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An Electrochemical Sensor Based on Electroreduction of Graphene Oxide on a Glassy Carbon Electrode Using Multiple Pulse Amperometry for Simultaneous Determination of L-Dopa and Benserazide

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Abstract

In this work, we described the development an electrochemical sensor based on electroreduction of graphene oxide (rGO) on a glassy carbon electrode (GCE) for simultaneous determination of L-Dopa and benserazide. For the elaboration of the GCE/rGO, the developed methodology was based on the electrochemical technique: multiple pulse amperometry (MPA). The MPA was more stable and efficient for the formation of rGO film, under optimum conditions (pH 6.00; concentration of rGO 2.00 mg mL^{-1} ; time 450 s; potentials -0.60 , -0.70 , -0.80 , -0.90 , -0.95 , -1.00 , -1.10 , -1.20 , and -1.30 V). After the film was formed, the cyclic voltammetry was used to detect LD and BZ in real samples and optimized conditions 0.05 mol L^{-1} PBS (pH 5.50). The linear range for the LD is $25\text{--}425 \text{ }\mu\text{mol L}^{-1}$ and the BZ of $5\text{--}80 \text{ }\mu\text{mol L}^{-1}$. The limit of detection calculated was 17.10 (LD) and $2.99 \text{ (BZ)} \text{ }\mu\text{mol L}^{-1}$.

Keywords: graphene oxide, electroreduction, simultaneous determination, L-Dopa, benserazide

1. Introduction

Parkinson's disease (PA) is an illness that affects about 1% of the world population, according to the World Health Organization (WHO), and this number tends to increase considerably, as demonstrated by recent studies conducted by the University of Rochester. Its estimated that the number of people affected by the PA in the 15 countries analyzed will more than double up to 2040 [1]. Thus, it becomes extremely important to develop research on this disease and the treatment employed. Mainly alternative routes for the quantification of compounds are present in the drug used in the treatment of BP, which provides an effective and lower cost treatment.

Parkinson's disease is a condition that attacks the central nervous system (CNS) and the brain, and this evil affects the amount of dopamine in the body. Dopamine (DA) is an existing neurotransmitter catecholamine in the CNS and in the mammalian brain. It has a vital role in maintaining the activities of the central nervous system, cardiovascular system, and hormonal system [2]. Patients suffering from BP have a considerable decay in the production OF DA in the brain. The medications for this disease have the design of elevating the dopamine index in the brain [3].

However, it is not possible to inject dopamine directly into the patient, because the blood-brain barrier does not allow the arrival of this hormone in the encephalon. With this, L-Dopa (LD) is used, a medicine that can overcome such a barrier and is converted into the encephalon in DA [4, 5]. Despite this, when there is an irregularity in the levels OF DA, there are some side effects such as nausea, vomiting, cardiac arrhythmia, schizophrenia, and dyskinesia [2, 6].

Unfortunately, Dopa decarboxylase (DDC) quickly converts LD into the blood-stream, so only a small percentage reaches the brain. By inhibiting the enzyme, higher amounts of L-Dopa administered can reach the brain [7]. In order not to be converted "early," the drug hydrochloride is introduced in the benserazide (BZ), which acts as a DDC inhibitor [8].

Electroanalytical methods emerged as an alternative line when compared to chromatographic methods that demand the use of expensive instruments, which are experimentally complex and require a lot of time to analyze with various pretreatments of the sample. These methods are based on the different electroanalytical and electrochemical techniques. These in turn have excellent sensitivity, selectivity, speed, reduced costs, and possibility of miniaturization of the system, making the electrochemical sensors a promising tool to supplement the existing techniques. Therefore, the development of new strategies with the aim of perfecting and improving the electrochemical techniques is promising [9–11].

Solid electrodes of different materials are employed in the electroanalytical methods; one of the most widely used materials is those based on carbonaceous materials, such as carbon graphite, pyrolytic carbon, and glass carbon. In addition to serving as a base electrode, carbonaceous materials are also used as modifiers, in order to catalyze the redox process and/or increase selectivity. Some examples that can be found in the literature are the use of reduced graphene oxide (RGO), graphene, nanotubes (single or multiple wall), etc.

For this work the glass carbon electrode (GCE) was chosen as the base electrode and the reduced graphene oxide (rGO) by electroreduction. The GO could be reduced also by thermal and chemical process; however the electrochemical reduction causes a homogeneity and stability of the surface of the work electrode; for this reason we opted for the use of electroreduction of GO.

The process of reduction of GO by electrochemical route is described in the literature in two ways, one using conventional amperometry (Amp) and the other using cyclic voltammetry (CV). The methodology employing the Amp consists in the application of a single potential in a specific time range, already the methodology employing CV focused on the application of a potential window at different scanning speeds and number of cycles.

In order to add value to this work, the use of multiple pulse amperometry (MPA) was tested, a technique derived from conventional amperometry, and there are still no reports in the literature of this technique used for the purpose of reducing GO. Since MPA consists in the application of different potentials in a certain time range, this process has as an advantage the application of activation potentials of the electrode surface and cleaning [12]. Therefore, these possibilities can generate better efficiency in the reduction process; the number of pulses of potential applicable

considering the software GPES reaches 10 and has the possibility of acquiring the current as a function of time to each potential pulse.

The glass carbon electrode has distinct properties of its allotropic forms, pyrolytic graphite, and diamond carbon, which are also used in electrochemistry. However, it presents the best synergism of physical properties, affinity with the modifier material, and its good affinity with the selected immobilization methodology, which is a prerequisite described in the literature [13].

The material that will carry out the chemical modification of the base electrode will be the GO, which will be reduced electrochemically in order to make it a conductive material, thus decreasing the resistivity of the electrode facilitating the transfer of electrons. The GO consists of a graphene sheet, a carbon structure consisting of sp^2 bonds, which has these connections transformed into sp^3 bonds by the substitutes groups, which removes its conductive characteristic. The reduction process causes this material to lose some functional groups and return to conductive characteristics and maintain the interaction from the functional groups [14–17].

The proposal of this work is the development of an electroanalytical method for the electrochemical reduction of graphene oxide and the simultaneous determination of LD and BZ in an electrochemical cell of three electrodes.

2. Construction of an electrochemical sensor based on electroreduction of graphene oxide on a GCE

2.1 Instrumentation

Electrochemical measurements and the formation of the work electrode modifier film were performed in a multi-potentiostat/galvanostat model PGSTAT101 coupled to a microcomputer containing the new 1.11 software and a microcomputer containing the GPES 4.1 software. As a system for obtaining electrochemical measurements, an electrochemical cell containing three electrodes was used: a saturated Ag/AgCl electrode containing 3.0 mol L^{-1} of KCl as a reference electrode, a platinum wire as an auxiliary electrode, and the GCE/rGO as a working electrode.

Sodium phosphate dibasic heptahydrate, monobasic sodium phosphate monohydrate obtained from the Synth[®], phosphoric acid, and 3,4-dihydroxy-L-phenylalanine were used; benserazide hydrochloride is acquired from Sigma-Aldrich[®]. All the chemical reagents used were analytical grade, and the buffer solutions were prepared with purified water by Millipore Milli-Q system.

The solution containing L-Dopa was prepared with a solution of phosphoric acid pH 2.00 and concentration 0.1 mol L^{-1} , and the solution of benserazide was prepared in phosphate buffer pH 5.50 and concentration 0.1 mol L^{-1} .

2.2 Pretreatment of glassy carbon electrode

The glassy carbon electrode was pretreated by mechanical polishing with Alumina, HNO_3 , and H_2O and also by electrochemical activation performed in a $0.5 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ solution for 20 scans at scan rate of 100 MV s^{-1} and a potential range -0.2 to 1.6 V .

2.3 Electroreduction of graphene oxide

The graphene oxide (GO) was synthesized based on previous works published [14]. The dispersion of GO was prepared in ethanol and Nafion 5% v/v. The construction of the proposed sensor was carried out by adding $5 \mu\text{L}$ of the GO suspension

Figure 1.
Process of modifying the surface of the glassy carbon electrode.

in the GCE. Then the electrode was kept in the oven at 60°C for 10 minutes. The electrochemical reduction of the GO was performed at multiple pulses to obtain the rGO. The reduction was carried out using a three-electrode setup: reference electrode (Ag/AgCl), auxiliary electrode (platinum wire), and working electrode (GCE) at PBS 0.1 mol L⁻¹ and pH 7.00. **Figure 1** summarizes the process of modifying of the GCE.

2.4 Multiple pulse amperometry

The electrochemical reduction of rGO by MPA is not described in the literature. However, based on the procedure via amperometry and by cyclic voltammetry [18–22], some conditions were adopted as a starting point. According to the literature review, [22] the application of a negative potential after the reduction process using cyclical voltammetry tends to improve the reduction and homogeneity of the film, with this in mind and in order to enjoy the best performance possible of the MPA technique was adopted 9 potentials for the process of reduction of GO, optimizing which would be these potentials, the time of application and the concentration of GO.

Considering that mechanisms may change according to the conditions used in electroreduction, some experimental parameters will be evaluated in the MPA reduction process. Initially, four parameters were optimized—applied potential, pulse application time, GO concentration, and pH effect. The objective of the optimization of these parameters is to develop a sensor with higher sensitivity in the determination of LD and BZ, and the conditions and results obtained in the optimization process will be discussed later.

3. Simultaneous determination of L-Dopa and benserazide

3.1 Electrochemical behavior of L-Dopa and benserazide

The electrochemical behavior of LD against different sensor configurations, bare GCE and GCE/rGO before being optimized and after optimization, is shown in **Figure 2A**. An associated oxidation peak around 0.45 V is observed from the oxidation of hydroxyls bound to the LD aromatic ring. This oxidation process involves two electrons, and it is a similarly reversible process [23–25]. The cyclic voltammogram obtained with GCE/rGO after optimizing exhibits a current variation of approximately 1300% over simple GCE, as shown in **Figure 2B**. The observed current gain for L-Dopa is also observed in benserazide, thus making rGO a viable material for GCE modification.

The increase in current observed in the oxidation process of LD and BZ is due to some characteristics of rGO. Reduced graphene oxide consists of a graphene sheet with the presence of some functional groups along its structure, which will make

the interaction between the base electrode and the analyte easier. In addition, rGO is a conductive material thus facilitating the exchange of electrons between the analyte and the WE.

It is noteworthy that the increase in observed current from the addition of rGO leads to an increase in the linear working range of the sensor and an increase in its sensitivity.

3.2 Optimization of experimental parameters

3.2.1 Effect of applied potential range

Initially we analyzed potential range to reduce the graphene oxide. These potentials were in the window of -0.50 to -1.40 V. **Figure 3** indicates the behavior of the analytes before the different potentials adopted, and the anodic current was adopted as the selection method of the best result and also took into account the separation of current peaks anodic of LD and BZ. This figure does not represent the process of reducing but the electrochemical behavior of analytes using the GCE/rGO after the reduction.

Among the potentials adopted, $(-0.50, -0.60, -0.70, -0.80, -0.90, -1.00, -1.10, -1.20, -1.30)$, $(-0.60, -0.70, -0.80, -0.90, -0.95, -1.00, -1.10, -1.20, -1.30)$, and $(-0.70, -0.80, -0.90, -1.00, -1.05, -1.10, -1.20, -1.30, -1.40)$, the

Figure 2. LD $0.1 \mu\text{mol L}^{-1}$ electrochemical behavior in the analytical response (A) GCE, GCE/rGO before optimization, and GCE/rGO after optimization (B).

Figure 3. (A) Cyclic voltammograms obtained at different electrodes prepared via MPA with different potentials (PBS 0.1 mol L^{-1} , pH 5.50) after addition of $1.0 \times 10^{-5} \text{ mol}$ (BZ) and $9.95 \times 10^{-5} \text{ mol L}^{-1}$ (LD). (B) Analytical response.

range of -0.60 to -1.30 V presented the best result; therefore this value was adopted as the optimum value, and the analysis process continued.

3.2.2 Effect of time

After determining the potentials to be applied in the electroreduction, the time of application of these potentials was evaluated. Three application times were analyzed: 400, 450, and 500. **Figure 4** represents the voltammograms obtained after the GO reduction process, and this analysis was performed in the presence and absence of analyte for electrodes prepared at different times of reduction.

Through the current difference, it was determined which would be the best fear of the application of the pulses, having as optimum time 450 s.

3.2.3 Effect of GO concentration

With the potentials and time of application well-defined, the evaluation of the GO concentration added to the electrode surface was performed, from 0.5 to 3 mg mL^{-1} . **Figure 5** shows the voltammograms for the different concentrations of the solutions added on the GCE, and the optimum condition was defined by means of the current difference. This condition was 2.00 mg mL^{-1} .

Figure 4. (A) Cyclic voltammograms obtained at different electrodes prepared via MPA with different times (PBS 0.1 mol L^{-1} , pH 5.50) after addition of $1.0 \times 10^{-5} \text{ mol}$ (BZ) and $9.95 \times 10^{-5} \text{ mol L}^{-1}$ (LD). (B) Analytical response.

Figure 5. (A) Cyclic voltammograms obtained at different electrodes prepared via MPA with different GO concentrations (PBS 0.1 mol L^{-1} , pH 5.50) after addition of $1.0 \times 10^{-5} \text{ mol}$ (BZ) and $9.95 \times 10^{-5} \text{ mol L}^{-1}$ (LD). (B) Analytical response.

3.2.4 Effect of pH

Finally, the pH influence of the electrolytic solution in the reduction process was evaluated, since it can cause interferences in the process of electroreduction, such as damage to the modifier material. Concerning this study, the optimal measurements previously evaluated in **Figure 6** can be observed, and the influence of the pH value of the support solution—phosphate buffer at 0.10 mol L^{-1} —in the formation of the rGO film. By means of the current difference, the best pH value was defined, which was 6.00.

It should be noted that when the dispersion concentration of the modified material was optimized due to the influence of the thickness of the modification and the number of functional groups, and the thicker and the smaller the number of groups, the lower the number of functional loads will be transported. The potential is applied to eliminate the excess of functional groups, transforming the GO into or returning to give conductive characteristics of this material. The reduction time in turn allows a better interaction of the film with the reduction process making its surface more homogeneous.

Therefore, it is extremely important that there is an optimization of the parameters of the technique and the concentration of modifier material to obtain a more efficient sensor, that is, with good sensitivity and selectivity.

3.3 Optimization of experimental conditions

After the optimization of the GO reduction process, it was evaluated which of these presented better conditions for the continuation of the analyses of LD and BZ. The choice of the best technique was based on the separation of the peaks of LD and BZ and the higher anodic current.

3.3.1 Influence of ionic force

It is known that the ionic force is directly linked to the feasibility of the analysis once the concentration of the ions influences in the transport of loads, which can generate an increase in the signal of the analyte.

The determination of the ionic strength of the solution was made by means of the difference of white current and the addition of the analytes obtained in a buffer system pH 5.50, and the electrolyte concentration support was varied from 0.025 to 0.200 mol L^{-1} . **Figure 7** illustrates the voltammograms obtained for these different concentrations, and through them it was possible to define that the ionic force that

Figure 6.
 (A) Cyclic voltammograms obtained at different electrodes prepared via MPA with different pH (PBS 0.1 mol L^{-1} , pH 5.50) after addition of $1.0 \times 10^{-5} \text{ mol}$ (BZ) and $9.95 \times 10^{-5} \text{ mol L}^{-1}$ (LD). (B) Analytical response.

Figure 7.

(A) Cyclic voltammograms obtained at different electrodes prepared via MPA with different electrolyte concentration (PBS pH 5.50) after addition of 1.0×10^{-5} mol (BZ) and 9.95×10^{-5} mol L⁻¹ (LD). (B) Analytical response.

best suited was 0.050 mol L⁻¹. The higher concentration may be happening with the competition between the electrolyte ions support and the analytes and concentrations below these there are not enough ions to carry out the transport of loads.

3.3.2 pH influence

Subsequently, the pH effect was evaluated, which may influence the electrode stability and provide secondary reactions of the analytes, such as LD, which in basic pH suffers secondary reactions which degrade it. This parameter was also evaluated by analyzing anodic current, and the higher these differences are the signal obtained, **Figure 8** illustrates the voltammograms for the different pH values of the electrolyte support, the optimum value for analysis was determined being 5.50.

3.4 Calibration curve

First, we calculated the amount of analyte needed to prepare a solution containing LD and BZ in the same proportion found in Prolopa[®], from 5.23 mol of LD to 1.00 mol of BZ, which would be analyzed. Through this solution, the voltammetry analysis was performed using proposed sensor (previously optimized) as shown in **Figure 9**. The analytical curves were constructed for LD (**Figure 10**) and BZ (**Figure 11**).

Figure 8.

(A) Cyclic voltammograms obtained at different electrodes prepared via MPA with different pH of the electrolyte (PBS 0.050 mol L⁻¹) after addition of 1.0×10^{-5} mol (BZ) and 9.95×10^{-5} mol L⁻¹ (LD). (B) Analytical response.

Having all the data optimized, it was possible to construct the final analytical curves by means of successive additions of LD 5.08 mmol L^{-1} and BZ 0.97 mmol L^{-1} in order to obtain the sensibility, the detection limit (LOD), and the limit of quantification (LOQ) for each analyte.

The sensibility for LD was $0.05148 \text{ } \mu\text{mol L}^{-1}$ and $0.10303 \text{ } \mu\text{mol L}^{-1}$ for BZ. For the analytical curve, to have an acceptable degree of reliability, the value of R must be close to 1. The values obtained for R are above 0.98, which indicates a considerable good degree of reliability.

The detection limit (LOD) represents the smallest amount of the analyte present in a sample that can be detected by the method. From the parameters of the analytical curve, the LOD can be expressed by $\text{LOD} = 3 \times (\text{S/B})$, where S is the standard deviation of the white and B is the slope of the analytical curve.

Figure 9. Voltammograms (PBS 0.05 mol L^{-1} pH 5.5) and calibration curve, using cyclic voltammetry, containing LD in the concentration range of $2.53 \times 10^{-5} \text{ mol L}^{-1}$ to $4.19 \times 10^{-4} \text{ mol L}^{-1}$ and BZ of $4.83 \times 10^{-6} \text{ mol L}^{-1}$ to $8.02 \times 10^{-5} \text{ mol L}^{-1}$.

Figure 10. Calibration curve of BZ in phosphate buffer, pH 5.50, and concentration 0.05 mol L^{-1} .

Figure 11.

Calibration curve of LD in phosphate buffer, pH 5.50, and concentration 0.05 mol L⁻¹.

The limit of quantification (LOQ) is the smallest amount of the analyte in a sample that can be determined with accuracy and accuracy acceptable under the established experimental conditions. The LOQ is established through the analysis of samples containing decreasing concentrations of the analyte up to the lowest determinable level and can be expressed by the equation: $LOD = 10 \times (S/B)$, where S and B have the same values found for the LOD, previously.

The limit of detection and the limit of quantification of LD and BZ obtaining was, respectively, 5.14 $\mu\text{mol L}^{-1}$ and 17.10 $\mu\text{mol L}^{-1}$ for LD and 8.96×10^{-1} $\mu\text{mol L}^{-1}$ and 2.99 $\mu\text{mol L}^{-1}$ and BZ. However, the detection limit can be adopted as the first point of the analytical curve, thus becoming 4.83 $\mu\text{mol L}^{-1}$ for BZ and 25.30 $\mu\text{mol L}^{-1}$ for LD.

After the calibration curve was constructed, reproducibility and repeatability tests were performed. Reproducibility was confirmed by inter-day testing, where analyses of the same sample were performed on different days, and by intra-day testing, where five electrodes were built and samples of the same concentration were analyzed, after these tests. A good reproducibility must have standard deviation of less than 5% which will occur.

Repeatability can be ratified by inter- and intra-day analysis, and it was made by intra-day analysis, where an electrode was built to analyze five samples of the same concentration, which presented a standard deviation below 5%, which statistically proves the repeatability of the method.

It is noteworthy that all analyses presented throughout the text were made in triplicate, where a relative error of less than 5% was observed, which statistically proves that the analyses presented are within an acceptable standard.

3.5 Application in pharmaceutical formulation

In order to evaluate the applicability of the proposed method, a solution of LD and BZ was prepared employing the sample of the drug (Prolopa[®]) at the concentration of 5.08 mmol L⁻¹ of LD and 0.97 mmol L⁻¹ of BZ, having the nominal value of each analyte present in the Prolopa[®] as a reference for calculating the indicated concentrations. This solution was analyzed by the proposed sensor.

Then, the recovery tests were performed. The results were obtained in triplicate and are presented in **Tables 1** and **2**.

Measure	[LD] added	[LD] recovered	Recovery (%)	E _R (%)
1	$2.53 \times 10^{-5} \text{ mol L}^{-1}$	$2.60 \times 10^{-5} \text{ mol L}^{-1}$	102.77	2.77
2	$2.87 \times 10^{-4} \text{ mol L}^{-1}$	$2.78 \times 10^{-4} \text{ mol L}^{-1}$	96.86	-3.14
3	$4.19 \times 10^{-4} \text{ mol L}^{-1}$	$3.99 \times 10^{-4} \text{ mol L}^{-1}$	97.91	-2.09

Table 1.
Results of the LD addition and recovery tests in a solution prepared with the drug.

Measure	[BZ] added	[BZ] recovered	Recovery (%)	E _R (%)
1	$4.83 \times 10^{-5} \text{ mol L}^{-1}$	$4.95 \times 10^{-5} \text{ mol L}^{-1}$	102.48	2.48
2	$5.50 \times 10^{-5} \text{ mol L}^{-1}$	$5.25 \times 10^{-5} \text{ mol L}^{-1}$	95.45	-4.55
3	$8.02 \times 10^{-5} \text{ mol L}^{-1}$	$8.41 \times 10^{-5} \text{ mol L}^{-1}$	104.86	4.86

Table 2.
Results of the addition and recovery tests of BZ in a solution prepared with the drug.

Through the results presented in **Tables 1** and **2**, it was possible to observe that the sensor proved to be promising in the simultaneous determination of the analytes with a maximum relative error of $\pm 5\%$. And the results found were analyzed in the same system with successive additions of the drug solution.

It is noteworthy that the methodology of modification of the surface of the GCE is rapid and that the data obtained when compared with those present in the literature [23, 26–33] has performance as good as the sensors described; however, the preparation methodology of some is more laborious than the proposal in this work.

4. Conclusion

The proposed methodology was successfully developed, since it proved to be effective in the simultaneous determination of LD and BZ. It is worth noting that the methodology employed uses a rapid analysis technique, when comparing the separation techniques (chromatography).

In short, the work proved to be efficient and innovative when compared to the literature but can have the methodology of reduction by MPA improved. Thus, this study enable an improvement in the methodology of analysis and the description of this technique with the purpose of electroreduction and/or electropolymerize in the literature.

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References

- [1] Dorsey ER, Sherer T, Okun MS, Bloem RB. The emerging evidence of the Parkinson pandemic. *Journal of Parkinson's Disease*. 2018;**8**(Suppl 1):S3-S8. DOI: 10.3233/JPD-181474
- [2] Venton BJ, Wightman RM. Psychoanalytical electrochemistry: Dopamine and behavior. *Analytical Chemistry*. 2003;**75**:414A-421A. DOI: 10.1021/ac031421c
- [3] Olanow CW, Obeso JA, Stocchi F. Continuous dopaminereceptor treatment of Parkinson's disease: Scientific rationale and clinical implications. *Lancet Neurology*. 2006;**5**:677-687. DOI: 10.1016/S1474-4422 (06) 70521-X
- [4] Hershey T, Black KJ, Carl JL, Mcgee-Minnich L, Snyder AZ. Long term treatment and disease severity change brain responses to levodopa in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2003;**74**:844-851. DOI: 10.1136/jnnp.74.7.844
- [5] Khor SP, Hsu A. The pharmacokinetics and pharmacodynamics of levodopa in the treatment of Parkinson's disease. *Current Clinical Pharmacology*. 2007;**2**(3):234-243. DOI: 10.2174/157488407781668802
- [6] Rezaei B, Shams-Ghahfarokhi L, Havakeshian E, Ensafi AA. An electrochemical biosensor based on nanoporous stainless steel modified by gold and palladium nanoparticles for simultaneous determination of levodopa and uric acid. *Talanta*. 2016;**158**:42-50. DOI: 10.1016/j.talanta.2016.04.061
- [7] Burkhard P, Dominici P, Borri-Voltattorni C, Jansonius JN, Malashkevich VN. Structural insight into Parkinson's disease treatment from drug-inhibited DOPA decarboxylase. *Nature Structural Biology*. 2001;**8**(11):963-967. DOI: 10.1038/nsb1101-963
- [8] Treseder SA, Rose S, Summo L, Jenner P. Commonly used L-amino acid decarboxylase inhibitors block monoamine oxidase activity in the rat. *Journal of Neural Transmission*. 2003;**110**(3):229-238. DOI: 10.1007/s00702-002-0778-4
- [9] Norouzi P, Larijani B, Faridbod F, Ganjali MR. A novel method for ultra trace measurement of bentazon based on nanocomposite electrode and continuous coulometric FFTCyclic voltammetry. *International Journal of Environmental Research*. 2015;**9**:101-108
- [10] Wang Y, Hasebe Y. Uricase-adsorbed carbon-felt reactor coupled with a peroxidase-modified carbon-felt-based H₂O₂ detector for highly sensitive amperometric flow determination of uric acid. *Journal of Pharmaceutical and Biomedical Analysis*. 2012;**57**:125-132. DOI: 10.1016/j.jpba.2011.08.021
- [11] Trojanowicz M. Recent developments in electrochemical flow detections—A review: Part II. Liquid chromatography. *Analytica Chimica Acta*. 2011;**688**:8-35. DOI: 10.1016/j.aca.2010.12.024
- [12] de Miranda JAT. Simultaneous determination of aspirin and ascorbic acid in medications using flow injection analysis with amperometric detection [master's thesis]. Uberlândia: Federal University of Uberlândia—Graduate Program in Chemistry; 2011. DOI: 10.1002/elan.200804262
- [13] Pereira AC. Electrochemical behavior of some electroactive organic compounds immobilized in inorganic media aiming at the development of sensors for NADH [PhD thesis]. Unicamp: Institute of Chemistry; 2003

- [14] Oliveira AEF, Braga GB, Tarley CRT, Pereira AC. Thermally reduced graphene oxide: Synthesis, studies and characterization. *Journal of Materials Science*. 2018;**53**:12005-12015. DOI: 10.1007/s10853-018-2473-3
- [15] Li Y, Martens I, Cheung KC, Bizzotto D. Electrodeposition of reduced graphene oxide onto gold electrodes: Creating thin electrochemically active and optically transparent overlayers. *Electrochimica Acta*. 2019;**319**:649-656. DOI: 10.1016/j.electacta.2019.07.004
- [16] Yao SY, Cai WL, Liu L, Liao XQ, Tao KL, Feng F, et al. Electrochemical behavior of eriocitrin and highly sensitive determination based on an electrochemically reduced graphene oxide modified glassy carbon electrode. *Analytical Methods*. 2016;**18**:3722-3729. DOI: 10.1039/C6AY00064A
- [17] Wang X, Shen W, Zhang X, Guo S, Gao Y, Li X, et al. Indirect electrochemical determination of ribavirin using boronic acid-diol recognition on a 3-aminophenylboronic acid-electrochemically reduced graphene oxide modified glassy carbon electrode (APBA/ERGO/GCE). *Analytical Letters*. 2019;**52**:1-14. DOI: 10.1080/00032719.2019.1576716
- [18] Motta R, Schrebler R, Zanoni R, Dalchiele EA. Insights from experiment and theory into the electrochemical reduction mechanism of graphene oxide. *Electrochimica Acta*. 2019;**304**:231-238. DOI: 10.1016/j.electacta.2019.02.108
- [19] de Camargo MNL et al. Tuning the electrochemical reduction of graphene oxide: Structural correlations towards the electrooxidation of nicotinamide adenine dinucleotide hydride. *Electrochimica Acta*. 2016;**197**:194-199. DOI: 10.1016/j.electacta.2015.09.022
- [20] Kumar DR, Kesavan S, Bayonosa ML, Shin JJ. 3,5-Diamino-1,2,4-triazole@electrochemically reduced graphene oxide film modified electrode for the electrochemical determination of 4-nitrophenol. *Electrochimica Acta*. 2017;**246**:1131-1140. DOI: 10.1016/j.electacta.2017.06.116
- [21] Palakollu VN et al. Electrochemically reduced graphene oxide/poly-glycine composite modified electrode for sensitive determination of L-dopa. *Materials Science and Engineering: C*. 2017;**77**:394-404. DOI: 10.1016/j.msec.2017.03.173
- [22] García-Argumánez A, Llorente I, Caballero-Calero O, González Z, Menéndez R, Escudero ML, et al. Electrochemical reduction of graphene oxide on biomedical grade CoCr alloy. *Applied Surface Science*. 2019;**465**:1028-1036. DOI: 10.1016/j.apsusc.2018.09.188
- [23] Sunder GSS, Rohanifar A, Devasurendra AM, Kirchhoff JR. Selective determination of L-DOPA at a graphene oxide/yttrium oxide modified glassy carbon electrode. *Electrochimica Acta*. 2019;**301**:192-199. DOI: 10.1016/j.electacta.2019.01.098
- [24] Hassanvand Z, Jalali F. Simultaneous determination of L-DOPA, l-tyrosine and uric acid by cysteine acid-modified glassy carbon electrode. *Materials Science and Engineering C*. 2019;**98**:496-502. DOI: 10.1016/j.msec.2018.12.131
- [25] Prabhu P, Babu RS, Narayanan SS. Amperometric determination of L-dopa by nickel hexacyanoferrate film modified gold nanoparticle graphite composite electrode. *Sensors and Actuators B: Chemical*. 2011;**156**(2):606-614. DOI: 10.1016/j.snb.2011.02.006
- [26] Sufredini HB, Pedrosa VA, Codognoto L, Machado SAS, Filho RCR, Avaca LA. Enhanced electrochemical response of boron-doped diamond electrodes brought on by a cathodic

surface pre-treatment. *Electrochimica Acta*. 2013;**49**:4021-4026

[27] Naushad M, Gupta VK, Wabaidur SM, Alothman ZA. Simultaneous determination of benserazide and levodopa in pharmaceutical tablet, human serum and urine sample by differential pulse voltammetry using modified glassy carbon electrode. *International Journal of Electrochemical Science*. 2013;**8**:297-311

[28] Kul D, Brett CMA. Electrochemical investigation and determination of levodopa on poly(Nile blue-a)/multiwalled carbon nanotube modified glassy carbon electrodes. *Electroanalysis*. 2014;**26**:1320-1325. DOI: 10.1002/elan.201400071

[29] Zhou YZ, Alany RG, Chuang V, Wen J. Studies of the rate constant of L-DOPA oxidation and decarboxylation by HPLC. *Chromatographia*. 2012;**75**:597-606. DOI: 10.1007/s10337-012-2229-1

[30] Ensafi AA, Bahmari H, Rezaei B, Maleh KH. Application of ionic liquid-TiO₂ nanoparticle modified carbon paste electrode for the voltammetric determination of benserazide in biological samples. *Materials Science and Engineering*. 2013;**33**(2):831-835. DOI: 10.1016/j.msec.2012.11.008

[31] Fouladgar M. A high sensitive square wave voltammetric sensor based on ZnO nanoparticle ionic liquid paste electrode for determination of benserazide in biological samples. *Measurement*. 2016:141-147. DOI: 10.1016/j.measurement.2016.02.057

[32] Rabinca AA et al. Voltammetric method for simultaneous determination of L-Dopa and benserazide. *Current Analytical Chemistry*. 2017;**13**:218-224. DOI: 10.2174/1573411012666160601161703

[33] Rahmanifar E, Yoosefian M, Maleh KH. Application of CdO/

SWCNTs nanocomposite ionic liquids carbon paste electrode as a voltammetric sensor for determination of benserazide. *Current Analytical Chemistry*. 2017;**13**:46-51. DOI: 10.2174/1573411012666160601145809