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ELECTROCHEMICAL DETERMINATION OF L-DOPA IN PHARMACEUTICAL SAMPLES USING METALLOPHTALOCYANINES MODIFIED CARBON NANOTUBES PASTE ELECTRODES

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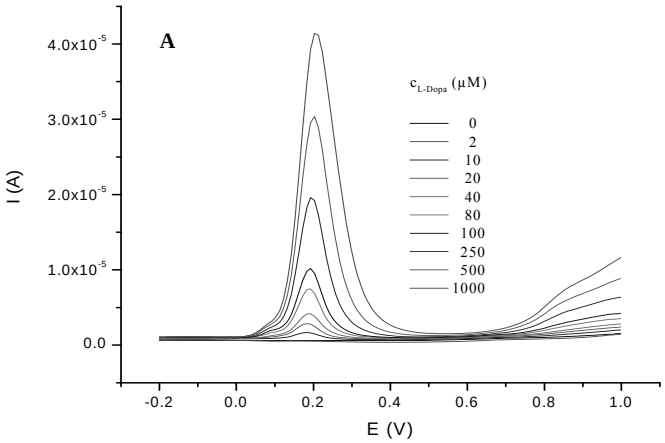
Levodopa (L-dopa), the biological precursor of catecholamines, is the most widely prescribed drug in the treatment of Parkinson's disease. The present work presents an application of a carbon nanotubes paste electrode (CNTPE) modified with metallo-phthalocyanines as an electrochemical sensor for L-dopa detection. Using the electrooxidation of L-dopa at +0.2 V in phosphate buffer solution of pH 7.0 at the FePc modified CNTPE a linear calibration curve was obtained from $10 \cdot 10^{-6}$ to $80 \cdot 10^{-6}$ M and a detection limit of $5.04 \cdot 10^{-7}$ M. The method was successfully applied for the determination of L-dopa in commercial dosage forms without any pre-treatment.



INTRODUCTION

Levodopa [(–)-3-(3,4-dihydroxylphenyl)-L-alanine], an unusual amino acid, is an important neurotransmitter, and has been used for the treatment of neural disorders such as Parkinson's disease (PD).[1] PD is believed to be related to low levels of dopamine (DA) in certain parts of the brain. Levodopa (L-dopa) is considered the most effective treatment available for PD. When L-dopa is taken orally, it crosses through the "blood–brain barrier"; once it crosses, it is converted to DA. The resulting increase of DA concentration in brain is believed to improve nerve conduction and assist the movement disorders in PD. Therefore the success of DA replacement therapy by its precursor, L-dopa, is a major landmark in the field of neurology.[2] L-dopa can alleviate the symptoms of Parkinson's disease and can also decrease muscular rigidity and tremors.[3,4] So the research about levodopa has an important practical significance.

Nevertheless, elevated levels of DA also cause adverse reactions such as psychosis nausea, emesis, hypotension and dyskinesia, vomiting and cardiac arrhythmias.[5-7] Therefore, in order to

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achieve a better curative effect and a lower toxicity, it is very important to rapidly control the content of L-dopa and its inhibitors and impurities in biological fluids and pharmaceutical formulations. In vitro, L-dopa is a lethal toxin to the culture of neurons and a few animal studies have shown that chronic L-dopa may be toxic in vivo, too.[8-11] Chronic L-dopa treatment in PD patients is frequently associated with some side effects such as nausea and vomiting results from the increases of plasma L-dopa level. Clearly the process of L-dopa detection and its concentration determination is an important feature in pharmaceutical and clinical procedures.[12,13]

Several techniques have been reported in the literature for the determination of L-dopa in pharmaceutical formulations and biological fluids such as spectrophotometry,[14-20] spectrofluorimetry,[21] ion-selective electrode,[9] NMR spectroscopy,[22] flow injection analysis (FIA),[10,14,23,24] high performance liquid chromatography (HPLC)[25-31] and capillary electrophoresis.[32-34] Nevertheless, each technique has often suffered from diverse disadvantages with regard to cost and selectivity, the use of organic solvents, complex sample preparation procedures or long analysis time.

Electrochemical methods are powerful techniques to follow the oxidation of catecholamines.[35] The two hydroxyl groups present in L-dopa can be electrochemically oxidized at a glassy carbon electrode and this is the basis for its determination.[9] Although the electrochemical behavior of L-dopa on glassy carbon electrodes is complex, its determination by voltammetric and amperometric methods is reported in the literature.[9,31, 36-44] Beside the fact that the methods based on electrochemical sensors have many advantages over the other methods, there are few papers on the determination of L-dopa in pharmaceutical formulations using the modified electrodes.[45-48]

Carbon nanotubes can be used to promote electron transfer reactions when used as electrode material in electrochemical devices, electrocatalysis and electroanalysis processes due to their significant mechanical strength, high electrical conductivity, high surface area, good chemical stability, as well as relative chemical inertness in most electrolyte solutions and a wide operation potential window.[49] The electronics properties of these nanomaterials have been exploited as means of promoting the electron transfer reaction for a wide range of molecules and biological species including: insulin,[50] carbohydrates,[51] hydrogen peroxide,[52] glucose,[53] norepinephrine,[54] aminophenol,[55] morin,[56] *cyto-chrome C*,[57] promethazine,[58] thiols,[59] methyldopa,[60] epinephrine[61] and nicotinamide adenine dinucleo-tide.[62]

The incorporation of electroactive materials into a carbon nanotubes paste electrode is advantageous and has been widely applied in the electroanalytical community.[63,64] Recently, immobilization of phthalocyanines and metallophthalocyanines at the surface of carbon nanotubes has been achieved.[65] The resulting phthalocyanine-nanotube complexes (nanocomposites) possess the catalytic properties of phthalocyanine without any destruction of the electrical properties and structures of the nanotubes and thus noncovalent functionalization of carbon nanotubes is important for developing new nanomaterials.

To the best of our knowledge, the electrochemical determination of L-dopa using a carbon nanotubes paste electrode modified with iron(II) phthalocyanine (FePc) has not been reported. Thus, in continuation of our studies concerning the preparation of chemically modified electrodes,[66,67] in this paper, we described the preparation and suitability of a FePc modified carbon nanotube paste electrode (FePcCNTPE) for the determination of L-dopa in an aqueous buffer solution. The analytical performance of the modified electrode has been evaluated for the determination of L-dopa in commercial dosage forms (Madopar) without any pre-treatment with satisfactory results.

EXPERIMENTAL

1. Reagents and solutions: Levodopa, Co(II) phthalocyanine and Fe(II) phthalocyanine were analytical grade all purchased from Sigma-Aldrich, Germany. L-dopa working solutions were prepared just before use by diluting the stock solutions in phosphate buffer solution of pH 7.0 with double distilled water. Potassium hexacyanoferrate(III) (Merck, Germany) was used to prepare a redox probe solution of $1.0 \cdot 10^{-3}$ mol L^{-1} $K_3[Fe(CN)_6]$ in 1.0 mM phosphate buffer solution (PBS) of pH=7.0. The buffer solution was prepared from phosphate salts: Na_2HPO_4 and NaH_2PO_4 (Merck) in double distilled water. All solutions were freshly prepared using twice distilled water. Carbon powder and paraffin oil (d_4^{20} = 0.88 g·cm^{-3}) were obtained from Fluka. Short multi-wall carbon nanotubes powder (main range of diameter 40–60 nm; length 1–2 μm; purity p > P95.0%; ash < 0.2 wt.%; special surface area 40–300 m^2 g^{-1}; amorphous carbon <3%), was purchased from China, Shenzhen Nanotech Part Co., Ltd. Tablets of Madopar® Roche were used as pharmaceutical samples.

2. Instrumentation: All electrochemical experiments were performed using a potentiostat/galvanostat system Autolab PGStat 12 controlled by General Purpose Electrochemical

System (GPES) electrochemical interface for Windows (version 4.9.007). A conventional three electrodes in one-compartment cell (10 mL) was used with the electrodes system consisted of an unmodified or a FePc chemically modified carbon nanotubes paste electrode as working, Ag|AgCl|KCl 3M as reference and a platinum wire as auxiliary electrode. A pH-multimeter was also used for pH measurements.

3. Preparation of the electrodes: Firstly an unmodified carbon paste electrode (CPE) was prepared by introducing a carbon paste into a plastic tube (1.0 mL polyethylene disposable syringe). The carbon paste consists in 70:30 (w/w) mixture of carbon powder with paraffin oil blended by hand for 15 minutes until it became homogenous (an uniformly wetted paste). Electrical contact was made by inserting a copper wire into the plastic tube filled with carbon paste. This electrode was used in as conducting substrate for preparing the following modified electrodes CP/CNTs, CP/ FePcCNTP and CP/CoPcCNTP also.

Multi-wall CNTPEs were prepared by thoroughly hand mixing the CNTs powder with paraffin oil (70:30 w/w) in a mortar and pestle. Chemically modified CNTPEs were prepared in a similar fashion except that the CNTs powder was first mixed with the desired weight of modifier (CoPc or FePc). The content of the metallo-phthalocyanine (MePc) in the CME is described on a percent basis as the weight of the MePc added to the 1 g of CNTs powder. Both pastes deposited onto the top of the already prepared carbon paste and packed into 1.0 mL polyethylene disposable syringes with a copper wire being used for electrical contact. The MePc content of the modified electrodes was varied from 0.25% to 2%, the best activity was obtained at 2% for MePc and this content was used for all the reported experiments. The initial CMEs activity could always be immediately restored by simply removing the outer layer of paste by briefly smoothing the newly exposed portion. Mixed CNTPEs renewed in this manner exhibited stable and reproducible electrochemical behavior over weeks.

4 Analytical procedure: The unmodified and modified CNTPEs were pretreated by performing 10 cyclic voltammetric scans from -0.5 V to +1.5 V at a scan rate of 50 $mV \cdot s^{-1}$, in phospahate buffer solution pH = 7.0, this step assuring the electrode activation and stabilization. A 10 mL solution containing appropriate amounts of L-dopa in PBS (pH 7.0) was transferred into the voltammetric cell. Voltammograms were recorded by scanning from -0.2 to +1.4 V. The modified electrode was regenerated by successive washing with 0.1 M PBS (pH 7.0) and running several CV scans between -0.5 to +1.5 V in the buffer solution. Finally the electrode washed with double distilled water to remove all adsorbates from the surface and to provide a fresh surface before running the next experiments. All electrochemical experiments were carried out at room temperature.

5. Pharmaceutical sample solution preparation: 10 Madopar tablets (Roche South Korea) containing 50 mg of benserazide (as hydrochloride) and 200 mg of L-dopa were finely powdered in a mortar with pestle. Calculated amounts of the tablets required for $5.0 \cdot 10^{-3}$M L-dopa were separately transferred into 25 mL volumetric flask and were dissolved in PBS of pH 7.0. The content of the flask were sonicated for 5 min to obtain complete dissolution of the analytes. The sample solution was filtered and suitable aliquot of the clear filtrate was collected, diluted in order to obtain the concentration of 5.0 10^{-5} M L-dopa and stored in the refrigerator for further use.

RESULTS AND DISCUSSION

1. Electrochemical activation process

Initially the electrochemical pretreatment of unmodified and modified CPEs was examined. Fig. 1 shows the CVs obtained for $K_3[Fe(CN)6]$ at both untreated and pretreated modified CPEs.

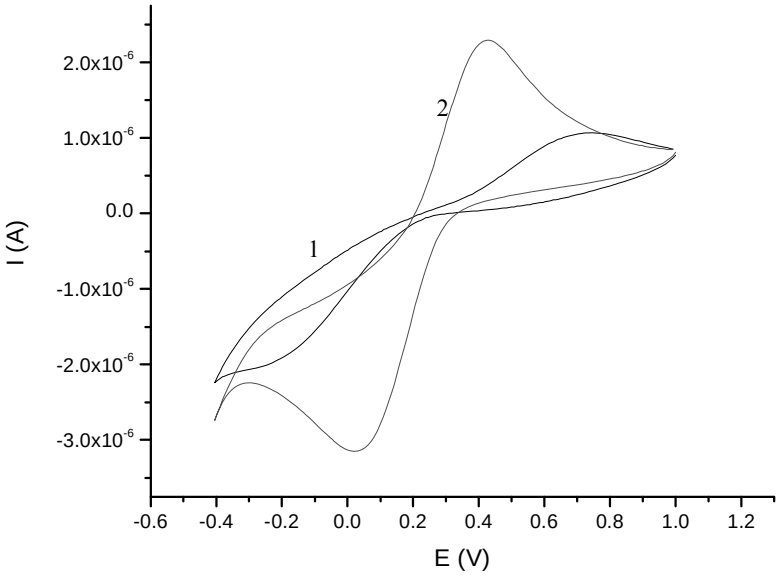
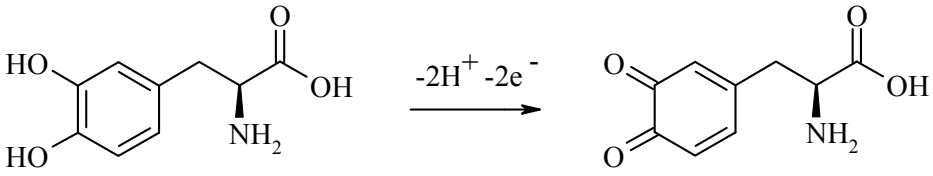


Fig. 1 – Cyclic voltammograms of 1.0 x 10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ in PBS pH=7.0 obtained at CPE: (black) before and (red) after electrochemical pretreatment; scan rate 50 mV s^{-1}. Pretreatment parameters: 10 CV scans, potential range -0.5 V to +1.50 V, scan rate 50 mV s^{-1}.



Scheme 1 – Redox mechanism of L-dopa.

It can be observed that the activation of CPE improves the shape of the cyclic voltammogram as it is expected for a reversible behavior, increases the peak currents recorded and reduces the peak-to-peak separation to the theoretical value for reversible redox couple.

It is known that the surface of a CPE is practically hydrophobic because of a layer created by the paraffin oil from the composition of the carbon paste. Activating the surface by multiscan-10 or 20 cycles (by CV) or by polarization at a certain potential value (*e.g.* chronoamperometry), the hydrophobic layer of the paraffin oil is interrupted by functional groups that contain carbon and oxygen. These groups can be hydrated thus the hydrophobic character of the surface of the electrode is changed. These is important specially when organic compounds are analyzed, the effect being the modification of the kinetics of the reaction that occurs at the electrode surface, the elimination of the undesired products from the surface of the electrode (for instance secondary products that block the active surface of the electrode), but also the increase of the sensibility [68,69] of the electrode.

As concerns the L-Dopa study using the CPE, a passivation process was recorded (as well for the case of classic bare electrodes of GC or Au). There was recorded a decrease of peak currents cycle by cycle that can be explained by the adsorption of the oxidation products of L-Dopa at the surface of the electrode. Thus a cleaning process is needed before any experiment: classical cleaning for GC or Gold-bare electrode or activation for the CPE.

L-Dopa suffers an irreversible oxidation process at the surface of electrodes (CPE, GC, Au) that involves 2 electrons and 2 protons as shown in Scheme 1.

In previous studies, it was shown that the anodic peak currents corresponding to the oxidation of L-Dopa increase with increasing pH until 6.5 - 7 but at higher values of pH the peak current tends to decrease.[7] Thus, a solution pH=7 was chosen for all the experiments, this value being also close to physiological pH.

2. Electrode surface influence

Preliminary experiments were carried out to realize the electrochemical behavior of L-dopa at different electrode surfaces including the MePcMWCNTs modified electrodes and unmodified electrode. We compared the voltammograms obtained with the CPE with the voltammograms recorded using GC and gold bare electrodes. From Figure 2, it can be observed that the results obtained in case of GC or Au are comparable (approximately same intensity of peak currents and closed peak potentials) meanwhile the oxidation peak for L-Dopa at CPE seems to appear at lower peak current and the corresponding peak potential is shifted towards left.

The behavior of the new electrode obtained, CP/CNTPE, was examined in $K_3Fe(CN)_6$ following the same protocol used in case of CPE. As we expected, by activating the surface using the same method of multiscan, the same result obtained in case of CPE was recorded (not shown here). The peak currents increase and the ΔE_p decrease obtaining voltammograms that show the reversible character of the reaction of $K_3Fe(CN)_6$ at the electrode surface.

3. Electrochemical behavior of L-dopa at the MWCNTs modified electrode

As far as concerns the behavior of L-Dopa at the surface of the new electrode, we compared it firstly with the behavior at the surface of CPE as shown in Fig. 3. A major improvement can be observed speaking about the peak current (an increase of peak currents was obtained). The influence of CNTs introduced was the increase of sensibility of the electrode.

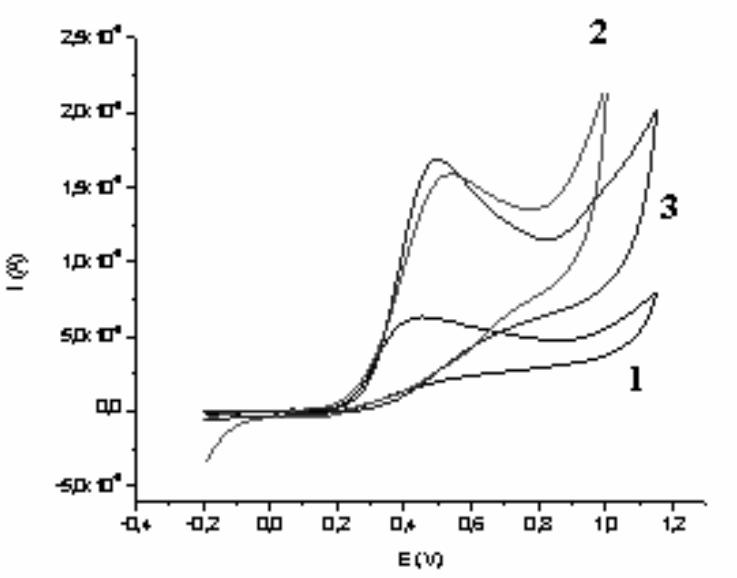


Fig. 2 – Cyclic voltammograms obtained for the oxidation of a solution of $1.0 \cdot 10^{-3}$ mol·L^{-1} L-dopa in 0.1 mol·L^{-1} PBS, pH 7.0 on a CPE (1), conventional gold electrode (2) and a CP electrode (3). Scan rate (ν) = 50 mV·s^{-1}, potential range -0.2 to +1.2 V.

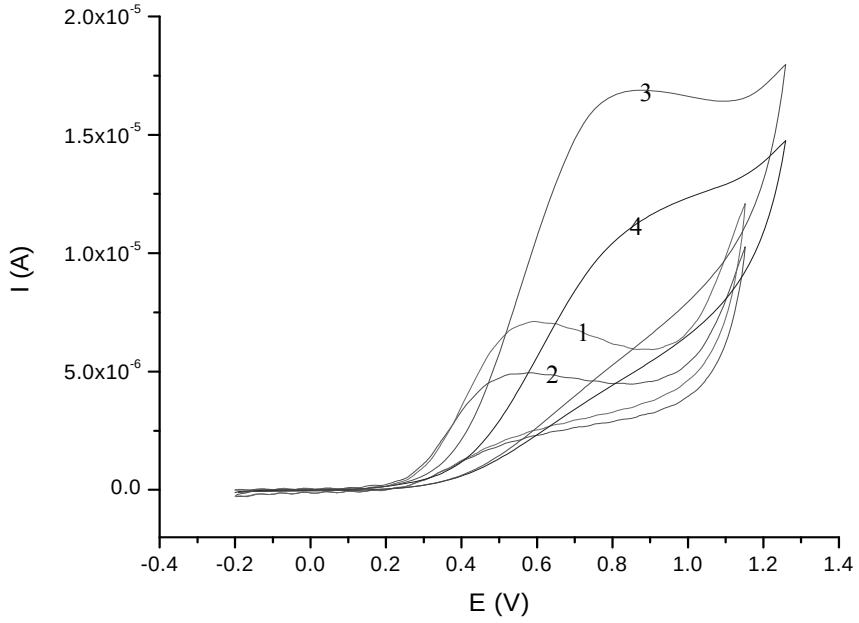


Fig. 3 – Cyclic voltammograms of L-Dopa $1.0 \cdot 10^{-3}$ mol·L^{-1} in 0.1 mol·L^{-1} PBS, pH 7.0 ,CE: Pt, RE: Ag/AgCl/KCl 3M WE: **CPE** (scan 1 - curve 1 and scan 2 – curve 2), **CP/CNTE** (scan 1 – curve 3 and scan 2 – curve 4); scan rate 50 mV·s^{-1}, potential range -0.2 to +1.2 V.

We also compared the voltammograms recorded for L-Dopa using CP/CNTPE with the voltammograms recorded using classic electrodes. In Fig. 4 it can be observed that the intensity of oxidation peak current at CP/CNTPE is now comparable with the intensity of peak currents obtained with GC (the voltammogram at gold is not shown here because as it was observed in Fig. 2 it is similar to the one recorded using GC). This means that the modification of CPE with CNTs led at the amplification of the redox signal of L-Dopa but in the figure it can be emphasized that the effect was also the displacement of the peak potential towards more positive values and this is undesirable. It is well known that not only the increase of the intensity of the signal increases the sensibility of the determination but also the decrease of potential values applied (the effect being the decrease of the noise signal).

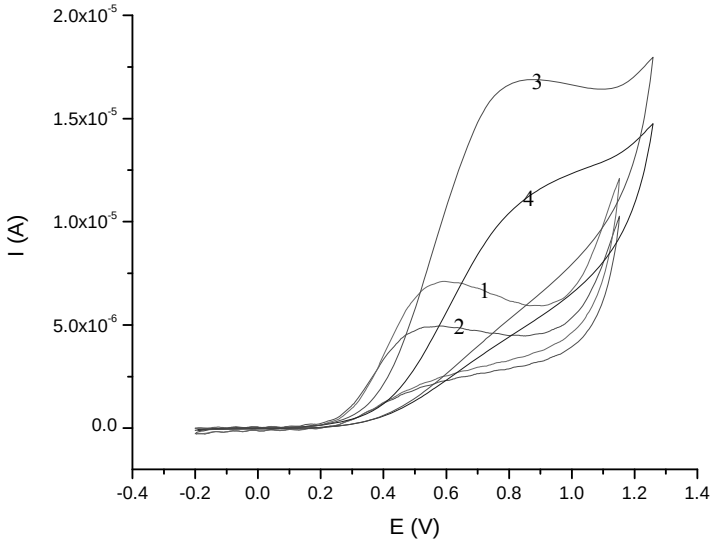


Fig. 4 – Cyclic voltammograms of L-Dopa $1.0 \cdot 10^{-3}$ mol L^{-1} in 0.1 mol·L^{-1} PBS, pH 7.0 CE: Pt, RE: Ag/AgCl/KCl 3M, WE: CPE (scan 1 - curve 1 and scan 2 – curve 2), CP/CNTE (scan 1 - curve 3 and scan 2 – curve 4) and GC (scan 1 - curve 5 and scan 2 - curve 6); scan rate 50 mV·s^{-1}, potential range -0.2 to +1.2 V.

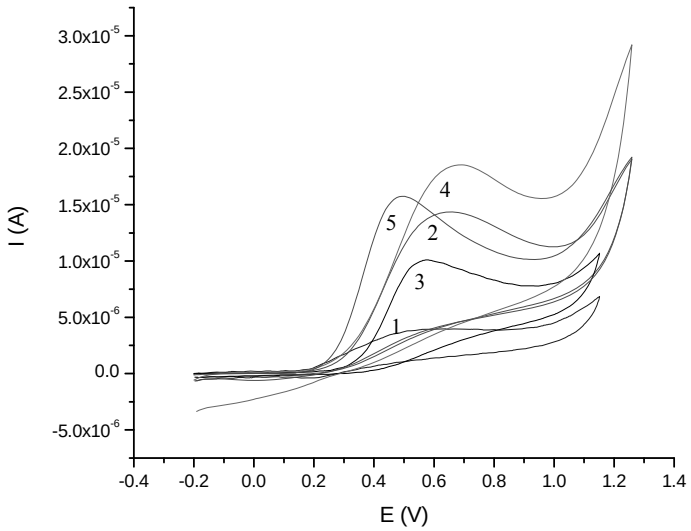
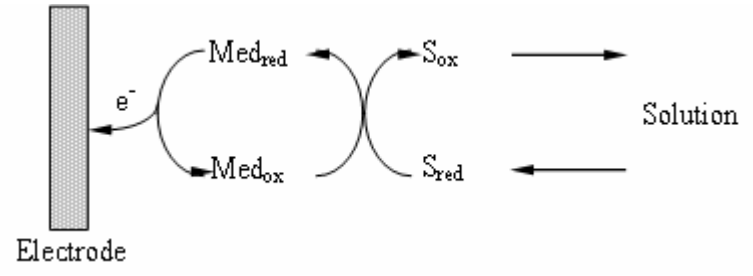


Fig. 5 – Cyclic voltammograms (scan 1) of L-Dopa $1.0 \cdot 10^{-3}$ mol·L^{-1} in 0.1 mol·L^{-1} PBS, pH 7.0; CE: Pt, RE: Ag/AgCl/KCl 3M, WE: **GC** (curve 1), **CPE** (curve 2), **CP/CNTE** (curve 3), **CP/CNT-CoPcE** (curve 4), and **CP/CNT-FePcE** (curve 5); scan rate 50 mV·s^{-1}, potential range -0.2 to +1.2 V.

This result also led to the idea of trying to improve the sensibility of the electrode thus we constructed the electrode based on carbon paste and carbon nanotubes with the addition of a chemical mediator. We chose the Fe-phtalocyanine and the Co-phtalocyanine as mediators and we constructed two different electrodes. We followed again the same protocol used for the other two electrodes constructed, the CPE and the CP/CNTE, described above. The main result is represented in Fig. 5 where we compared the oxidation signal of L-Dopa recorded using the electrodes: GCE, CPE, CP/CNTPE, CP/CNTPE-CoPc, CP/CNTPE-FePc.

As we expected we obtained an increased response of L-Dopa at the chemically modified electrodes comparing with the classic electrodes or with the unmodified carbon paste electrodes. As shown in Fig. 5 the signal on CPE remains the smallest one, followed by the signal on GCE, on CP/CNTE, the biggest ones being on CP/ FePc CNT and CP/ CoPc CNT. Considering this result we used the chemically modified electrodes to determine de content of L-Dopa in pharmaceutical samples.

The amplification of the signal is due to an electrocatalytic process represented in Scheme 2.



Scheme 2 – Oxidation mechanism of L-Dopa (denoted as A_{red})
by metallo-phthalocyanines (denoted as M_{red}) at the surface of the modified electrode.

4. Calibration plot and limit of detection

DPV technique was employed to determine the concentration of L-dopa. There were used both modified electrodes described above. Here there are represented only the results obtained using the electrode modified with Fe(II) phthalocyanine. In order to obtain the regression curve I_p vs. $c_{L\text{-dopa}}$ (Figure 6 right) we recorded the differential pulse voltammograms (represented in Fig. 6 left) using the CP/FePc CNTPE for the following concentrations of L-Dopa: 0 (only 0.1 mol·L^{-1} PBS pH=7.0), 2, 10, 20, 40, 80, 100, 250, 500, 1000 μM.

It can be observed that the plot Ip vs. $c_{L\text{-Dopa}}$ is constitued of two liniar segments corresponding to two different ranges of substrate concentration (0-200 μM for first linear segment and 200-1000 μM for second one). This comportment may be due to kinetic limitation.

We considered a narrower segment of the lower range of concentrations as regression curve, between 10 and 80 μM. The plot is represented in Fig. 7 and the characteristic of the plot are: R= 0.9982, SD = $1.68\cdot10^{-7}$, equation $I_p = 2.35\cdot10^{-7} + 7.45\cdot10^{-8}\ c_{L\text{-dopa}}$. The detection limit (3σ) is $5.04\cdot10^{-7}$ M, comparable with the values reported in literature[7, 9, 47, 48] and the sensitivity (slope) $7.454\cdot10^{-8}$ A/μM.

5. Analysis/determination of L-dopa in pharmaceutical formulations

The voltammetric proposed method based on CP/FePcCNTP electrode was applied to the determination of L-dopa in one pharmaceutical formulation commercialized as Madopar®. Recording the DPVs, reading the current of the peaks and interpolating by using the equation of the linear range chosen above we obtained the recovery of the L-Dopa in the sample being 93% (n=3). Table 1 gives the results obtained using the amperometric method, as well as the label values of the samples analyzed.

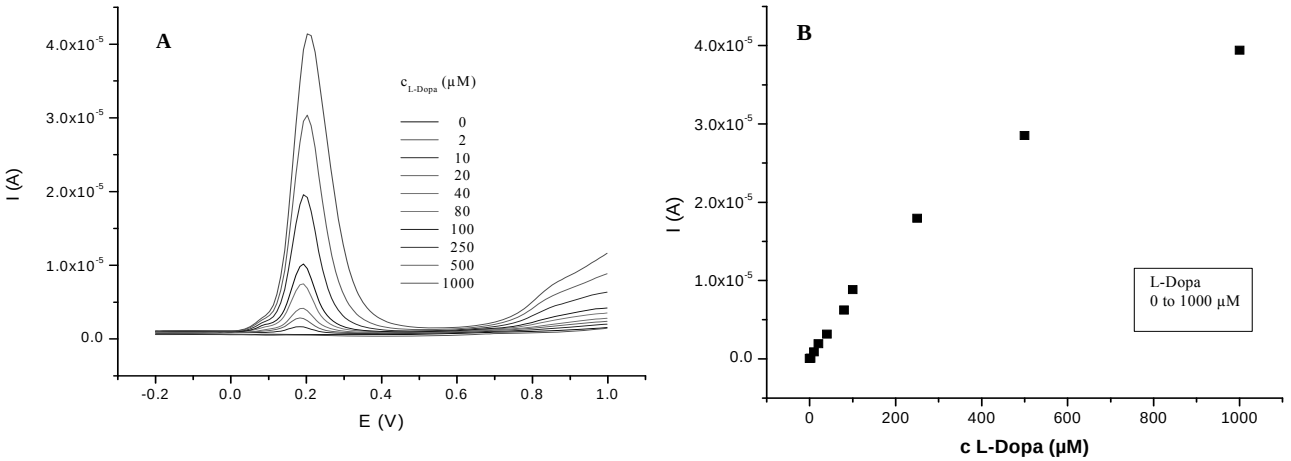


Fig. 6 – **A**: DP-Voltammograms for different concentrations of L-Dopa (0-1000 μM), PBS pH=7, CE: Pt, RE: Ag/AgCl/KCl 3M, WE: CP/CNT-FePcE. DPV parameters: MA=50 mV, SP=10 mV. **B**: Plot of intensity of peak currents recorded with DPV technique vs. $c_{L\text{-dopa}}$ (0, 10, 20, 40, 80, 100, 250, 500 nad 1000 μM).

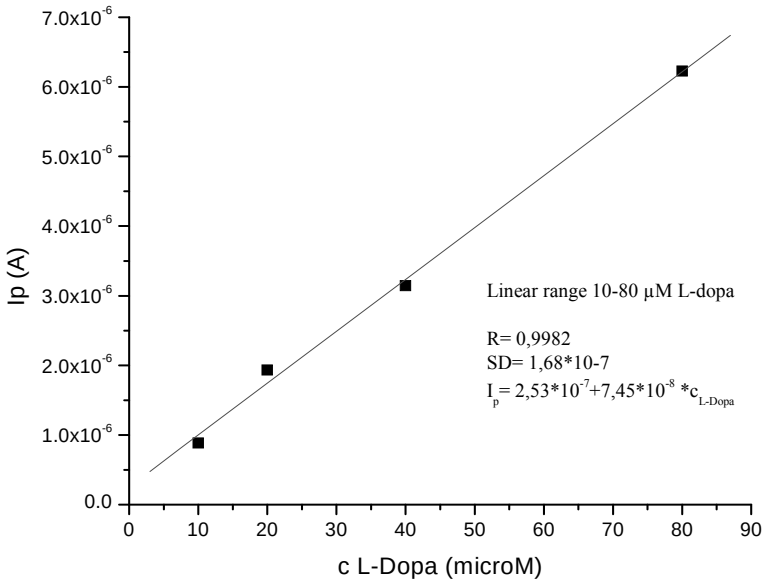


Fig. 7 – Calibration plot I_p vs. $c_{L\text{-}dopa}$, concentration domain10-80 µM L-dopa in 0.1 mol·L^{-1} PBS, pH 7.0.

Table 1

Determination of L-dopa content in pharmaceutical samples using CP/FePcCNTP electrode

Sample	Expected (µM)	Found (µM)	Recovery (%)	RSD (%)
Madopar®	50	46,4	93	2,76

CONCLUSIONS

A carbon nanotubes paste electrode modified with iron-phthalocyanine was used for the sensitive voltammetric determination of L-dopa. The measurements were carried out using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results showed an efficient catalytic activity of the electrode for the electrooxidation of L-dopa, which leads to lowering its potential by more than 200 mV. Under the optimum conditions the electrode provides a linear response versus L-dopa concentrations in the range of 10 µM and 80 µM using DPV. The modified electrode was used for determination of L-dopa in commercial dosage forms (Madopar) without any pre-treatment with satisfactory results.

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