

Simultaneous determination of levodopa and tryptophan using a modified glassy carbon electrode

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Abstract

This work discusses the development of a new sensor for simultaneous determination of levodopa and tryptophan. The proposed sensor was a glassy carbon electrode modified with V₂O₅ nanoparticles. Based on the excellent electrochemical properties of the modified electrode, a sensitive voltammetric method was used for individual and simultaneous determination of the levodopa and tryptophan in the aqueous solutions. The proposed method under the optimized conditions was utilized to determine the levodopa with linear range of 0.06–250.0 μM and detection limit of 0.02 μM (S/N=3). Finally, the applicability of the proposed sensor was verified by evaluation of levodopa and tryptophan in real sample.

Keywords: Levodopa; tryptophan; V₂O₅ nanoparticles; glassy carbon electrode.

Introduction

Levodopa ((-)-3-(3,4-dihydroxyphenyl)-L-alanine, L-dopa) is an important chemical substance and a naturally occurring dietary supplement and psychoactive drug found in certain kinds of food and herbs and is synthesized from the essential amino acids L-phenylalanine and L-tyrosine in the mammalian body and brain [1]. Levodopa medication has been used for the most commonly prescribes for

Parkinson's disease. People with Parkinson's disease have depleted levels of dopamine, causing tremor, muscle stiffness or rigidity, slowness of movement (bradykinesia) and loss of balance. Since Dopamine cannot be administered directly as it does not cross the blood brain barrier readily, while after oral administration, levodopa is absorbed and converted into dopamine by decarboxylase. Therefore, levodopa can alleviate the

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symptoms of Parkinson's disease, decreasing the muscular rigidity and tremors [1].

Tryptophan (TRP, 2-amino-3-(1H-indol-3-yl)-propionic acid), a vital constituent of proteins and a precursor for biologically important molecules such as the neurotransmitter serotonin, dopamine and the neurohormone melatonin. Moreover, it is an indispensable component in human nutrition for building and keeping a positive nitrogen balance. Since TRP is not synthesized by the human organism and is not found in significant amounts in vegetables, it may be administered in the form of medications and food supplements [2]. TRP is one of the main components of drugs used to treat disorders such as depression and hypertension. Nonetheless, its improper metabolization has been suggested to be a possible cause of schizophrenia, hallucinations, and delusions, due to the formation of a toxic metabolite in the brain. Furthermore, high dosages of TRP can cause nausea, dizziness, and loss of appetite [3].

According to the above mentioned content and since supplementation may be beneficial for patients receiving levodopa, competition between tryptophan and levodopa may result in tryptophan malabsorption, leading to depression and other side effects of levodopa treatment. So, it is necessary and important for simultaneous determination of the levodopa and tryptophan in pharmaceutical and biological samples.

A variety of analytical methods such as spectrophotometry [4,5], capillary electrophoresis [6,7], and chromatography methods [8,9], have been developed to measure the levodopa and tryptophan levels in different sample matrices. However, these methods are sometimes unsuitable

for routine analysis due to the requirement of expensive instruments, long analysis time, and sample pretreatment. Moreover, due to the electroactive properties of levodopa and tryptophan, those can be determined by electrochemical methods. Electrochemical technique is a good alternative to the aforementioned methods due to the faster response, higher selectivity, higher sensitivity, reproducibility, easy operation and lower cost [10-14].

Glassy carbon electrodes (GCEs) are very versatile as electrode material for trace level determination of organic molecules as they provide high sensitivity, negligible porosity, and good mechanical rigidity. Bare glassy carbon electrodes have always suffered from serious problems such as interference and electrode fouling, which can result in poor selectivity and reproducibility [15-17]. To resolve these problems, chemically modified electrodes (CME) with various materials have emerged as efficient and versatile approach and attracted considerable interest over the past two decades due to the advantages in terms of reduced costs, automatic and fast analysis, high sensitivity and selectivity [18-21].

The unprecedented achievements in nanoscience and nanotechnology in the recent era has strongly urged the researchers to integrate the nanomaterial in electrode fabrication to achieve an ultrasensitive surface with improved selectivity. The reasons for their growing applications relates to their unique physical and chemical properties which entirely different from their bulk counterparts. Through different synthetic routes, it is now possible to develop nano-scaled materials with highly controlled shape,

surface area, size, charge and physicochemical properties [23,24].

Vanadium oxide (V_2O_5) is the most stable oxide with semiconductor property and the energy gap of ~ 2.2 eV at room temperature. V_2O_5 is of instance interest due to its layered structure, various phases, great catalytic, good electrical and optical properties. Layered structure and special characteristics (chemical sensing and electrochemical properties) of V_2O_5 has attracted much interest. The good catalytic activity is the result of easy reduction and oxidation between the multiple oxidation states of vanadium in the V_2O_5 . It was recently reported that V_2O_5 possess electrocatalytic activity, where V^{5+} ions play the dominant role for the electrooxidation reaction [25-27]. In the light of the above information, this work aims at developing a modified electrode for the sensitive determination of levodopa and tryptophan, using V_2O_5 nanoparticles to enhance the properties of glassy carbon electrodes. The resulting electrode is successfully applied for the determination of levodopa and tryptophan in the real samples.

Experimental

Apparatus and Chemicals

An Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was applied to measure the electrochemicals. General Purpose Electrochemical System (GPES) software was employed to control the conditions of experiments. A conventional three-electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode (Azar Electrode, Urmia, Iran), a platinum wire (Azar Electrode, Urmia, Iran), and the V_2O_5 /GCE were used as the reference, auxiliary and working electrodes, respectively. pH was measured by a

Metrohm 710 pH meter. Levodopa, tryptophan, and all the remaining reagents had an analytical grade. They have been prepared *via* Merck (Darmstadt, Germany). Orthophosphoric acid and the related salts that were above the pH range of 2.0–9.0 were used for preparing the buffer solutions. V_2O_5 nanoparticles were synthesized in our laboratory as reported previously [27].

Preparing electrode

V_2O_5 nanoparticles have been used to coat the bare glassy carbon electrode. A stock solution of V_2O_5 /GCE in 1 mL of aqueous solution has been prepared by distributing 1 mg of V_2O_5 nanoparticles via ultra-sonication for 30 min, whereas 5 μ L of aliquots of the V_2O_5 nanoparticles suspension solution has been cast on carbon working electrodes. Then, we waited until the solvent evaporation at room temperature.

Preparing real samples

Samples of urine have been kept in a refrigerator directly after gathering. Ten millilitres of samples have been centrifuged for fifteen minutes at 2,000 rpm. The supernatant has been filtered by a 0.45 μ m filter. Next, various volumes of solution has been transported into a 25 millilitres volumetric flask and diluted to the mark with PBS (pH= 7.0). This diluted urine samples were anaesthetized with different amounts of levodopa and tryptophan. Content of levodopa and tryptophan have been analyzed using the suggested procedure by employing the standard addition method.

Result and discussion

Characterization of V_2O_5 /NPs

The morphology of V_2O_5 /NPs was assessed using scanning electron microscope (SEM). Figure 1 reveals the typical SEM images of the V_2O_5 /NPs sample. It was found that the

synthesized V_2O_5 /NPs have a spherical shape with diameter ~ 35 nm.

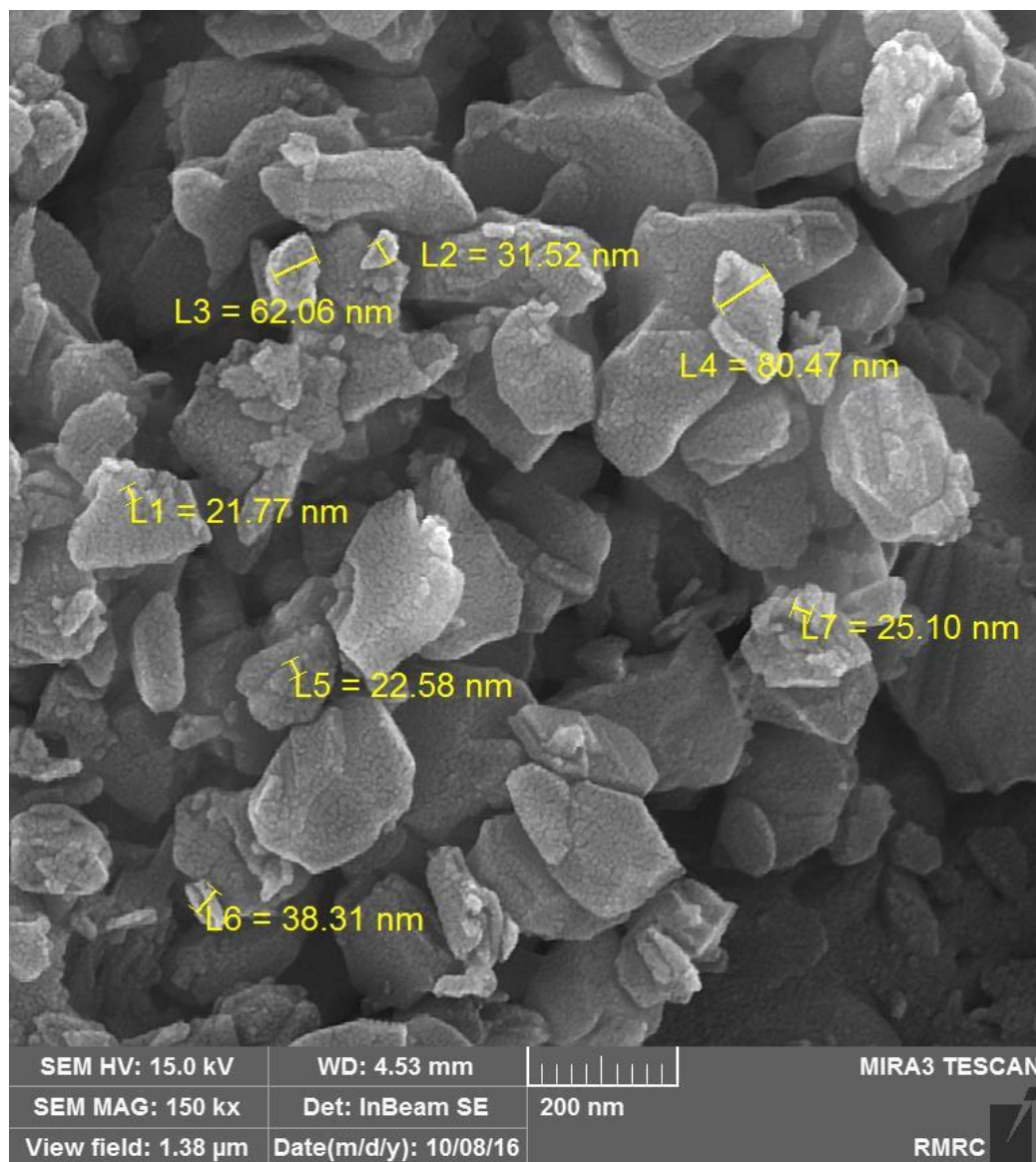


Figure 1. SEM image of V_2O_5 nanoparticles

Electrochemical properties of levodopa on V_2O_5 /GCE surface

The electrochemical behaviour of levodopa is dependent on the pH value of the aqueous solution (Figure 2). Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of levodopa. Thus the electrochemical behaviour of levodopa was studied in 0.1 M PBS in different pH values ($2.0 <$

$pH < 9.0$) at the surface of V_2O_5 /GCE by CV. It was found that the electrocatalytic oxidation of levodopa at the surface of V_2O_5 /GCE was more favoured under neutral conditions than in acidic or basic medium, because the obtained current was more than other pHs. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of levodopa oxidation at the surface of V_2O_5 /GCE.

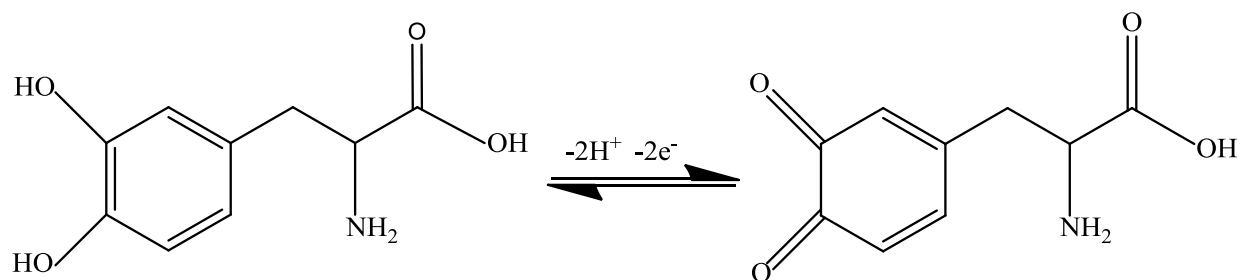


Figure 2. Electrochemical oxidation of levodopa

Figure 3 shows responses of CV to electro-oxidation of 100.0 μM levodopa at the unmodified GCE (curve b) and $\text{V}_2\text{O}_5/\text{GCE}$ (curve a). The peak potential occurs at 350 mV due to levodopa oxidation, which is around 300 mV more negative than the unchanged GCE. Furthermore,

$\text{V}_2\text{O}_5/\text{GCE}$ exhibits very high anodic peak currents for levodopa oxidation than that of the unchanged GCE. This showed a significant improvement of the electrode performance toward levodopa oxidation by changing the constant GCE with V_2O_5 nanoparticles.

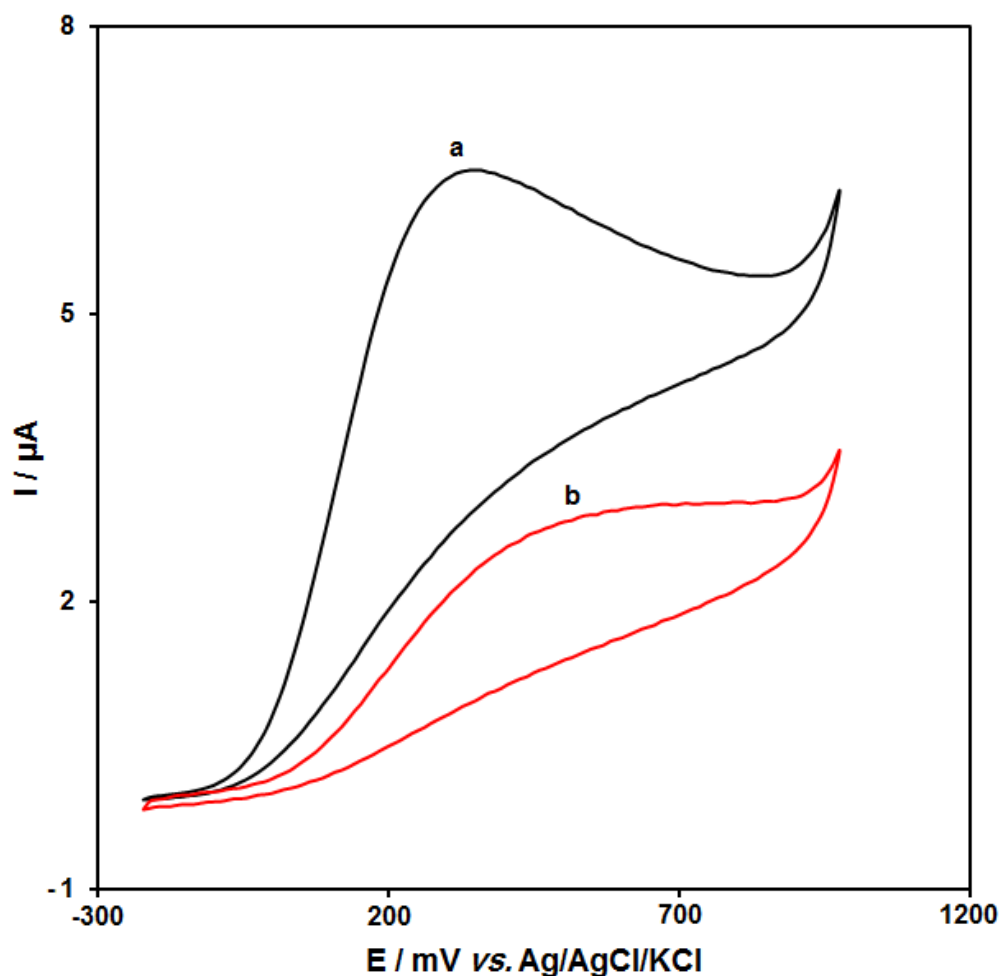


Figure 3. CVs of a) $\text{V}_2\text{O}_5/\text{GCE}$ and b) unmodified GCE in the presence of 100.0 μM of levodopa at pH 7.0. In all cases, the scan rate was 50 mV s^{-1}

Effect of scan rate

Researchers investigated the impact of the rates of potential scan on levodopa oxidation current (Figure 4). Findings indicated induction of enhancement in the current of the peak by the increased

potential scan rate. Additionally, diffusion in oxidation processes are monitored, as inferred by the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) [28].

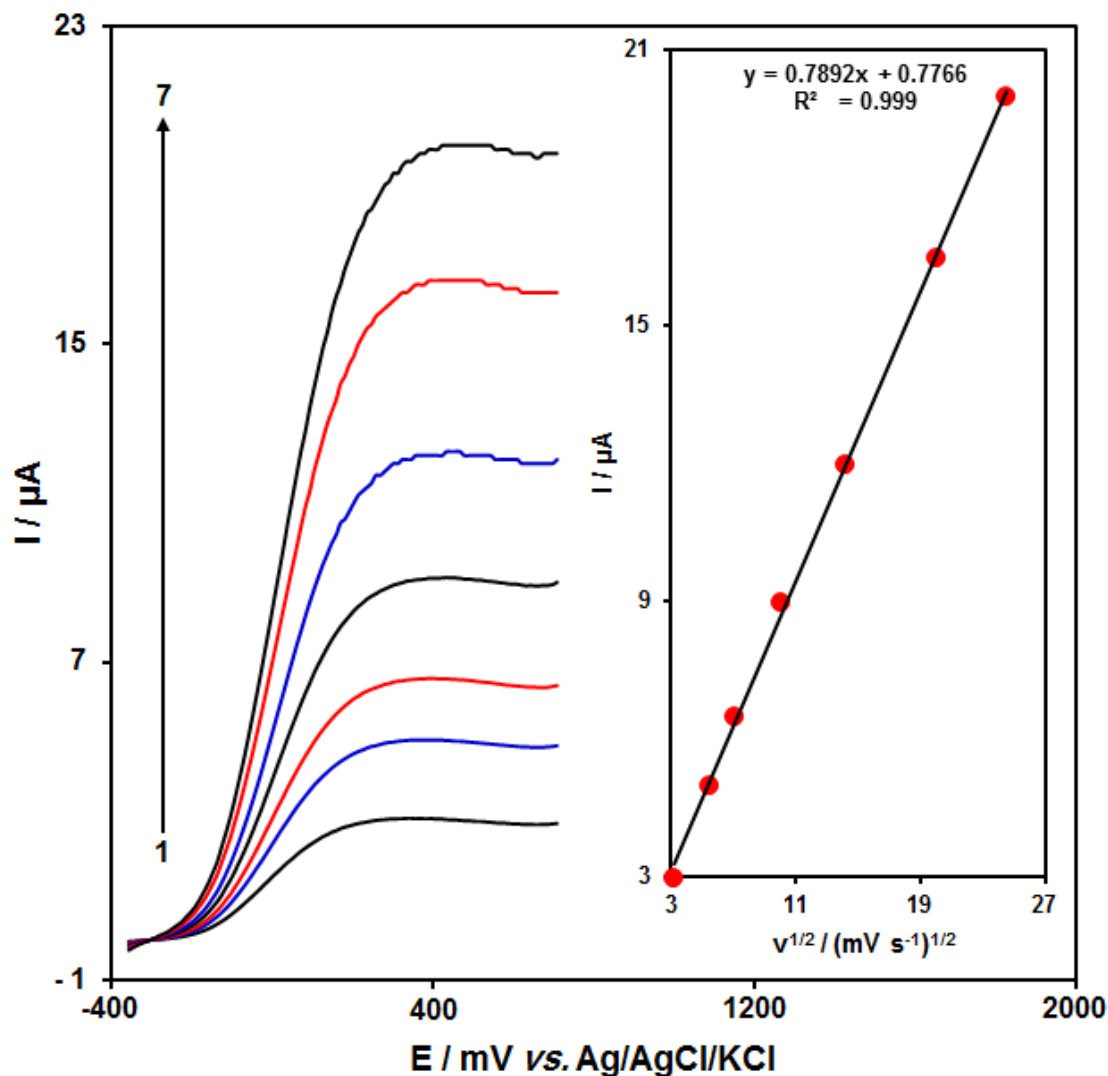


Figure 4. LSVs of $\text{V}_2\text{O}_5/\text{GCE}$ in 0.1 M PBS (pH 7.0) containing 100.0 μM of levodopa at various scan rates; numbers 1–7 correspond to 10, 30, 50, 100, 200, 400 and 600 mVs^{-1} , respectively. Inset: Variation of anodic peak current vs. square root of scan rate

Chronoamperometric analysis

Chronoamperometric measurements of levodopa at $\text{V}_2\text{O}_5/\text{GCE}$ were conducted by adjusting the working electrode potential at 0.4 V versus Ag/AgCl/KCl (3.0 M) for different concentrations of levodopa (Figure 5) in PBS (pH 7.0), respectively. For electroactive materials

(levodopa in this case) with a diffusion coefficient of D , the Cottrell equation describes current seen for electrochemical reaction at the mass transport limited condition [28].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

where D and C_b respectively represent diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and bulk concentration (mol cm^{-3}). Experimental plots of I versus $t^{-1/2}$ were used with the best fits for various concentrations of levodopa (Figure 5A). Then, the

resultant straight lines slopes were drawn against levodopa concentrations (Figure 5B). According to the resultant slope and the Cottrell equation, mean value of D was $4.6 \times 10^{-5} \text{ cm}^2/\text{s}$ for levodopa.

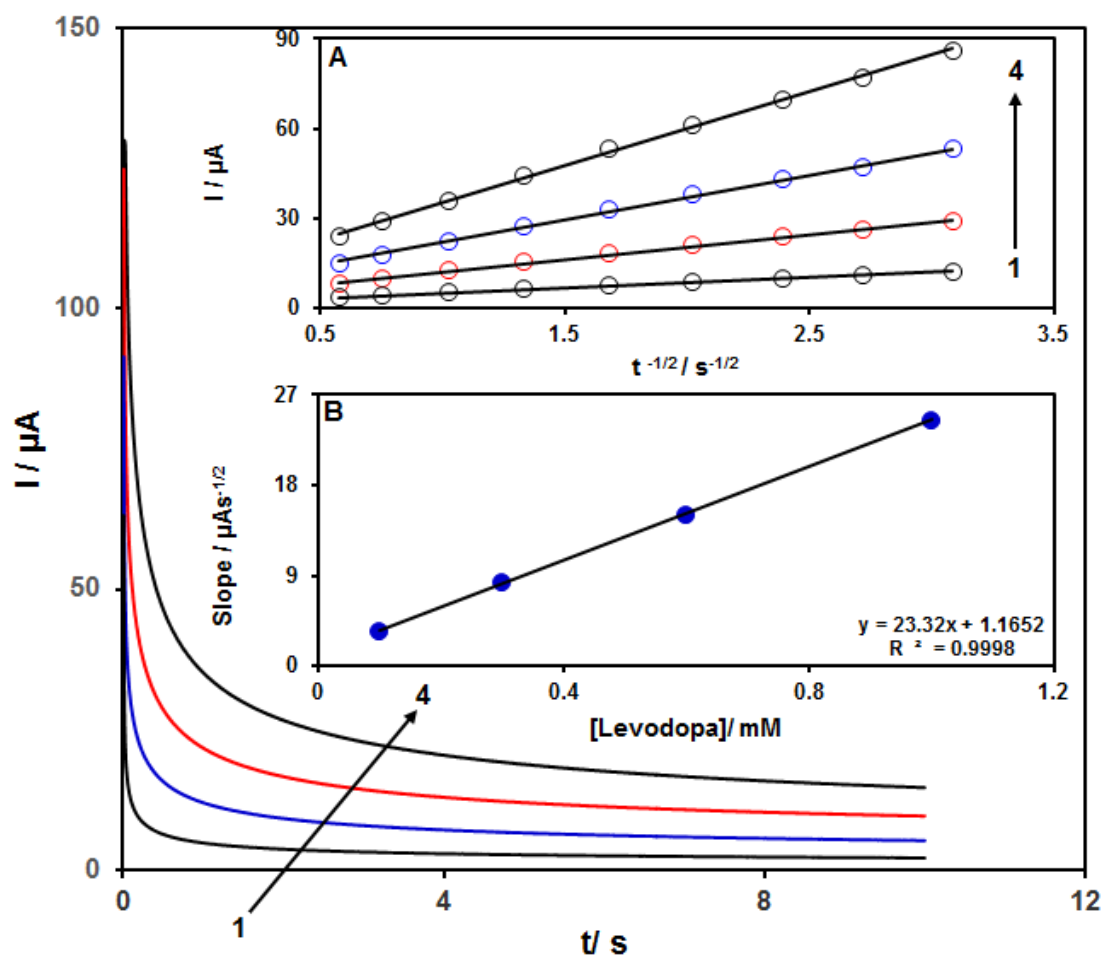


Figure 5. Chronoamperograms obtained at $\text{V}_2\text{O}_5/\text{GCE}$ in 0.1 M PBS (pH 7.0) for different concentrations of levodopa. The numbers 1-4 correspond to 0.1, 0.3, 0.6 and 1.2 mM of levodopa. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1-5. (B) Plot of the slope of the straight lines against levodopa concentrations

Calibration curve and LOD

The electro-oxidation peak currents of levodopa at $\text{V}_2\text{O}_5/\text{GCE}$ surface can be applied to define levodopa in the solution. Since the increased sensitivity and more suitable properties for analytical utilizations are considered as the benefits of differential pulse voltammetry (DPV), $\text{V}_2\text{O}_5/\text{GCE}$ in 0.1 M PBS consisting of different distinct concentrations of levodopa was used to

conduct DPV experiments (Figure 6). It was found that the electrocatalytic peak currents of levodopa oxidation at $\text{V}_2\text{O}_5/\text{GCE}$ surface linearly depended on levodopa concentrations above the range of 0.06-200.0 μM (with a correlation coefficient of 0.999), while determination limit (3σ) was achieved to be 0.02 μM . In the case of tryptophan electrocatalytic peak currents of tryptophan oxidation at the

surface of V_2O_5 /GCE were linearly dependent on the tryptophan concentrations, over the range of 5.0×10^{-6} M– 4.0×10^{-4} M (with a

correlation coefficient of 0.999) and the detection limit (3σ) was obtained 2.0×10^{-6} M.

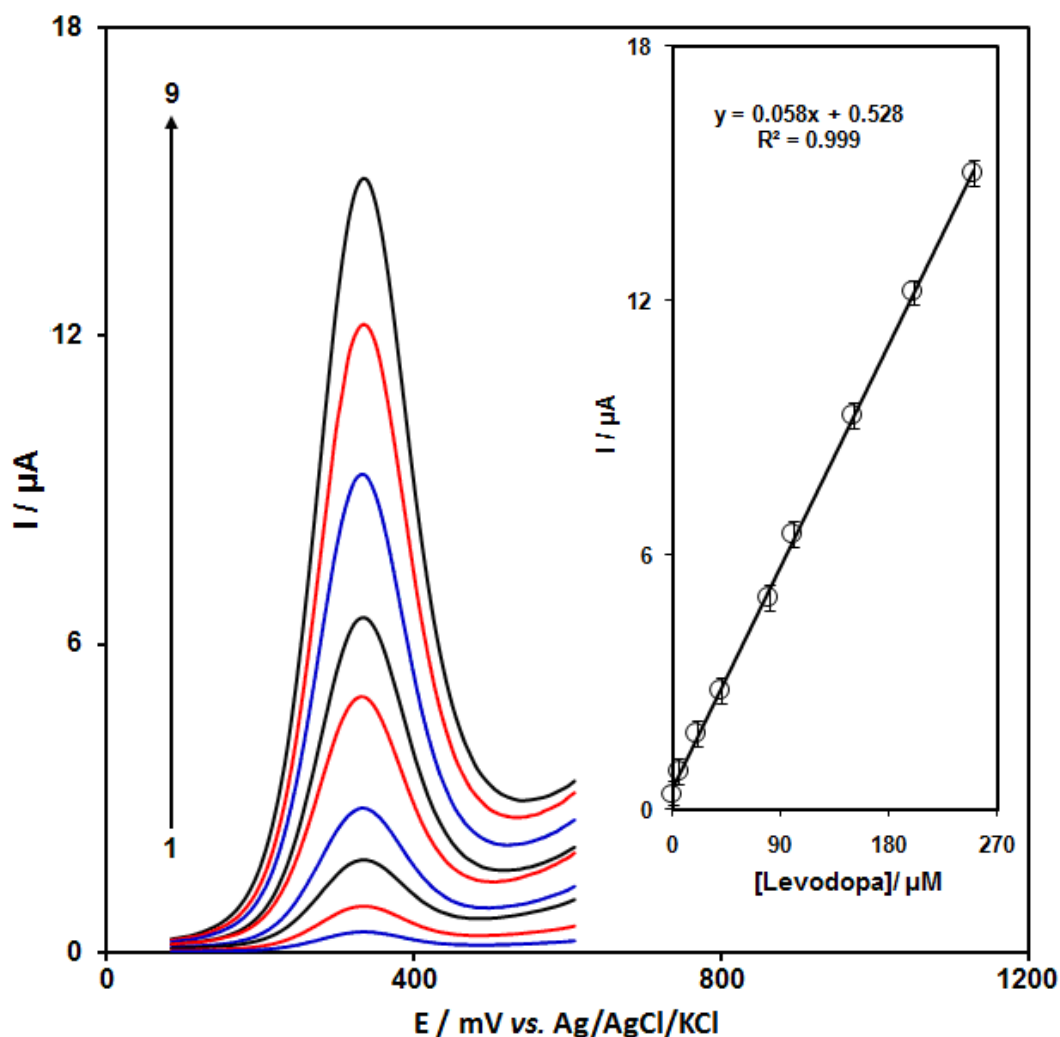


Figure 6. DPVs of V_2O_5 /GCE in 0.1 M PBS (pH 7.0) containing different concentrations of levodopa. Numbers 1-9 correspond to, 0.06, 5.0, 20.0, 40.0, 80.0, 100.0, 150.0, 200.0 and 250.0 μM of levodopa. The inset shows the plot of the peak current as a function of the levodopa concentration in the range of 0.06-250.0 μM

Simultaneous determination of levodopa and tryptophan

To the best of our knowledge, there is no report on using a GCE modified with V_2O_5 nanoparticles for determining levodopa and tryptophan. Moreover, due to reality that electrochemical detection of levodopa in the front of tryptophan with the help of un-modified electrodes has the caveat of interventions by tryptophan because of relative adjacent oxidation

capacities of the two specimens, it can be regarded a crucial phase. Such a phase has been conducted by simultaneous alterations of analytes concentrations and achieving DPVs (Figure 7). Findings reported certain anodic at 330 and 700 mV for levodopa and tryptophan oxidation, proving using the V_2O_5 /GCE, these two analytes can be detected without severe interventions from each another.

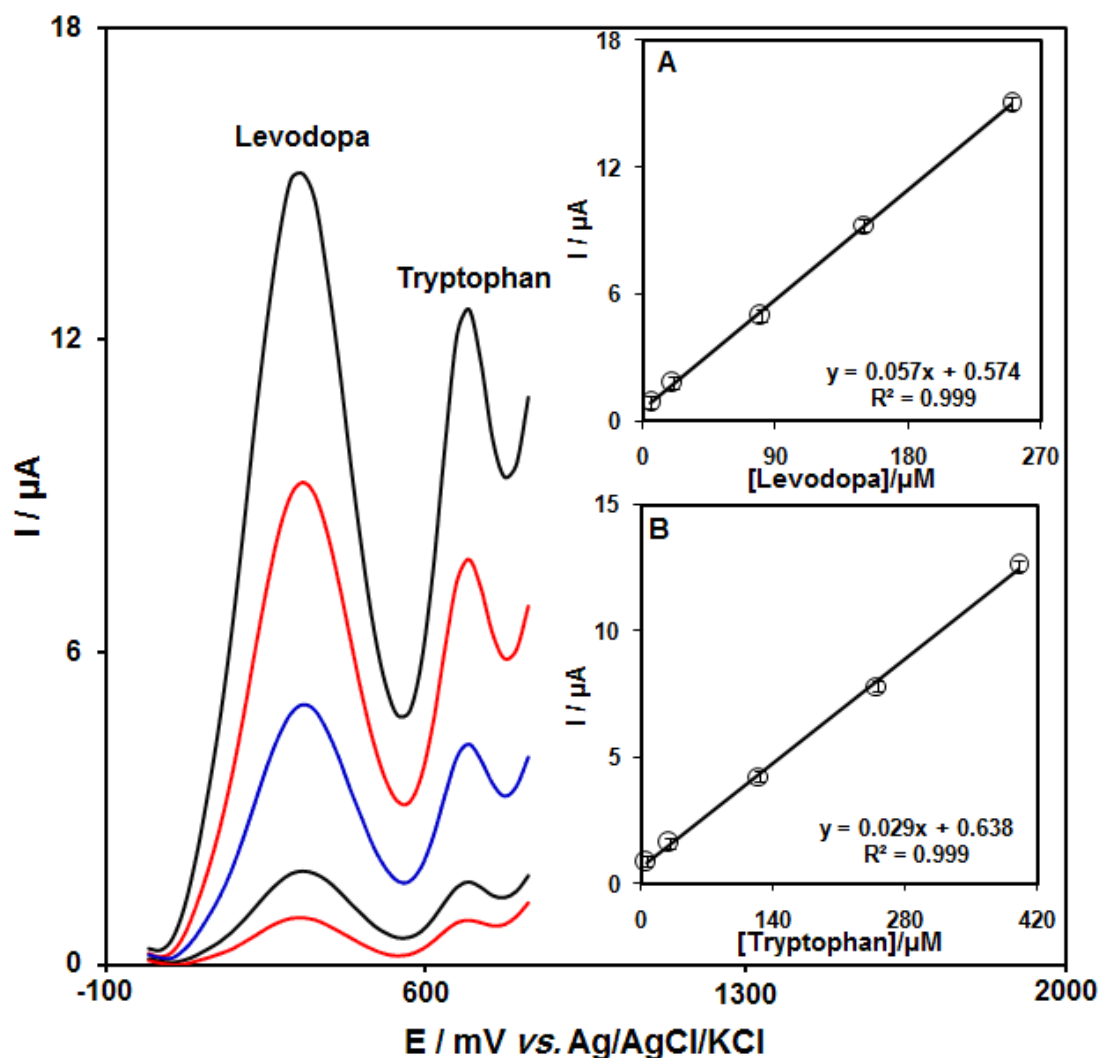


Figure 7. DPVs achieved at $\text{V}_2\text{O}_5/\text{GCE}$ surface in 0.1 M PBS (pH 7.0) consisting of various concentrations of levodopa and tryptophan. DPVs from internal to external respectively is corresponding to 5.0+5.0, 20.0+30.0, 80.0+125.0, 150.0+250.0 and 250.0+400.0 μM of levodopa and tryptophan. Insets: (a) plot of I_p versus levodopa concentration and (B) plot of I_p versus tryptophan concentration

Analysis of real samples

To assess the applicability of the modified electrode to determine the levodopa and tryptophan in real samples, the described method was applied to the determination of the levodopa and tryptophan in urine

sample. Therefore, the standard addition technique was applied (Table 1). Acceptable recoveries of levodopa and tryptophan were observed, and reproducible results were shown with regard to the mean relative standard deviation (R.S.D.).

Table 1. Comparison of the efficiency of some modified electrodes used in detection of levodopa

Method	Electrode	Modifier	Linear range (μM)	Detection limit (μM)	Ref
Voltammetry	Glassy carbon	rGO	2.0-100.0	1.13	29
Voltammetry	Carbon paste	FCMCNPE	2.0-500.0	1.2	30
Voltammetry	Carbon ionic liquid	Nafion/Co(OH) ₂ -MWCNTs	0.25-225.0	0.12	31
Voltammetry	Glassy carbon	poly(2,5-diamino benzene sulfonic acid)- β -cyclodextrine	1.0-200.0	0.41	32
Voltammetry	Carbon paste	BNH/TiO ₂	2.0-600.0	0.2	33
Voltammetry	Glassy carbon	V ₂ O ₅	0.06-250	0.02	This work

Conclusion

In this research study, a V₂O₅/GCE was developed for the electrochemical simultaneous determination of levodopa and tryptophan. In comparison with the previously reported methods for electrochemical determination of levodopa, the presented method has many advantages, such as simple fabrication, low detection limit and low cost. The V₂O₅/GCE shows a good electrochemical response for determination of levodopa with the wide linear range between 0.06 and 250.0 μM and the limit of detection was calculated to be 0.02 μM . Moreover, V₂O₅/GCE operated well in the simultaneous determination of levodopa and tryptophan in real samples with great accuracy and precision.

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