

Electrochemical platform for simultaneous determination of levodopa, acetaminophen and tyrosine using a graphene and ferrocene modified carbon paste electrode

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Abstract A carbon paste electrode (CPE) was modified with graphene and ethyl 2-(4-ferrocenyl-[1,2,3]triazol-1-yl) acetate (EFTA) and used for electrocatalytic oxidation of levodopa. Under optimum conditions and at pH 7.0, the oxidation of levodopa occurs at a potential about 280 mV (vs. Ag/AgCl) using square wave voltammetry (SWV) which is less positive than that of an unmodified CPE. The modified electrode can well resolve the voltammetric peaks of levodopa, acetaminophen and tyrosine. The peak current is linear in the 0.2 μ M to 0.4 mM levodopa concentration range. The respective figures for acetaminophen and tyrosine are 485 mV and 745 mV, respectively, and the analytical ranges are from 1.0 μ M to 0.15 mM for acetaminophen, and from 5.0 μ M to 0.18 mM for tyrosine.

Keywords Modified electrodes · Carbon paste electrode · Ferrocene mediator · Square wave voltammetry · Electrochemical sensor · Nanotechnology · Drug analysis

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Introduction

Parkinson's disease, PD, is one of the most common neurodegenerative disorders in humans leading to progressive deterioration of motor function due to loss of dopamine-producing brain cells [1]. Therapeutics with levodopa (levo-3,4-dihydroxyphenylalanine) will soon be entering its second half-century of use as the most effective symptomatic therapy for PD. Levodopa has provided revolutionary and lifelong benefits for averting disability and improving quality of life, as experienced by millions of PD patients [1]. Since dopamine cannot cross the protective blood–brain barrier, whereas levodopa can, levodopa is widely prescribed to increase dopamine concentration in the treatment of PD [1]. In human, levodopa can normally be prepared via biosynthesis from a nonessential amino acid called tyrosine. However, auto-oxidization of levodopa occurred in the peripheral system, can produce serious side effects, such as paranoia schizophrenia, nausea, vomiting and dyskinesia [1]. Therefore, determination of levodopa is crucial to control its dosage. Several methods have been reported for determination of levodopa in pharmaceutical and biological samples including chromatography [2], spectroscopy [3] and electroanalysis [4–8].

Acetaminophen or paracetamol, the most extensively used medication for pain and fever in both the United States and Europe, is an effective and safe agent, used worldwide for the relief of mild to moderate pain associated with headaches, backaches, arthritis and postoperative pain. It is also used for the reduction of fevers of bacterial or viral origin. Acetaminophen is a non-carcinogenic and an effective substitute for aspirin and used by the patients who are sensitive to aspirin, and it is safe up to therapeutic doses. When administered in suitable doses, acetaminophen has an excellent safety profile, with about 90% of absorption by the organism and

subsequently excreted via urine. Overdoses of acetaminophen can lead to the accumulation of toxic metabolites, causing severe and some-times fatal hepatotoxicity and nephrotoxicity [9]. Parkinson's disease patients chronically use opiates, acetaminophen and drugs treating neuropathic pain such as antidepressants and antiepileptics [9]. Also, it has been found that human absorption of acetaminophen is extremely dependent on gastric emptying. Other drugs that alter gastric emptying can change the pharmacokinetics of acetaminophen. It has been shown that levodopa can influence gastric emptying [10]. Then, simultaneous quantification of these two compounds can provide valuable evidences about the role of them in healthiness. Till now, a variety of methods have been introduced for acetaminophen determination such as, capillary electrophoresis, HPLC, spectroscopy and electrochemical techniques [11–15].

Tyrosine is one of the nonessential amino acids normally produced in human body from phenylalanine. Tyrosine also exists in dairy products, eggs, beans and meats. It has a vital role in production of several important neurotransmitters in brain that help nerve cell communicate. An increased level of tyrosine induces Parkinson's disease and increased sister chromatid exchange, while the lack of tyrosine may lead to albinism, alkaptonuria and other psychological diseases [16]. In human aqueous humor, tyrosine is the second highest in concentration next to ascorbic acid [16]. Tyrosine is often added to food products and to pharmaceutical formulations. It is critical to mental and physical health as it is needed to produce catecholamines through an iron-containing enzyme called tyrosine hydroxylase [17]. Both tyrosine and levodopa have very similar chemical structure and biological function and linked together by the process taking place in body to convert tyrosine to levodopa to meet its needs. Tyrosine and levodopa are necessary for producing dopamine and some other vital biological compound in our body. Therefore, simultaneous determination of levodopa, acetaminophen and tyrosine is highly demanded.

Electrochemical techniques are among the most appealing choices for biological and pharmaceutical compounds. Electrochemistry often offers analytical techniques which are characterized by instrumental simplicity, moderate cost, high sensitivity, portability and some other unique advantages. Due to similarity between redox reactions that taking place in electrochemical and biological systems, it can be assumed that oxidation-reduction reactions occurring in the body and at the surface of electrodes share similar principles [18–24].

Graphene, a one-atom-thick monolayer of graphite containing sp^2 -bonded carbon atoms with a honeycomb structure, has captured the attention of scientists, researchers and industry worldwide because of some exceptional properties like high electron mobility at room temperature, its large specific surface area, chemically inertness, high thermal conductivity

and ballistic transport. However, due to the strong π - π stacking interaction, the reported graphene materials especially those produced by chemical approaches are actually graphene nanoplatelets with a typical thickness of 2–10 graphene layers, which also exhibit similarly fascinating properties as graphene [25]. In the past decade, graphene has been the undisputed champion of the material world and has grabbed appreciable attention for the development of electrochemical sensors and biosensors, supercapacitors and batteries with enhanced performances thanks to its integration with various nanomaterials [26–31].

Literature survey show that all previously published electrochemical studies have dealt with individual determination of levodopa, acetaminophen and tyrosine, or simultaneous determination of them with other drugs but no studies have been reported on the simultaneous electrocatalytic determination of levodopa, acetaminophen and tyrosine by using modified electrodes. Now we wish report for the first time the preparation and suitability of an ethyl 2-(4-ferrocenyl-[1,2,3]triazol-1-yl) acetate / graphene modified carbon paste electrode (EFTAGCPE) as a new electrode in the electrocatalysis and in addition the determination of levodopa in an aqueous buffer. Then the analytical performance of the modified electrode in quantification of levodopa is evaluated in the presence of acetaminophen and tyrosine.

Experimental

Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands, www.metrohm-autolab.com). 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 (Azar Electrode, Urmia, Iran, www.azarelectrode.ir), a platinum wire (Azar Electrode, Urmia, Iran, www.azarelectrode.ir), and the EFTAGCPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter (www.metrohm.com) was employed for pH measurements.

All solutions were freshly prepared with double distilled water. Levodopa, acetaminophen, tyrosine and all other reagents were of analytical grade and were purchased from Merck chemical company (Darmstadt, Germany, www.merck.com). The buffers were prepared from orthophosphoric acid and its salts in the pH range of 2.0–9.0. Graphene was purchased from Sigma Aldrich Company (www.sigmaaldrich.com).

Synthesis of ethyl 2-(4-ferrocenyl-[1,2,3]triazol-1-yl)acetate

A clean, dry ball milling vessel (15 ml) was charged with 2 stainless steel grinding balls (10 mm diameter), ethyl 2-bromoacetate (0.05 ml, 0.5 mmol), ethynylferrocene (100 mg, 0.5 mmol), sodium azide (32 mg, 0.5 mmol), sodium ascorbate (45 mg, 0.23 mmol), copper acetate (10 mg, 0.05 mmol) and acidic alumina as milling auxiliary (1 g). The vessel was closed, and the milling was started (milling at 500 rpm). After completion of the reaction, as indicated by TLC, ethyl acetate (2 × 15 mL) was added to the resulting solid mixture. Then the reaction mixture was filtered and washed with additional ethyl acetate. The combined filtrate and washings were evaporated under reduced pressure to dryness and purified by layer chromatography on silica gel (n-hexane: ethyl acetate as eluent) to afford the pure product (134.9 mg, 79%).

Mp: 139–142 °C; $^1\text{H NMR}$ (400 MHz, DMSO-d_6): δ 1.23 (t, $J = 7.2$ Hz, 3H), 4.03 (s, 5H), 4.19 (q, $J = 7.2$ Hz, 2H), 4.31 (t, $J = 1.8$ Hz, 2H), 4.74 (t, $J = 1.8$ Hz, 2H), 5.39 (s, 2H), 8.17 (s, 1H); $^{13}\text{C NMR}$ (100 MHz, DMSO-d_6): δ 14.4, 50.8, 61.9, 66.8, 68.8, 69.7, 76.1, 122.4, 145.8, 167.7; MS: m/z (%): 339 (M^+ , 100), 311 ($\text{M}^+ - \text{N}_2$, 3), 224 ($\text{M}^+ - \text{C}_4\text{H}_7\text{N}_2\text{O}_2$, 27), 199 ($\text{M}^+ - \text{C}_5\text{H}_8\text{N}_3\text{O}_2$, 8), 121 ($\text{C}_5\text{H}_5\text{Fe}^+$, 65).

Preparation of the electrode

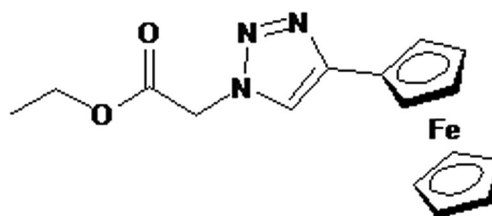
To obtain the best conditions in the preparation of the EFTAGCPEs, we optimized the ratio of EFTA, and graphene. The results of our studied showed that the maximum peak current intensity of levodopa could be obtained at the surface of EFTAGCPE with optimum ratio of EFTA and graphene.

The EFTAGCPEs were prepared by hand mixing 0.01 g of EFTA (Scheme 1) with 0.89 g graphite powder and 0.1 g graphene 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 prepared by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, EFTA modified CPE (EFTACPE) without graphene, graphene carbon paste electrode (GCPE) without EFTA and unmodified CPE in the absence of both EFTA and graphene were also prepared in the same way.

Procedure of real samples preparation

The acetaminophen oral solution was diluted 1000 times with deionized water; then, different volume of the diluted solution was transferred into a 25 mL volumetric flask and diluted to



Scheme 1 Chemical structure of EFTA

the mark with phosphate buffer (pH 7.0). The acetaminophen content was analyzed using the standard addition method.

Urine samples were stored in a refrigerator immediately after collection. 10 mL of the sample was centrifuged for 15 min at 2000 rpm. The supernatant was filtered out using a 0.45 μm filter. Then, different volume of the solution was transferred into a 25 mL volumetric flask and diluted to the mark with phosphate buffer (pH 7.0). The diluted urine sample was spiked with different amounts of levodopa, acetaminophen and tyrosine.

For preparing the plasma and serum of blood, 10 mL of blood was separated after putting the sample in an incubator at 37 °C for 30 min and centrifuging it. The serum and plasma were separated from each other. After filtering, the samples were diluted with phosphate buffer (pH 7.0) without any further treatment. The diluted samples were spiked with different amounts of levodopa, acetaminophen and tyrosine.

Results and discussion

Electrochemical properties of EFTAGCPE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of EFTA in an aqueous media. Therefore, EFTAGCPE was prepared and its electrochemical properties were studied in a phosphate buffer (pH 7.0) using cyclic voltammetry. It should be noted that one of the advantages of EFTA as an electrode modifier is its insolubility in aqueous media. Experimental results showed reproducible and well-defined cyclic voltammograms. Anodic and cathodic peak potentials were 0.33 and 0.21 V vs. Ag/AgCl/KCl (3.0 M) respectively. The observed peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 120 mV, was greater than the value of 59/n mV which is expected for a reversible system [32], suggesting that the redox couple of EFTA in EFTAGCPE has a quasi-reversible behavior in aqueous medium [32].

In addition, the long-term stability of the EFTAGCPE was tested over a 3-week period. When cyclic voltammograms were recorded after the modified electrode was stored in atmosphere at room temperature, the peak potential for levodopa oxidation was unchanged, and the current signals showed a

less than 2.0% decrease relative to the initial response. The antifouling properties of the modified electrode toward levodopa oxidation 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 mVs^{-1} . The peak potentials were unchanged, and the currents decreased by less than 2.1%. Therefore, at the surface of EFTAGCPE, not only did the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decreased.

Electrocatalytic oxidation of levodopa at an EFTAGCPE

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 phosphate buffer in different pH values ($2.0 < \text{pH} < 9.0$) at the surface of EFTAGCPE by cyclic voltammetry. It was found that the electrocatalytic oxidation of levodopa at the surface of EFTAGCPE 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 EFTAGCPE. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of levodopa oxidation at the surface of EFTAGCPE.

Figure 1 depicts the cyclic voltammogram responses for the electrocatalytic oxidation of $400.0 \mu\text{M}$ levodopa at unmodified CPE (curve a), GCPE (curve b), EFTACPE (curve d) and EFTAGCPE (curve e).

As it is seen, while the anodic peak potential for levodopa oxidation at the GCPE, and unmodified CPE are 610 and 660 mV, respectively, the corresponding potential at EFTACPE and EFTAGCPE is ~ 330 mV. These results indicate that EFTA can act as a good mediator and peak potential for levodopa oxidation at the EFTACPE and EFTAGCPE shift by ~ 280 and 330 mV toward negative values compared to GCPE and unmodified CPE, respectively. However, EFTAGCPE shows much higher anodic peak current for the oxidation of levodopa compared to EFTACPE, indicating that the combination of graphene and the mediator (EFTA) has significantly improved the performance of the electrode toward levodopa oxidation. In fact, EFTAGCPE in the absence of levodopa exhibited a well-behaved redox reaction (Fig. 1, curve c) in 0.1 M phosphate buffer (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of $400.0 \mu\text{M}$ levodopa (curve e).

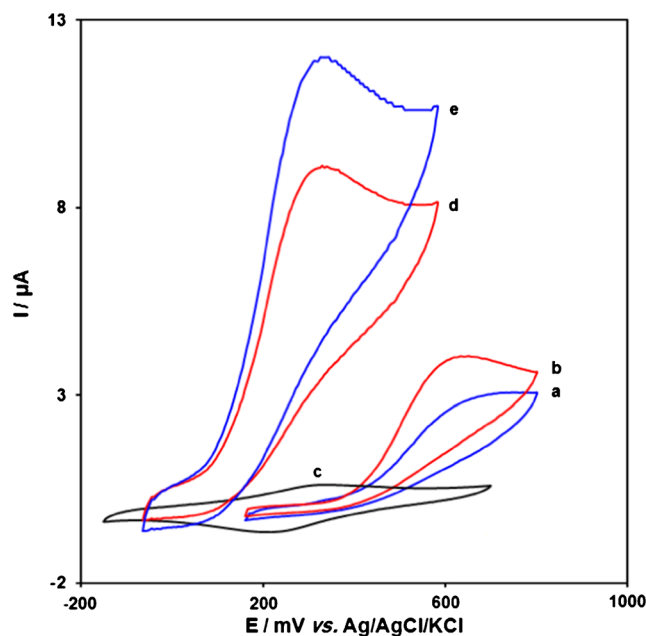
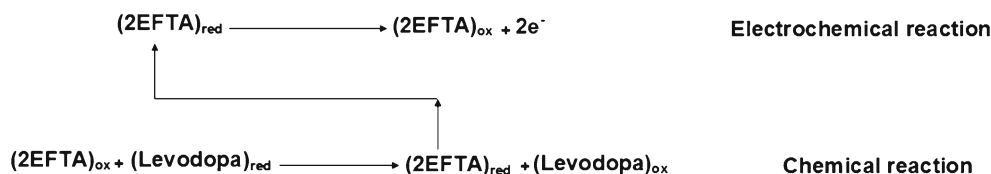


Fig. 1 Cyclic voltammograms of (a) unmodified CPE in 0.1 M phosphate buffer (pH 7.0) containing 0.4 mM levodopa; (b) GCPE in 0.1 M phosphate buffer (pH 7.0) containing 0.4 mM levodopa; (c) EFTAGCPE in 0.1 M phosphate buffer (pH 7.0); (d) EFTACPE in 0.1 M phosphate buffer (pH 7.0) containing 0.4 mM levodopa and (e) EFTAGCPE in 0.1 M phosphate buffer (pH 7.0) containing 0.4 mM levodopa

Based on these results, we propose an EC' catalytic mechanism, shown in Scheme 2, to describe the electrochemical oxidation of levodopa at EFTAGCPE. In this scheme, levodopa oxidized in the catalytic (C) reaction by the oxidized form of EFTA produced at the electrode surface via an electrochemical (E) reaction.

The effect of scan rate on the electrocatalytic oxidation of levodopa at the EFTAGCPE was investigated by linear sweep voltammetry (LSV) (Fig. 2). As can be seen in Fig. 2, 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 $2\text{--}20 \text{ mVs}^{-1}$, suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled (Fig. 2a). A plot of the scan rate-normalized current ($I_p/v^{1/2}$) vs. scan rate (Fig. 2b) exhibits the characteristic shape typical of an EC' process [32].

Figure 3c illustrates the Tafel plot for the sharp rising part of the linear sweep voltammogram at the scan rate of 2 mVs^{-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 0.1068 V was obtained which is consistent well with the involvement of one electron in the rate determining step of the electrode process [32], assuming a charge transfer coefficient, α of 0.45.

Scheme 2 Electrocatalytic oxidation of levodopa at the EFTAGCPE

Chronoamperometric measurements

Chronoamperometric measurements of levodopa at EFTAGCPE were carried out by setting the working electrode potential at 0.4 V (first potential step) and at 0.1 V (second potential step) for the various concentrations of levodopa in phosphate buffer (pH 7.0) (Fig. 4). 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 eq. [32]. Experimental plots of I vs. $t^{1/2}$ were employed, with the best fits for different concentrations of levodopa (Fig. 4a). The slopes of the resulting straight lines were then plotted vs. levodopa concentration (Fig. 4b). From the resulting slope and Cottrell equation the mean value of the D was found to be $2.3 \times 10^{-6} \text{ cm}^2/\text{s}$.

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k , for the reaction between levodopa and the EFTAGCPE according to the method described by Galus [33]:

$$I_C / I_L = \gamma^{1/2} \left[\pi^{1/2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma) / \gamma^{1/2} \right] \quad (1)$$

where I_C is the catalytic current of levodopa at the EFTAGCPE, I_L is the limited current in the absence of levodopa and $\gamma = kC_b t$ is the argument of the error function (C_b is the bulk concentration of levodopa). In cases where γ exceeds the value of 2, the error function is almost equal to 1 and therefore, the above equation can be shortened to:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \quad (2)$$

where t is the time elapsed. The above equation can be used to calculate the rate constant, k , of the catalytic process from the slope of I_C / I_L vs. $t^{1/2}$ at a given levodopa concentration. From the values of the slopes (Fig. 4c), the average value of k was found to be $1.2 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$.

Calibration plot and limit of detection

Square wave voltammetry method was used to determine the concentration of levodopa (Fig. 5) (Initial potential = 0 V, End potential = 0.6 V, Step potential = 0.001 V, Amplitude = 0.02 V, Frequency = 10 Hz). The plot of peak current vs. levodopa concentration consisted of two linear segments with slopes of 1.082 and 0.222 $\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$ in the concentration ranges of 0.2 to 30.0 μM and 30.0 to

400.0 μM , respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. The detection limit (3σ) of levodopa was found to be 0.07 μM .

In the case of acetaminophen peak currents at the surface of EFTAGCPE were linearly dependent on the acetaminophen concentrations, over the range of 1.0×10^{-6} – 1.5×10^{-4} M and the detection limit (3σ) was obtained 5.0×10^{-7} M and for tyrosine, peak current at the surface of EFTAGCPE were linearly dependent on the tyrosine concentrations, over the range of 5.0×10^{-6} – 1.8×10^{-4} M and the detection limit (3σ) was obtained 2.0×10^{-6} M.

These values are comparable with values reported by other research groups for electrocatalytic oxidation of levodopa, acetaminophen and tyrosine at the surface of chemically modified electrodes (Table 1).

Simultaneous determination of levodopa, acetaminophen and tyrosine

It is pertinent to note that the use of modified graphene electrode for simultaneous determination of levodopa,

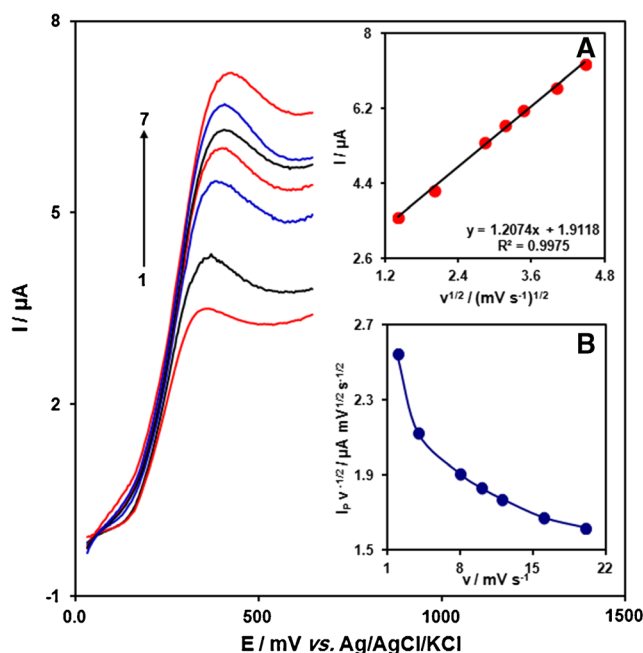


Fig. 2 Linear sweep voltammograms of EFTAGCPE in 0.1 M phosphate buffer (pH 7.0) containing 100.0 μM levodopa at various scan rates; numbers 1–7 correspond to 2, 4, 8, 10, 12, 16 and 20 mVs^{-1} , respectively. Insets: Variation of (a) anodic peak current vs. $v^{1/2}$; (b) normalized current ($I_p/v^{1/2}$) vs. v

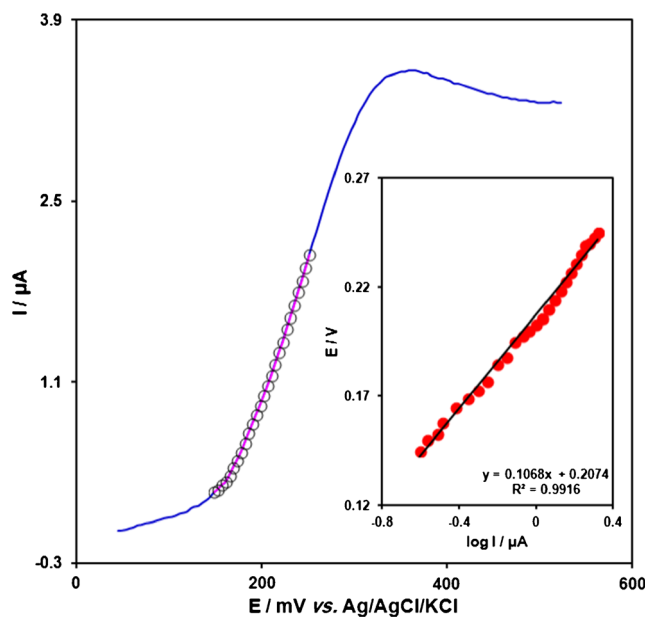


Fig. 3 Linear sweep voltammogram (at 2 mV s^{-1}) of an EFTAGCPE in 0.1 M phosphate buffer (pH 7.0) containing $100.0 \text{ } \mu\text{M}$ levodopa. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the linear sweep voltammogram

acetaminophen and tyrosine have not reported so far and we believe that this work is the first report on the simultaneous determination of levodopa, acetaminophen and tyrosine using EFTAGCPE.

This was performed by simultaneously changing the concentrations of levodopa, acetaminophen and tyrosine, and also

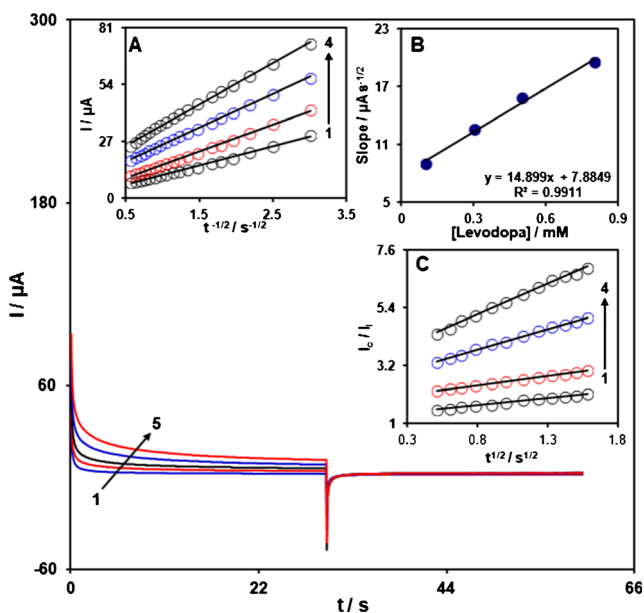


Fig. 4 Chronoamperograms at EFTAGCPE in 0.1 M phosphate buffer (pH 7.0) for different concentration of levodopa. The numbers 1–5 correspond to $0.0, 0.1, 0.3, 0.5$ and 0.8 mM of levodopa. Insets: **a** Plots of I vs. $t^{-1/2}$ resulting from chronoamperograms 2–5. **b** Plot of the slope of the straight lines against levodopa concentration. **c** dependence of I_{cat}/I_1 on $t^{1/2}$ derived from the data of chronoamperograms 1–5

at the same time recording the square wave voltammograms. The voltammetric results showed well-defined anodic peaks at potentials of 275, 485 and 745 mV , which are corresponding to the oxidation of levodopa, acetaminophen and tyrosine, respectively, indicating that simultaneous determination of these compounds is feasible at the EFTAGCPE, as shown in Fig. 6 (Initial potential = -0.1 V , End potential = 0.95 V , Step potential = 0.001 V , Amplitude = 0.02 V , Frequency = 10 Hz).

The sensitivity of the modified electrode towards the oxidation of levodopa was found to be $1.092 \text{ } \mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$, which is very close to the value obtained in the absence of acetaminophen and tyrosine ($1.082 \text{ } \mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$) (Fig. 6). These results are indicative that the oxidation processes of these compounds at the EFTAGCPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

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

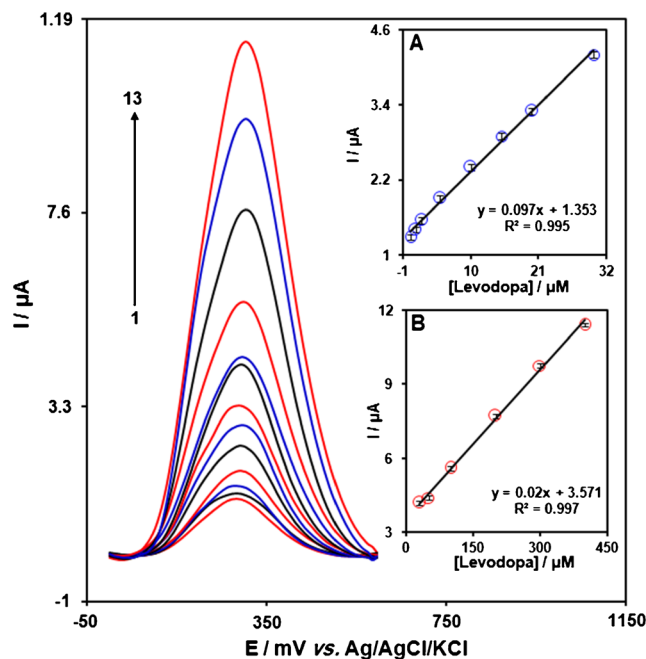


Fig. 5 Square wave voltammograms of EFTAGCPE in 0.1 M phosphate buffer (pH 7.0) containing different concentrations of levodopa. Numbers 1–13 correspond to $0.2, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0, 30.0, 50.0, 100.0, 200.0, 300.0$ and $400.0 \text{ } \mu\text{M}$ of levodopa. Insets: **a** The plots of the electrocatalytic peak current as a function of levodopa concentration in the range of $0.2\text{--}30.0 \text{ } \mu\text{M}$ and **b** The plots of the electrocatalytic peak current as a function of levodopa concentration in the range of $30.0\text{--}400.0 \text{ } \mu\text{M}$

Table 1 An overview on recently reported nanomaterial-based methods for determination of levodopa or acetaminophen or tyrosine

| Analyte | Electrode | Modifier | Method | LDR | LOD | Ref. |
|---------------------------|-------------------------|--|-------------|--|----------------------------|------|
| Levodopa | Glassy carbon | Radish peroxidase/organic nucleophilicfunctionalized carbon nanotube composite | Voltammetry | 0.1 μ M - 1.9 μ M | 40 nM | 5 |
| Levodopa | Carbon paste | Hemoglobin electrostatic immobilized on WO ₃ nanoparticles | Voltammetry | 60 μ M –1070 μ M | 0.25 μ M | 7 |
| Levodopa | - | Gold nanoparticles doped on titanium dioxide nanotubes | Voltammetry | 10 μ M –70 μ M | - | 8 |
| Acetaminophen Tyrosine | Glassy carbon | Tetra ruthenated cobalt(II) porphyrin intercalated into a smectite clay | Voltammetry | 1 μ M - 50 μ M 1 μ M - 24 μ M | 0.1 μ M 0.5 μ M | 12 |
| Acetaminophen | Glassy carbon disk | Poly(Alizarin Violet 3B), multiwalled carbon nanotubes and graphene | Voltammetry | 0.2 μ M - 100 μ M | 0.01 μ M | 13 |
| Acetaminophen | Glassy carbon electrode | Polymer and multi-walled carbon nanotubes | Voltammetry | 5.0 μ M –1000.0 μ M | 3.5 μ M | 14 |
| Acetaminophen | carbon paste | Pt-Co nanoparticles and a dopamine derivative | Voltammetry | 1.0 μ M- 850 μ M | 0.6 μ M | 15 |
| Levodopa | carbon paste | Graphene and ethyl | Voltammetry | 0.2 μ M - 0.4 mM | 0.07 μ M | This |
| Acetaminophen | | 2-(4-ferrocenyl-[1,2,3]triazol-1-yl) acetate | | 1.0 μ M to 0.15 mM | 0.5 μ M | work |
| Tyrosine | | | | 5.0 μ M to 0.18 mM | 2.0 μ M | |

concentration of the interfering substance that caused an error of less than $\pm 5\%$ in the determination of levodopa. According to the results, L-lysine, glucose, NADH, acetaminophen, uric acid, L-asparagine, L-serine, L-threonine, L-proline, L-histidine, L-glycine, L-tryptophan, L-phenylalanine, lactose,

tyrosine, saccharose, fructose, benzoic acid, methanol, ethanol, urea, caffeine, Mg^{2+} , Al^{3+} , NH_4^+ , F^- , SO_4^{2-} and S^{2-} did not show interference in the determination of levodopa, but dopamine, isoproterenol, epinephrine and norepinephrine can cause interference in the determination of levodopa.

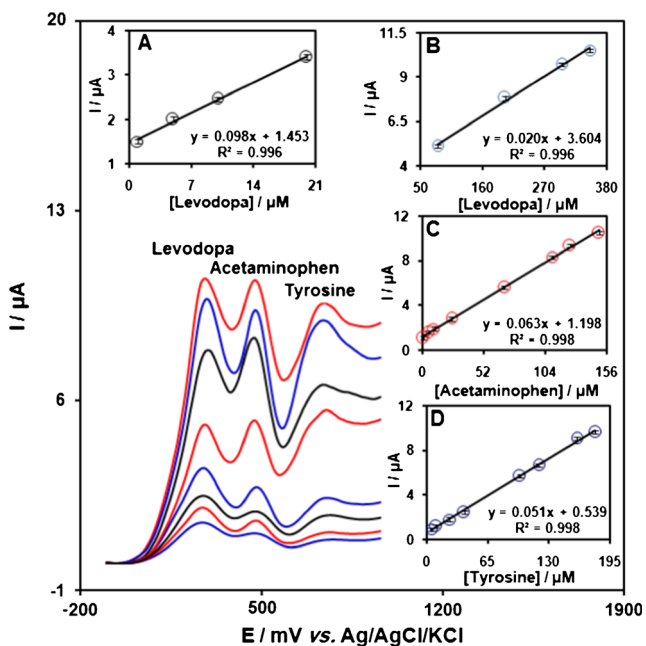


Fig. 6 Square wave voltammograms of EFTAGCPE in 0.1 M phosphate buffer (pH 7.0) containing different concentrations of levodopa + acetaminophen + tyrosine in μ M, from inner to outer: 1.0 + 1.0 + 5.0, 5.0 + 5.0 + 10.0, 10.0 + 10.0 + 25.0, 20.0 + 25.0 + 40.0, 80.0 + 70.0 + 100.0, 200.0 + 110.0 + 120.0, 300.0 + 125.0 + 160.0 and 350.0 + 150.0 + 180.0 respectively. Insets: **a** Plot of I_p vs. levodopa concentration in first linear segment, **b** plot of I_p vs. levodopa concentrations in second linear segment, **c** plot of I_p vs. acetaminophen concentrations and **d** plot of I_p vs. tyrosine concentrations

Real sample analysis

In order to evaluate the analytical applicability of the method, also it was applied to the determination of levodopa, acetaminophen and tyrosine in acetaminophen oral solution, human blood serum, human blood serum and urine samples. The results for determination of the three species in real samples are given in table 1S. Satisfactory recovery of the experimental results was found for levodopa, acetaminophen and tyrosine. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Conclusions

In the present study, a modified carbon paste electrode is constructed. The modified electrode was applied for levodopa determinations in the presence of acetaminophen and tyrosine for the first time. Excellent features, like a wide linear range, low detection limit, high reproducibility and repeatability and long time stability proved the successful application of this sensor for the determinations of levodopa, acetaminophen and tryptophan. Real sample analysis was done and good results were obtained.

Compliance with ethical standards The author(s) declare that they have no competing interests.

References

- Sadikovic M, Nigovic B, Juric S, Momar A (2014) Voltammetric determination of ropinirole in the presence of levodopa at the surface of a carbon nanotubes based electrochemical sensor in pharmaceuticals and human serum. *J Electroanal Chem* 733:60–68. doi:10.1016/j.jelechem.2014.09.020
- Mantena BPV, Rao SV, Rao KMCA, Ramakrishna K, Reddy RS (2015) Rapid separation technique for the determination of potential impurities present in levodopa, carbidopa, and entacapone in fixed dose combination drug product using trifunctionally bonded phase ethylene bridged sorbent column with smaller ion-pair reagent. *J Liq Chromatogr Relat Technol* 38:1073–1087. doi:10.1080/10826076.2015.1020166
- Yue HY, Zhang H, Huang S, Lin XY, Gao X, Chang J, Yao LH, Guo EJ (2017) Synthesis of ZnO nanowire arrays/3D graphene foam and application for determination of levodopa in the presence of uric acid. *Biosens Bioelectron* 89:592–597. doi:10.1016/j.bios.2016.01.078
- Tajik S, Taher MA, Beitollahi H (2014) First report for electrochemical determination of levodopa and cabergoline: application for determination of levodopa and cabergoline in human serum, urine and pharmaceutical formulations. *Electroanalysis* 26:796–806. doi:10.1002/elan.201300589
- Shoja Y, Rafati AA, Ghodsi J (2016) Glassy carbon electrode modified with horse radish peroxidase/organic nucleophilic-functionalized carbon nanotube composite for enhanced electrocatalytic oxidation and efficient voltammetric sensing of levodopa. *Mater Sci Eng C* 58:835–845. doi:10.1016/j.msec.2015.09.028
- Beitollahi H, Garkani Nejad F (2016) Graphene oxide/ZnO Nano composite for sensitive and selective electrochemical sensing of levodopa and tyrosine using modified graphite screen printed electrode. *Electroanalysis* 28:2237–2244. doi:10.1002/elan.201600143
- Ghodsi J, Rafati AA, Shoja Y (2016) First report on hemoglobin electrostatic immobilization on WO₃ nanoparticles: application in the simultaneous determination of levodopa, uric acid, and folic acid. *Anal Bioanal Chem*. doi:10.1007/s00216-016-9480-5
- Hosseini MG, Faraji M, Momeni MM, Ershad S (2011) An innovative electrochemical approach for voltammetric determination of levodopa using gold nanoparticles doped on titanium dioxide nanotubes. *Microchim Acta* 172:103–108. doi:10.1007/s00604-010-0471-5
- Bateman DN, Vale A (2016) Paracetamol (acetaminophen). *Medicine* 44:190–192. doi:10.1016/j.mpmed.2015.12.014
- Wang Y, Wu T, Bi CY (2016) Simultaneous determination of acetaminophen, theophylline and caffeine using a glassy carbon disk electrode modified with a composite consisting of poly(Alizarin Violet 3B), multiwalled carbon nanotubes and graphene. *Microchim Acta* 183:731–739. doi:10.1007/s00604-015-1688-0
- Beitollahi H, Nekooei S (2016) Application of a modified CuO nanoparticles carbon paste electrode for simultaneous determination of Isoperrenaline, acetaminophen and N-acetyl-L-cysteine. *Electroanalysis* 28:645–653. doi:10.1002/elan.201500249
- Kemmegne-Mbouguen JC, Toma HE, Araki K, Leopoldo Constantino VR, Ngameni E, Angnes L (2016) Simultaneous determination of acetaminophen and tyrosine using a glassy carbon electrode modified with a tetra-ruthenated cobalt(II) porphyrin intercalated into a smectite clay. *Microchim Acta* 183:3243–3253. doi:10.1007/s00604-016-1985-2
- Wang Y, Wu T, Bi CY (2016) Simultaneous determination of acetaminophen, theophylline and caffeine using a glassy carbon disk electrode modified with a composite consisting of poly(Alizarin Violet 3B), multiwalled carbon nanotubes and graphene. *Microchim Acta* 183:731–739. doi:10.1007/s00604-015-1688-0
- Liu R, Zeng X, Liu J, Luo J, Zheng Y, Liu X (2016) A glassy carbon electrode modified with an amphiphilic, electroactive and photo-sensitive polymer and with multi-walled carbon nanotubes for simultaneous determination of dopamine and paracetamol. *Microchim Acta* 183:1543–1551. doi:10.1007/s00604-016-1763-1
- Karimi-Maleh H, Hatami M, Moradi R, Khalilzadeh MA, Amiri S, Sadeghifar H (2016) Synergic effect of Pt-co nanoparticles and a dopamine derivative in a nanostructured electrochemical sensor for simultaneous determination of N-acetylcysteine, paracetamol and folic acid. *Microchim Acta* 183:2957–2964. doi:10.1007/s00604-016-1946-9
- Zhou S, Wu H, Wu Y, Shi H, Feng X, Huang H, Li J, Song W (2013) Large surface area carbon material with ordered mesopores for highly selective determination of L-tyrosine in the presence of L-cysteine. *Electrochim Acta* 112:90–94. doi:10.1016/j.electacta.2013.08.134
- Taei M, Hasanpour F, Salavati H, Banitaba SH, Kazemi F (2016) Simultaneous determination of cysteine, uric acid and tyrosine using Au-nanoparticles/poly(E)-4-(p-tolyldiazene)benzene-1,2,3-triol film modified glassy carbon electrode. *Mater Sci Eng C* 59:120–128. doi:10.1016/j.msec.2015.10.004
- Beitollahi H, Tajik S, Jahani S (2016) Electrocatalytic determination of hydrazine and phenol using a carbon paste electrode modified with ionic liquids and magnetic Core-shell Fe₃O₄@SiO₂/MWCNT nanocomposite. *Electroanalysis* 28:1093–1099. doi:10.1002/elan.201501020
- Alizadeh T, Ganjali MR, Akhondian M, Norouzi P (2016) Voltammetric determination of ultratrace levels of cerium(III) using a carbon paste electrode modified with nano-sized cerium-imprinted polymer and multiwalled carbon nanotubes. *Microchim Acta* 183:1123–1130. doi:10.1007/s00604-015-1702-6
- Mahmoudi Moghaddam H, Beitollahi H, Tajik S, Soltani H (2015) Fabrication of a nanostructure based electrochemical sensor for Voltammetric determination of epinephrine, uric acid and folic acid. *Electroanalysis* 27:2620–2628. doi:10.1002/elan.201500166
- Beitollahi H, Gholami A, Ganjali MR (2015) Preparation, characterization and electrochemical application of AG-ZnO nanoplates for voltammetric determination of glutathione and tryptophan using modified carbon paste electrode. *Mater Sci Eng C* 57:107–112. doi:10.1016/j.msec.2015.07.034
- Molaakbari E, Mostafavi A, Beitollahi H, Alizadeh R (2014) Synthesis of ZnO nanorods and their application in the construction of a nanostructure-based electrochemical sensor for determination of levodopa in the presence of carbidopa. *Analyst* 139:4356–4364. doi:10.1039/C4AN00138A
- Beitollahi H, Karimi-Maleh H, Khabazzadeh H (2008) Nanomolar and selective determination of epinephrine in the presence of norepinephrine using carbon paste electrode modified with carbon nanotubes and novel 2-(4-Oxo-3-phenyl-3,4-dihydroquinazoliny)-N'-phenyl-hydrazinecarbothioamide. *Anal Chem* 80:9848–9851. doi:10.1021/ac801854j
- Kalambate PK, Rawool CR, Srivastava AK (2016) Voltammetric determination of pyrazinamide at graphene-zinc oxide nanocomposite modified carbon paste electrode employing differential pulse voltammetry. *Sensors Actuators B Chem* 237:196–205. doi:10.1016/j.snb.2016.06.019
- Lei W, Si W, Xu Y, Gu Z, Hao Q (2014) Conducting polymer composites with graphene for use in chemical sensors and biosensors. *Microchim Acta* 181:707–722. doi:10.1007/s00604-014-1160-6
- Jahani S, Beitollahi H (2016) Selective detection of dopamine in the presence of uric acid using NiO nanoparticles decorated on graphene Nanosheets modified screen-printed electrodes. *Electroanalysis* 28:2022–2028. doi:10.1002/elan.201501136

27. Zhang L, Hui KN, Hui KS, Chen X, Chen R, Lee H (2016) Corrigendum to role of graphene on hierarchical flower-like NiAl layered double hydroxide-nickel foam-graphene as binder-free electrode for high-rate hybrid supercapacitor. *Int J Hydrog Energ* 41:9443–9453. doi:10.1016/j.ijhydene.2016.06.156
28. Novikov S, Lebedeva N, Satrapinski A, Walden J, Davydov V, Lebedev A (2016) Graphene based sensor for environmental monitoring of NO₂. *Sensors Actuators B Chem* 236:1054–1060. doi:10.1016/j.snb.2016.05.114
29. Ziolkowski R, Gorski L, Malinowska E (2017) Carboxylated graphene as a sensing material for electrochemical uranyl ion detection. *Sensors Actuators B Chem* 238:540–547. doi:10.1016/j.snb.2016.07.119
30. Yagati AK, Choi Y, Park J, Choi JW, Jun HS, Cho S (2016) Silver nanoflower-reduced graphene oxide composite based micro-disk electrode for insulin detection in serum. *Biosens Bioelectron* 80: 307–314. doi:10.1016/j.bios.2016.01.086
31. Gholipour-Ranjbar H, Ganjali MR, Norouzi P, Naderi HR (2016) Synthesis of cross-linked graphene aerogel/Fe₂O₃ nanocomposite with enhanced supercapacitive performance. *Ceram Int* 42:12097–12104. doi:10.1016/j.ceramint.2016.04.140
32. Bard AJ, Faulkner LR (2010) *Electrochemical Methods Fundamentals and Applications*. second ed, (Wiley, New York).
33. Galus Z (1976) *Fundamentals of electrochemical analysis*. Ellis Horwood, New York