. Bioanal. Electrochem. 6 (2014) 151.
[51] H. Beitollahi, F. Garkani Nejad, S. Tajik, Sh. Jahani, and P. Biparva, Int. J. Nano Dimens. 8 (2017) 197.
[52] M. Khatami, S. Kharazi, Z. Kishani Farahani, H. Azizi, and M. A. Lima Nobre, Tehran Univ. Med. J. 75 (2017) 72.
[53] G. Zhu, Z. Azizi, S. Pourseyedi, M. Khatami, and H. Mohammadi, J. Cluster Sci. 27 (2016) 1613.
[54] M. Baniasadi, Sh. Jahani, H. Maaref, and R. Alizadeh, Anal. Bioanal. Electrochem. 9 (2017) 718.
[55] M. Khatami, R. Mehnipor, M. H. Sobhani Poor, and G. Salehi Jouzani, J. Cluster Sci. 27 (2016) 1061.
[56] E. Zare, S. Pourseyedi, M. Khatami, and E. Darezereshki, J. Mol. Struct. 1146 (2017) 96.
[57] A. Soklic, M. Tasbihi, M. Kete, and U. Lavrencic-Stangar, Catal. Today 252 (2015) 54.
[58] R. Chand, E. Obuchi, K. Katoh, H. N. Luitel, and K. Nakano, Catal. Commun. 13 (2011) 49.
[59] G. Li, N. M. Dimitrijevic, L. Chen, T. Rajh, and K. A. Gray, J. Phys. Chem. C 112 (2008) 19040.
[60] M. Hussein, and D. Hanaor, Appl. Surf. Sci. 387 (2016) 682.
[61] M. Mazloum-Ardakani, R. Arazi, H. Beitollahi, and H. Naeimi, Anal. Methods 2 (2010) 1078.

[62] J. Q. Sun, B. F. Ye, G. M. Xia, X. H. Zhao, and H. M. Wang, Sens. Actuators B 233 (2016) 76.
[63] Q. L. Sheng, J. B. Zheng, X. D. Shang-Guan, W. H. Lin, Y. Y. Li, and R. X. Liu, Electrochim. Acta 55 (2010) 3185.
[64] S. Park, and R. J. Kazlauskas, Curr. Opin. Biotechnol. 14 (2003) 432.
[65] A. Salmanipour, M. A. Taher, and H. Beitollahi, Anal. Methods 4 (2012) 2982.
[66] A. J. Bard, and L.R. Faulkner, Electrochemical Methods Fundamentals and Applications, second ed, Wiley, New York (2001).
[67] M. Mazloum-Ardakani, S. H. Ahmadi, Z. Safaei Mohmoudabadi, and A. Khoshroo, Measurement 91 (2016) 162.
[68] M. F. Bergamini, A.L. Santos, N. R. Stradiotto, and M. V. B. Zanoni, J. Pharm. Biomed. Anal. 39 (2005) 54.
[69] H. Yaghoubian, H. Karimi-Maleh, M. A. Khalilzadeh, and F. Karimi, Int. J. Electrochem. Sci. 4 (2009) 993.
[70] M. F. S. Teixeira, M. F. Bergamini, C. M. P. Marques, and N. Bocchi, Talanta 63 (2004) 1083.
[71] M. F. S. Teixeira, L. H. Marcolino-Junior, O. Fatibello-Filho, E. R. Dockal, and M.F. Bergamini, Sens. Actuators B 122 (2007) 549.
[72] P. Daneshgar, P. Norouzi, M. R. Ganjali, A. Ordikhani-Seyedlar, and H. Eshraghi, Colloids Surf. B 68 (2009) 27.