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Scientific paper

Rapid Electrochemical Method for the Determination of L-DOPA in Extract From the Seeds of *Mucuna Prurita*

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Abstract

This work presents the electrochemical behavior of levodopa (L-DOPA), at boron-doped diamond (BDD) electrodes, using cycling voltammetry (CV), in Britton-Robinson (BR) buffer solution, and application of the proposed electrode for the determination of L-DOPA in extracts from the seeds of velvet bean (*Mucuna prurita* Hook or *Mucuna pruriens* (L.) DC.). L-DOPA provides a well-defined and single oval-shape oxidation peak at +0.8 V vs. Ag/AgCl (3 M KCl) reference electrode in BR buffer solution at pH 3.0. Experimental parameters, such as pH of supporting electrolyte and square wave voltammetry (SWV) operating parameters (frequency and modulation amplitude) were optimized. The effect of possible interferences was evaluated. Under optimal conditions the detection limit of the developed method was 0.8 μM and the calibration curve for L-DOPA was linear in the range from 2 to 100 μM. The proposed method was successfully applied to the determination of L-DOPA in an extract from the seeds of *Mucuna prurita*. The obtained result was in good agreement with obtained by photometry with 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The developed approach can be beneficial for the quantification of L-DOPA using a BDD electrode as up-to-date potential alternative sensor for electroanalytical applications.

Keywords: L-DOPA, boron-doped diamond electrode, square wave voltammetry

1. Introduction

Mucuna prurita is a medicinal plant which is mentioned in the Indian system of medicines including folk medicines mostly due its management of diabetes, which is effective either individually or in combinations with other plants.[1] The seeds of this plant, which also possess anti-inflammatory effect, have been used as a tonic and aphrodisiac for male virility.[2] Anti-Parkinsonism effects have been reported for the seed, and this can be attributed to the L-DOPA presence. Levodopa shows high importance in the brain neurotransmission processes. The precursor of dopamine can cross the blood brain barrier and at the action site be converted to dopamine.[3] According to these statements reliable, a cost-effective, and sensitive analytical procedure for its quantification can play an important role in this field.

L-DOPA (levodopa, 3,4-dihydroxy-l-phenylalanine) is widely used as a source of dopamine in the treatment of most patients with Parkinson's disease and epilepsy.[4] This drug can be principally metabolized by an enzymatic reaction (dopa decarboxylase) to dopamine compensating for the deficiency of dopamine in the brain.[5] Together with the positive effect, several side effects are attributed to the long term use of this drug, such as paranoia and

dyskinesia.[6–7] Based on these facts, different analytical methods, usually based on chromatographic techniques and spectrophotometry, are employed for the quantification of this compound.[8–17] They possess satisfactory sensitivity and selectivity but the disadvantage of these methods lies in expensive instrumentation and time consuming sample preparation. Also, electroanalytical methods have been presented for the quantification of levodopa.[18–21] Some of the described methods require long time electrode preparation and limited life time which can strongly influence the reproducibility of these electrodes. Usage of novel solid electrode materials can be beneficial in the field of electroanalysis.

Boron doped diamond electrodes, nowadays, belong to the group of one of the best solid electrode materials. This electrode is widely investigated and has been found as powerful tool for the quantification of biologically active compounds.[22, 23]

Based on all these facts, the aim of this work was to develop a novel, simple, rapid and sensitive electrochemical method for the determination of L-DOPA in extracts from the seeds of *Mucuna prurita* with a boron-doped diamond electrode as electrochemical sensor. The voltammetric behavior of L-DOPA was investigated. After optimization of the experimental procedure and investigation of the influence of some interferents the method was successfully applied to the determination of L-DOPA in the real sample.

2. Experimental

2. 1. Chemicals and Solutions

3,4-Dihydroxy-L-phenylalanine (L-DOPA), ascorbic acid (AA), dopamine (DOP), uric acid (UA), boric acid, sodium hydroxide, ethanol, acetic acid and phosphoric acid were of analytical grade, purchased from Sigma Aldrich, USA.

Britton-Robinson buffer solution was used as supporting electrolyte and it was prepared by mixing aqueous solutions (0.04 M) of boric, phosphoric and acetic acid. The pH values were adjusted with sodium hydroxide (0.2 M).

All solutions were prepared using Millipore water. Stock solution of the L-DOPA (10^{-3} M) was prepared in 50% aqueous ethanol. Calibration standard solutions were prepared from the stock solution by appropriate dilution with supporting electrolyte.

2. 2. Apparatus

Cyclic voltammetric (CV) and square-wave voltammetric (SWV) measurements were performed using an electrochemical system AUTOLAB PGSTAT 302N, Methrom Autolab B.V. (The Netherlands) controlled by the corresponding software (NOVA 1.10). The cell (10 mL) consisted of a three-electrode system, the boron-doped diamond electrode (inner diameter: 3 mm; Windsor Scientific Ltd., Slough, Berkshire, United Kingdom), an Ag/AgCl (saturated KCl) reference electrode and a Pt counter electrode. All potentials reported in this paper are given vs. the Ag/AgCl (3 M KCl) reference electrode at an ambient temperature. All pH values were measured with a pH meter model Orion 1230, equipped with a WTW combined electrode.

The potential was swept over the range from 0 to +1.2 V (vs. Ag/AgCl) at different scan rates for CV, and from 0.3 to + 1.2 V at the optimized instrumental parameters (step potential 5 mV, frequency 30 Hz, and modulation amplitude 60 mV) for square-wave voltammetry.

2. 3. ABTS Method

$ABTS^{\bullet+}$ radical solution was prepared by mixing equal volumes of the ABTS stock solution (7 mM in water) with 2.45 mM of potassium persulfate. This mixture was allowed to stand for 12–16 h at room temperature in the dark. For ABTS determinations different concentrations of L-DOPA solutions were prepared in the same range as for SWV (2 – 100 μM). The standard solutions of L-DOPA were mixed with the $ABTS^{\bullet+}$ radical solution (to made $ABTS^{\bullet+}$ concentration about 65 μM), and the absorbance was measured at 734 nm. The absorbance of $ABTS^{\bullet+}$ was adjusted to 0.7 by dissolving with ethanol (blank), and 10 μM of standard L-DOPA solution was added in 1 mL of $ABTS^{\bullet+}$. Five min was allowed to produce inhibition of the blank absorbance. The same procedure was applied for sample analysis and the results were calculated from the calibration curve.

2. 4. Sample Preparation

Dried seeds of *Mucuna prurita* were purchased from the store Magic Garden Seeds, Regensburg, Germany, in the year 2012. The extraction procedure was done according to previously described literature data.[24] The powdered plant material (3.2270 g) was soaked with 0.1 M HCl (10 mL) for 1 hour. The prepared mixture was placed in a water bath (80 °C) for 5 min, and cooled to room temperature. After the addition of ethanol (10 mL) and shaking (10 minutes), the mixture was centrifuged (10 min, 4000 rpm, Rotine 420R, Hittech, Germany). The supernatant was collected and the extraction with ethanol was repeated. Finally, the combined supernatants were diluted with ethanol to a volume of 50 mL and were stored in the fridge at 4 °C.

3. Results and Discussions

3. 1. Effect of pH and Scan Rate on the Voltammetric Behavior of L-DOPA at a Boron-doped Diamond Electrode

The effect of pH on the peak current (CV) for the oxidation of L-DOPA was evaluated with BR buffer solu-

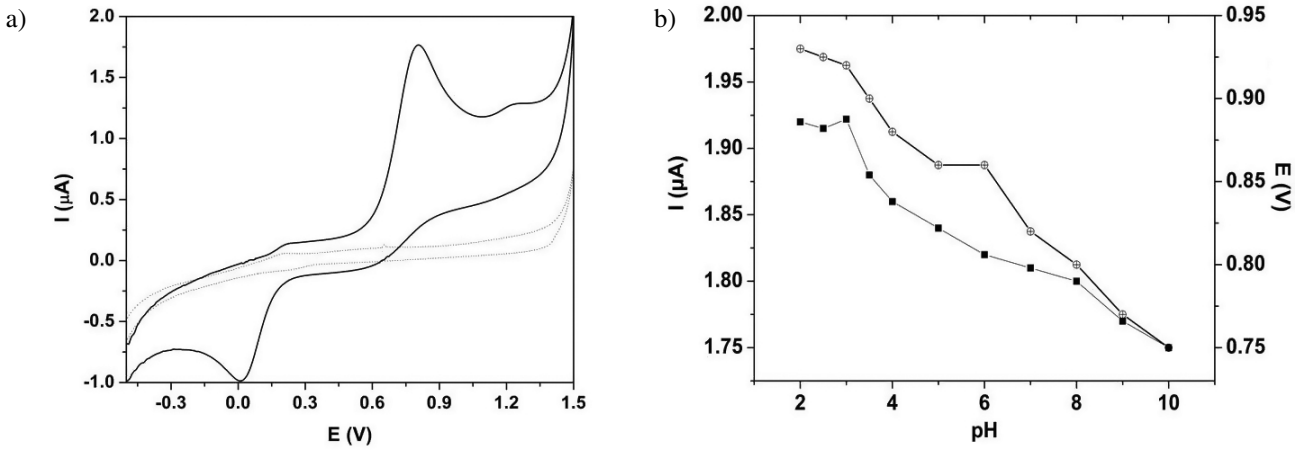

Figure 1. a) Cyclic voltammograms of L-DOPA in BR buffer solution (pH 3.0) at a boron doped diamond electrode; dotted line – blank; L-DOPA concentration 0.1 mM. b) Dependence of peak current (full dots) and peak potential from the pH of the supporting electrolyte.

tion as a supporting electrolyte. L-DOPA (0.1 mM) provides a well-defined peak in acidic medium at a potential of around + 0.8 V (Fig. 1. A). The highest oxidation peak current was obtained in a supporting electrolyte solution at pH 3 (Fig. 1. B). With increasing pH the obtained signal becomes broader with a concomitant decrease of the peak current. For all further experiments pH 3.0 was chosen as supporting electrolyte. On the reverse scan the analyte provides a reduction peak at a potential around 0.0 V. With a potential difference between the anodic and cathodic peaks of around 0.8 V, the electrochemical redox behavior of L-DOPA at BDD electrode can be considered as a quasi-reversible process.

In order to investigate the nature of the electrochemical reaction of L-DOPA at a BDD electrode, the effect of the scan rate was evaluated in BR buffer solution at pH 3.0. From Fig. 2 A and B it is obvious that an increase of the scan rate is followed with a linear increase of both peak currents. The current is linearly dependent on the square root of the scan rate. The corresponding equations obtained from these measurements are $I_a\,(\mu A) = 0.2469 \times v^{1/2}(V\ s^{-1})^{1/2} + 0.0531$, ($R^2$ = 0.9961) and $I_c\,(\mu A) = -0.1570 \times v^{1/2}(V\ s^{-1})^{1/2} + 0.1331$, ($R^2$ = 0.9992), where I_a presents the oxidation peak current and I_c the corresponding reduction peak current.

Both currents are linearly dependent on the square root of the scan rate, which suggests that both processes, oxidation and reduction are diffusion controlled. Small shifts in the peak potentials when varying the scan rate confirm that the electrochemical reactions are quasi-reversible.

3. 2. Optimization of Square Wave Voltammetric Parameters

Electrochemical methods due to their characteristics, offer rapid, low-cost, sensitive and selective procedures for

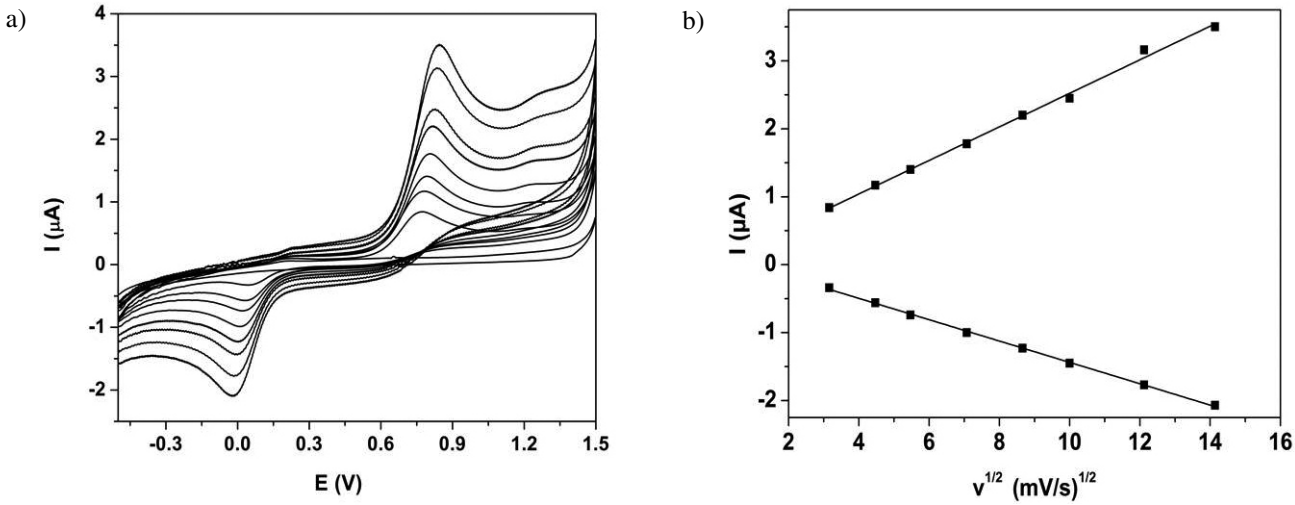

Figure 2. a) Cyclic voltammograms of L-DOPA (0.1 mM) at a BDDE ; supporting electrolyte BR buffer solution pH 3.0; scan rates 10, 20, 30, 50, 75, 100, 150 and 200 mV/s. b) Dependences of the oxidation and reduction peak currents from the square root of the scan rate.

the quantification of numerous biologically active compounds. Square wave voltammetry with optimized instrumental parameters can become a suitable method for the determination of these compounds, due to its low background current and low detection limit. According to this, the most important SWV instrumental parameters, such as modulation amplitude and frequency were investigated (data not shown). All experiments were done with 0.1 mM L-DOPA in Britton-Robinson buffer solution at pH 3.0.

The influence of the modulation amplitude on the oxidation peak current of L-DOPA was studied in the range from 10 to 100 mV. Other parameters were at fixed value, i.e., the frequency was 10 Hz and the step potential 5 mV. With increase of the modulation amplitude, the peak current increased rapidly to a value of 60 mV. Further increase of the modulation amplitude produced a wider peak shape and a decrease of the current. Thus, a value of 60 mV was selected as optimum for the determination of L-DOPA, and was used for all further experiments. When varying the frequency from 10 to 100 Hz with modulation amplitude of 60 mV, the peak current increased up to a value of 30 Hz. At higher frequency the peak current leveled off to a constant value and finally even decreased. The most suitable peak was observed at a frequency of 30 Hz. Taking into account peak current and peak resolution the optimized experimental conditions (amplitude of 60 mV and frequency of 30 Hz) were used for all further experiments.

3. 3. Calibration Curve

Calibration curves were constructed by plotting the oxidation peak current against the concentration of the analyte. The dependence was linear in the range from 2 to 100 μM (Fig. 3 A and B). The corresponding regression equation obtained from these measurements can be expressed as I_a *(μA) = 0.0294 c (μM) + 0.0068* (R^2 = 0.9989), with a detection limit 0.8 μM. Under optimized experimental conditions the repeatability from 6 measurements (c = 10 μM) was 2.3 %. This value indicates that the proposed method has excellent repeatability. Characteristics of the developed methodology are comparable or better than those proposed in the literature. [12] The advantages of the developed method lie in the use of a solid electrode without any modification, wide dynamic range and possible application in complexes matrices such as extracts from plants containing L-DOPA.

3. 4. Effect of Possible Interferences

Ascorbic acid (AA), uric acid (UA) and dopamine (DOP) are frequently accompanying L-DOPA in drugs and/or in urine samples; their effect as possible interferents on the peak current obtained for L-DOPA (c = 0.1 mM) was examined. For this purpose the mixed solution method was used with a concentration of all three tested interfering compounds of 0.1 mM. From Fig. 4 it can be concluded that L-DOPA shows an oxidation peak potential similar to the interferences, which results in an increase of the current. In the presence of ascorbic acid and uric acid (c_{AA} = c_{UA}) the peak current for L-DOPA increased for 10–15 %. In the case of dopamine (c_{DOP} = 0.1 mM) the peak current obtained for L-DOPA increased for 45 %. The resulting voltammograms of L-DOPA are presented in Fig. 4 B-D.

3. 5. Analytical Application

The proposed method was applied for the determination of L-DOPA in extracts from the seeds of *Mucuna*

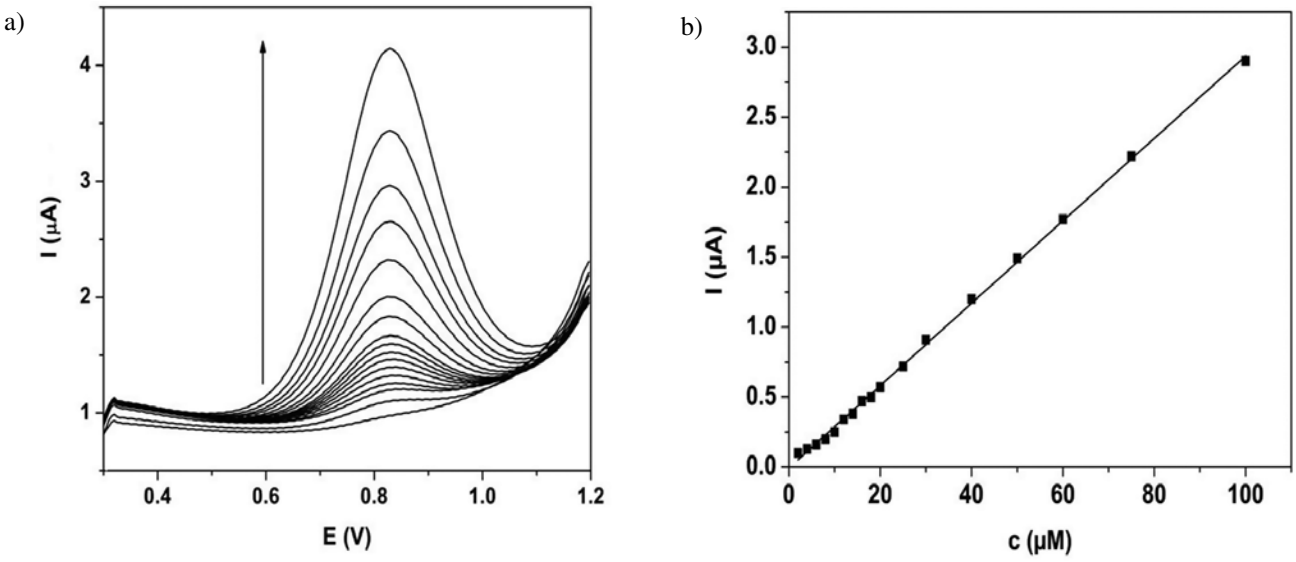

Figure 3. a) SW voltammograms of L-DOPA at a BDDE obtained for different concentrations (2 to 100 μM) under optimized experimental conditions. b) Calibration curve obtained from these measurements.

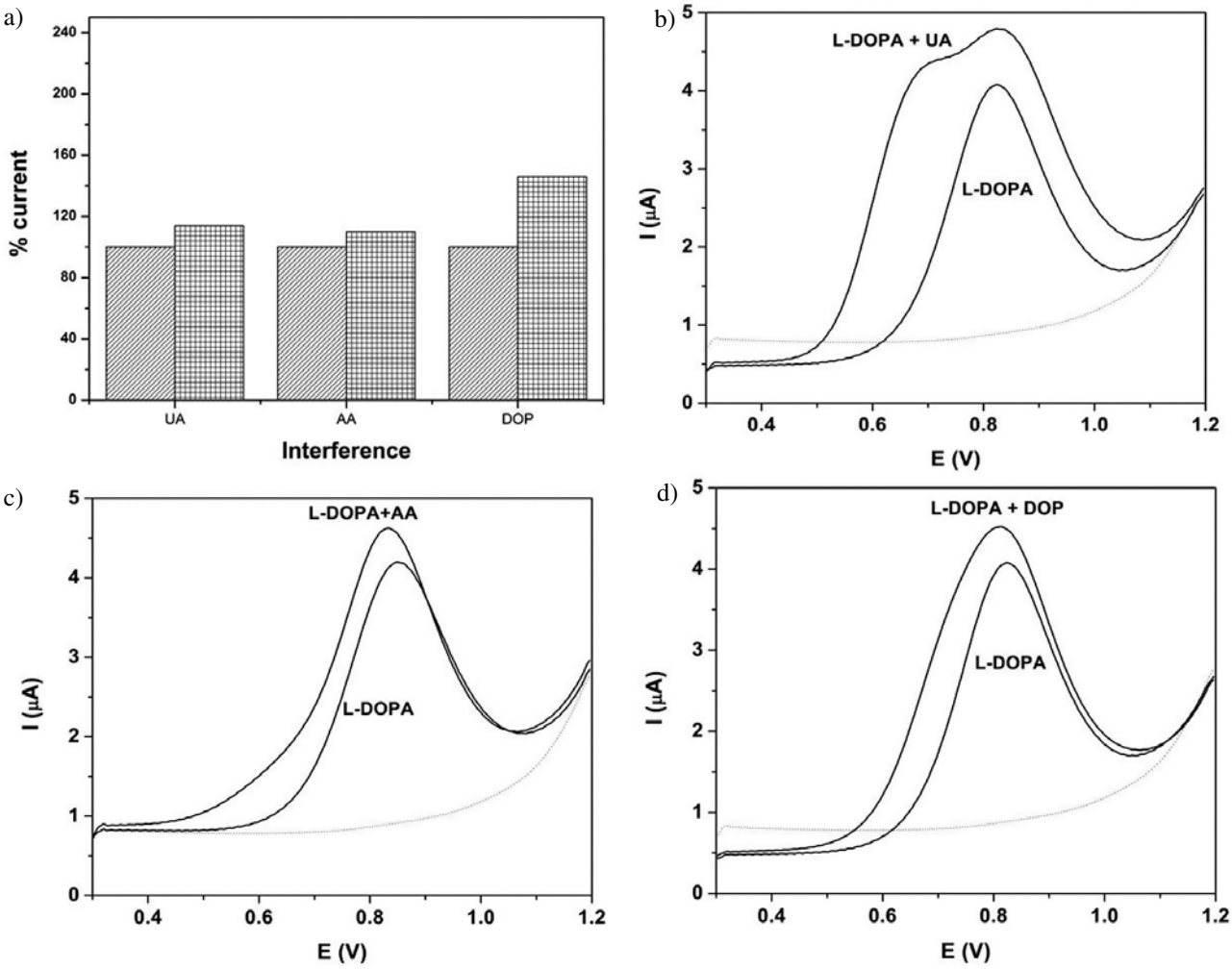

Figure 4. a) Relative peak currents of L-DOPA (0.1 mM) in the absence (left bars) and in the presence (right bars) of interferents (0.1 mM) under optimized experimental conditions. B–d) presents voltammograms from these measurements, for uric acid (UA), ascorbic acid (AA) and dopamine (DOP), respectively.

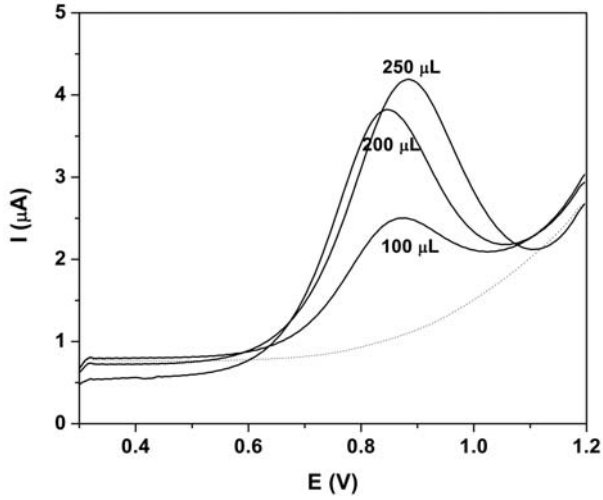

Figure 5. SW voltammograms of a velvet bean extract recorded with a BDDE for different sample volumes (100, 200 and 250 μL in 10 mL of total volume) by the proposed electroanalytical method under optimized conditions.

prurita. Three different volumes of sample of 100, 200 and 250 μL were diluted with supporting electrolyte to make final volume of the 10 mL and signals were recorded with SWV at BDD electrode, under optimized experimental conditions. Representative voltammograms are presented in Fig. 5. All measurements were repeated three times and concentrations of L-DOPA were calculated from the calibration curve. ABTS method was used as a

Table 1. Determination of the L-DOPA content in extract from the seeds of *Mucuna prurita*

Sample	Proposed method L-DOPA (%)	Reference method L-DOPA (%)	Literature data[24] L-DOPA (%)
Extract from seeds of *Mucuna prurita*	5.1 ± 0.1	5.1 ± 0.1	3.1–6.1

reference method for comparison of the results obtained for the contents of L-DOPA in the extract. According to the data presented in Table 1, it can be concluded that the result obtained with the proposed method is in good agreement with the value obtained by the spectrophotometric method and corresponds to the literature data.[24]

4. Conclusions

Using a boron-doped diamond electrode in combination with square wave voltammetry we developed a fast, low-cost, simple and sensitive method for the determination of L-DOPA in extracts from the seeds of velvet beans (*Mucuna prurita*). A detection limit of 0.8 μM and a dynamic working concentration range from 2 to 100 μM were obtained. It was found that dopamine strongly interfers with L-DOPA quantification and that the presence of ascorbic and uric acid, at the same concentration level as the analyte, cause an increase of the oxidation peak increase of 10–15%. The method was successfully applied to the determination of L-DOPA in a real sample. The results indicated that the developed methodology can be beneficial for the field of electroanalytical chemistry.

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Povzetek

Z uporabo ciklične voltametrije smo študirali elektrokemijsko obnašanje levodope (L-DOPA) na z borom dopirani diamantni elektrodi v Britton-Robinsonovem (BR) pufru. Proučili smo tudi možnost uporabe omenjene elektrode za določanje L-DOPA v ekstraktih iz semen rastlin *Mucuna prurita* Hook ali *Mucuna pruriens* (L.) DC. Optimizirali smo eksperimentalne parametre kot so pH nosilnega elektrolita in parametri (frekvenca in modulacija amplitude) voltametrije s kvadratnim spreminjanjem potenciala (SWV). Proučevali smo tudi vpliv možnih interferenc. Pri optimalnih pogojih je meja zaznave razvite metode 0,8 μM, umeritvena krivulja za L-DOPA pa linearna v območju od 2 do 100 μM. Metodo smo uspešno uporabili za določanje L-DOPA v izvlečkih pripravljenih iz semen *Mucuna prurita*.