NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY POLITEHNICA BUCHAREST

Doctoral School of Chemical Engineering and Biotechnologies

PhD THESIS SUMMARY

Electrochemical sensors with applications in the analysis of biologically active compounds

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1. INTRODUCTION

Motivation, importance and relevance of the research topic

Neurological disorders represent a major global issue due to the rapid increase in the number of cases. Early diagnosis is essential for improving the quality of life and reducing the progression of neurological pathologies. For this purpose, portable devices capable of detecting biological compounds through minimally invasive procedures have been developed. These devices can be used directly at the site of patient care.

The general objective of this study represents the development of electrochemical sensors via original methods based on sinusoidal voltages (E_{sin}) and sinusoidal currents (I_{sin}) for the rapid determination of biologically relevant compounds in urine samples. Therefore, electrochemical sensors consisting of electrodes modified with composite materials based on conductive polymers (PEDOT, PEDOT-PDA, PEDOT/PB) and metallic nanoparticles (nano-Au, nano-Pt, nano-Ag) were proposed. Original methods based on the use of sinusoidal currents and sinusoidal voltages were developed for the synthesis of composite materials because they provide an increase in the electrochemically active surface area, electrocatalytic properties and analytical sensitivity.

Objectives of the doctoral thesis

The general objective is the development of new electrochemical sensors designed on electrochemical detection platforms based on conventional electrodes modified with composite materials with applications in the detection of biologically active compounds. The main analytes investigated are the neurotransmitters epinephrine and serotonin, the antioxidant quercetin and potassium ions. The overall objective of the thesis represents the development of sensors for the detection of biologically active compounds in real samples (urine samples) through minimally invasive sampling procedures.

The specific objectives of the doctoral thesis were established based on the general objective, in correlation with the activities included in the individual scientific research program. These objectives were fully achieved through scientific documentation (preparation of a review describing the electrochemical determination of neurotransmitters using electrochemical sensors based on composite materials), the preparation and presentation before the doctoral supervisory committee of seven scientific research reports, the publication of scientific papers with data presented in the doctoral thesis (five ISI-indexed journal articles of which three in Q1 journals, one in Q3 and one in Q4) and the participation in 14 national/international conferences, including four oral presentations and 10 poster presentations.

Research methodology

The research methodology enabled the monitoring of progress indicators and the full achievement of the specific objectives of the doctoral thesis: the establishment and optimization of the experimental parameters for the original methods based on sinusoidal currents (I_{sin}) and sinusoidal voltages (E_{sin}), the selective functionalization of conventional electrode surfaces with composite materials based on conductive polymers (PEDOT, PDA), metallic nanoparticles (Au, Pt, Ag) and inorganic materials (Prussian Blue), the electrochemical characterization of the modified electrodes in the presence of a redox probe

to determine the electrochemical properties, the morphological characterization of the modified electrodes, the study of the influence of potential scan rate on the electrochemical responses of the developed sensors in the presence of redox probes, the voltammetric sensors testing in synthetic samples with known analyte composition, the estimation of the analytical performance of the developed sensors, the interference study, the development of calibration curves, testing and validation of the developed sensors in real samples.

Structure of the doctoral thesis

The doctoral thesis is structured in three main parts: Introduction (Chapter 1), Part A-The critical review of literature data (Chapters 2 and 3), Part B-Original contributions (Chapters 4, 5, 6, 7, 8 and 9) and Part C-General conclusions, Main achievements and original contributions and Future directions for development and improvement of analytical performance (Chapter 10). The final sections include: References (Chapter 11) and Annexes (Chapter 12).

Chapter 1 describes the motivation, the importance of the research topic, the objectives of the doctoral thesis and the research methodology.

Chapter 2 describes the main types of electrochemical sensors based on conventional electrodes functionalized with conductive polymers, metallic/inorganic nanoparticles and composite materials. The principal aspects regarding synthesis, characterization, and analytical performance of the functionalized electrodes are also discussed.

Chapter 3 includes a concise description of the main biologically active compounds investigated in the thesis, namely neurotransmitters and antioxidants. This chapter highlights the properties, the role and the possibilities for analytical determination of biologically active compounds.

The original contributions are described in Chapters 4, 5, 6, 7, 8 and 9.

Chapter 4 includes the description of the experimental activities related to the instrumentation used, the reagents, the electrodes, the synthesis procedures, the investigation techniques, the analytical testing protocols and the validation procedures for the electrochemical sensors developed within the thesis.

Chapter 5 describes the development of original methods for preparing electrochemical sensors and the optimization of experimental parameters for the synthesis of conductive polymers, metallic and inorganic nanoparticles along with the composite materials.

Chapter 6 describes the development of electrochemical detection platforms based on conventional electrodes functionalized with organic conductive polymers (PEDOT, polydopamine) and gold nanoparticles.

The electrochemical sensor consisting of a PEDOT and nano-Au layer, prepared *in-situ* via the I_{sin} method was implemented for the analytical determination of epinephrine and serotonin. The research objectives include the optimization of the electrochemical parameters for the synthesis of PEDOT and nano-Au, the electrochemical and morphological characterization of PEDOT and the PEDOT/nano-Au composite material, as well as the validation of the electrochemical sensors through the evaluation of the analytical performance criteria.

6.1.1. *The* electrochemical synthesis of conducting polymer and gold nanoparticles

The *in-situ* synthesis of the conductive polymer PEDOT was carried out using the electrogravimetric method with a quartz crystal coated with gold (Au). The results shown in the figure below illustrate the sinusoidal current applied during deposition, the resulting potential as well as the variation in the resonance frequency of the quartz crystal during the polymerization process (***Figure 31***).

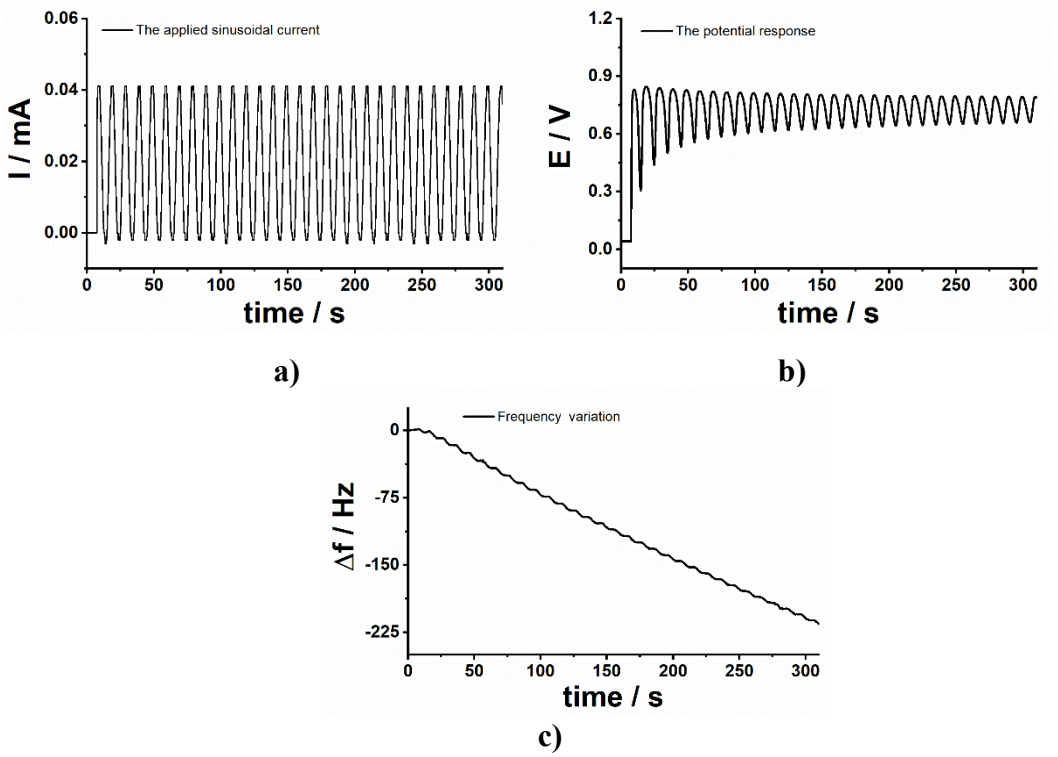


Figure 1. *The synthesis of the PEDOT layer with the electrogravimetric method combined with I_{sin} method:* ***a)*** *The sinusoidal current applied;* ***b)*** *The potential response;* ***c)*** *The frequency variation. Image adapted under the CC BY 4.0 licence from reference [1], DOI: 10.3390/bios14070320.Text translated into Romanian and figures rearranged.*

According to the experimental data from ***Figure 31***, PEDOT is deposited by modulating the potential between 0.30 and 0.85 V, demonstrating the involvement of anodic potential ranges above 0.80 V in the electrochemical polymerization process. During the polymerization process, the expulsion of counter-ions and/or solvent molecules also occurs, the process being correlated with the increase in the quartz crystal frequency at the end of each synthesis cycle (one synthesis cycle represents one complete cycle of the applied sinusoidal signal) [1].

The mass of the deposited polymer can be estimated with Sauerbrey equation:

$$\Delta f = -\frac{2 \times f_0^2 \times \Delta m}{A \times \rho^{\frac{1}{2}} \times \mu^{\frac{1}{2}}} \qquad (24)$$

Where:

Δf – the variation in the resonant crystal (Hz), f_0 – the resonance frequency (Hz), Δm – the mass variation ($\mu g/cm^2$), A – the active area of the quartz crystal (cm^2), ρ – the density of the quartz crystal (g/cm^3) and μ – the shear modulus of the quartz crystal ($g \times cm^{-1} \times s^2$).

In this way, the mass of the polymer was found to be 5.2 μg cm^{-2}. From the experimental data with the electrochemical polymerization of the monomer, the mass of the deposited polymer can be estimated using the electric charge and the number of electrons transferred per monomer molecule (2.25 electrons) [2,3]. A value of approximately 3.1 μg cm^{-2} was obtained, assuming that no side reactions occur. This lower value compared to that obtained from the electrogravimetric measurements is attributed to the neglect of the incorporation of counter-ions and/or solvent molecules into the polymer layer.

6.1.3.3. Assessment of analytical parameters. Construction of calibration curves.

The analytical performance of the PEDOT/nano-Au sensor developed with optimized synthesis parameters for epinephrine was evaluated (***Figure 44a***). As shown in ***Figure 44b***, the sensor exhibits a linear response over a concentration range from 1 μM to 100 μM. The linear regression equation is expressed as follows: I_{pa} (μA) = 1.2 + 0.060 [EP] (μM), with a coefficient of determination $R^2 = 0.9974$. The sensor sensitivity is 0.845 μA × μM^{-1} × cm^{-2}. The limit of detection (LOD) and the limit of quantification (LOQ) were determined to be 0.5 μM and 1.7 μM, respectively [1]. The experimental results demonstrate an improved analytical performance of the sensor developed using the new I_{sin} method parameters.

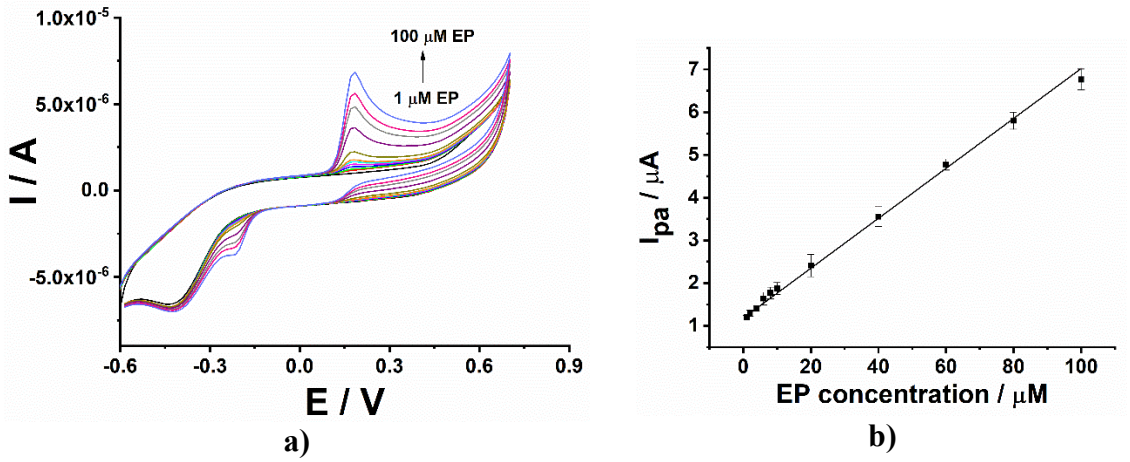


***Figure 2**. The analytical response: **a)** CV for PEDOT-nano Au in 0.1 M PBS (pH=7) and 1-100 μM EP, v = 0.05 V/s; **b)** The calibration curve with error bars (n=3). Image adapted under the CC BY 4.0 licence from reference [1], DOI: 10.3390/bios14070320. Text translated into Romanian language and figures rearranged.*

6.1.4.1. Electroanalysis of epinephrine

The PEDOT/nano-Au electrochemical sensor developed with the intermediate synthesis parameters was employed for the detection of epinephrine in a tap water sample and in a real urine sample diluted 1:10 with 0.1 M phosphate buffer (pH = 7) (***Figure 46***). The study performed in the tap water sample aimed to evaluate the analytical performance of the sensor in media with high electrical resistance, being added a supporting electrolyte, in this case a phosphate buffer solution with a final concentration in the analyzed sample of 0.04 M. In this case, a potential value of 0.25 V at which epinephrine oxidation occurs was

observed (***Figure 46a***). Based on the linear regression model of the dependence of the anodic peak current on analyte concentration, a coefficient of determination R^2 = 0,9751 was obtained, with a sensitivity of 0.277 µA × $µM^{-1}$ × cm^{-2}. From the comparison between the sensitivity values obtained for this sample and the phosphate buffer solution it results that the sensor can be efficiently used in samples with high electrical resistance [2].

The electrochemical sensor PEDOT/nano-Au developed with the implementation of intermediate synthesis parameters was used for the detection of epinephrine in a real urine sample diluted 1:10 with 0.1 M phosphate buffer, pH 7 (***Figure 46b***). Epinephrine oxidation occurs at a potential of 0.32 V. The peak current associated with the epinephrine oxidation shows a linear increase, directly proportional to the analyte concentration. The analytical recovery values were determined for the concentrations of 40 µM, 80 µM, and 160 µM, yielding values of 90.8%, 101.3%, and 104.2%, respectively [2].

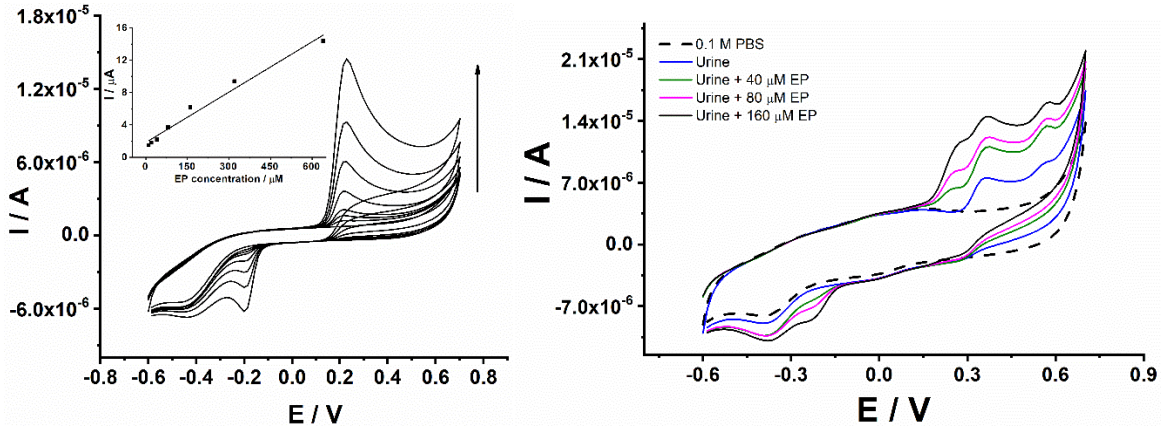


Figure 3. *CV with the analytical response of PEDOT/nano-Au sensor in:* ***a)*** *tap water sample. Inset: the calibration curve and* ***b)*** *urine sample spiked with EP 40-160 µM; v = 0,05 V/s. Images adapted under the CC BY 4.0 licence from ref. [2], DOI:10.3390/bios13080781. Text translated into romanian language.*

6.1.5. Comparison of the analytical performance of the developed sensors with literature data

The analytical performance of the PEDOT/nano-Au sensor synthesized with the I_{sin} method is comparable to the results reported in the literature for sensors developed using conventional synthesis methods (***Table 15***).

Table 1*. Examples of sensors reported in the literature used for the electroanalysis of EP.*

Electrode and selective material	Analyte	Detection method	LOD (µM)	Linear response range (µM)	Sensitivity (µAµM^{-1})	Ref.
GCE-CNF-AuNPs	EP	SWV	1.7	50-1 × 10^3	7.27 × 10^{-1}	[3]
Au/ZnO/PPy/RGO/GCE	EP	DPV	6 × 10^{-2}	6 × 10^{-1}-5 × 10^2	-	[4]
MIP/AuNPs/GCE	EP	DPV	7.6 × 10^{-2}	9 × 10^{-2}-1 ×10^2	-	[5]

Electrode and selective material	Analyte	Detection method	LOD (μM)	Linear response range (μM)	Sensitivity ($\mu A \mu M^{-1}$)	Ref.
PEDOT/ nano-Au	**EP**	**CV**	**5×10^{-1}**	**1-1 × 10^2**	**6×10^{-2}**	**This study**
	ST	**CV**	**5.7**	**10-320**	**4.9×10^{-2}**	**[2]**

RGO-reduced graphene oxide; MIP-molecularly imprinted polymer.

From the analysis of the literature results, it can be stated that the electrochemical sensor developed in this work, PEDOT/nano-Au, exhibits an analytical performance comparable to the analytical performances of the sensors reported in the literature. The developed electrochemical sensor can be efficiently used for the determination of epinephrine.

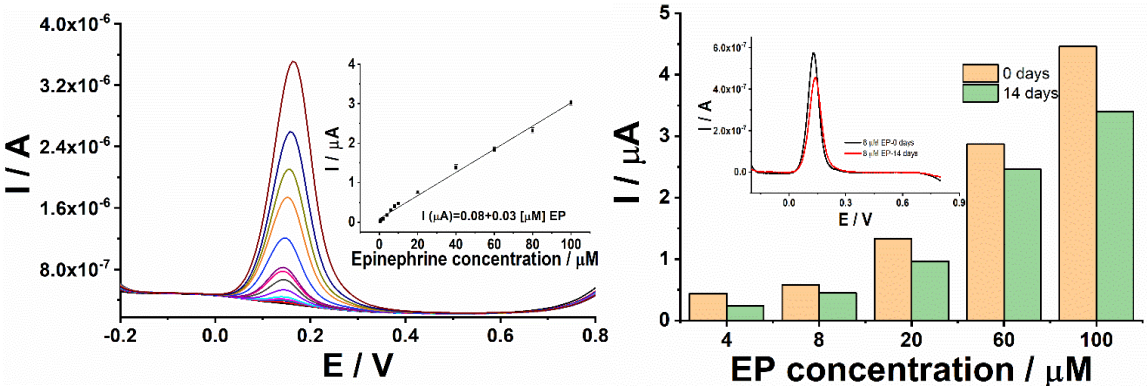
6.2. Electrochemical sensors based on PEDOT-PDA and gold nanoparticles

The main objective of the present study is the development of electrochemical sensors based on modified electrodes with composite materials that include a hybrid polymeric material consisting of a biomimetic organic polymer, polydopamine (PDA), a conducting organic polymer (PEDOT) and gold nanoparticles (nano-Au), synthesized with the sinusoidal current method (I_{sin}) [6–8]. The electrochemical sensor was implemented for the analytical determination of epinephrine in synthetic and real samples.

6.2.3.3. Optimization of analytical parameters

The electrochemical sensor PEDOT-PDA/nano-Au was investigated in 0.1 M PBS aqueous solution (pH = 7.4) for the analytical determination of epinephrine. The analytical response of the PEDOT-PDA/nano-Au sensor was evaluated by differential pulse voltammetry through the construction of a calibration curve in 0.1 M PBS (pH = 7.4) with successive additions of epinephrine in the concentration range of 0.4–100 μM (***Figure 57a***). A linear relationship was obtained between the anodic peak current associated with the epinephrine oxidation and its concentration over the range of 0.4–100 μM, described by the following regression equation: I_{pa} (μA) = 0.082 + 0.029 [EP] (μM), with R^2 = 0.9941. The oxidation of epinephrine at the surface of the PEDOT-PDA/nano-Au sensor occurs at an anodic peak potential of 0.141 V, a value significantly more negative compared to the anodic peak potential recorded for the electrode modified with the hybrid material deposited in two sequential steps. The limit of detection and the limit of quantification were calculated as 0.062 μM and 0.21 μM, respectively. The sensor sensitivity was determined as the ratio between the slope of the anodic peak current vs. epinephrine concentration plot (0.029 μA × μM^{-1}) and the geometric area of the electrode (0.071 cm^2), resulting in a sensitivity of 0.41 μA × cm^{-2} × μM^{-1}. The repeatability was evaluated through replicate measurements (n = 3) for epinephrine concentrations of 4 μM, 20 μM, 60 μM, and 100 μM and expressed as relative standard deviation. Values of 5.9%, 4.6%, 3.2%, and 3.4% were obtained, indicating acceptable repeatability values within the allowed limits. The reproducibility was assessed by measuring epinephrine concentrations of 4 μM, 20 μM, 60 μM, and 100 μM using three independently prepared electrochemical sensors under identical experimental conditions. The corresponding values were 7.05%, 4.9%, 3.5%, and 4.1%, respectively. These results

demonstrate good reproducibility for the prepared electrochemical sensor [8]. The stability of the PEDOT-PDA/nano-Au voltammetric sensor was evaluated for five concentration levels, from 4 µM to 100 µM epinephrine, over a period of 14 days (***Figure 57b***). At the lowest concentration, a decrease in the anodic peak current of 45.4% is observed. At intermediate concentrations (8 µM, inset graph showing sensor response) and high concentrations (60 µM), the decrease in sensor response is smaller (22.4% and 22.7%). In addition, for the 20 µM concentration level, a decrease in the anodic peak current of 27.8% can be observed. The results highlight good stability of the voltammetric sensor over the mentioned time period [8].



a) **b)**

Figure 4. *Analytical applications:* ***a)*** *DPV for PEDOT-PDA/nano-Au in solution of 0.1 M PBS and 0.4-100 µM EP. Inset: the calibration curve (I_{pa} vs. EP concentration);* ***b)*** *Evaluation of the stability of the PEDOT-PDA/nano-Au sensor over a 14-day period at five concentration levels. Inset: Sensor response to 8µM EP. Images adapted from reference [8], © Elsevier.*

6.2.4. Applications of sensors in the electroanalysis of epinephrine in real samples

The PEDOT-PDA/nano-Au electrochemical sensor was implemented for the detection of epinephrine in a real urine sample spiked with known concentrations of analyte. The urine sample was diluted at a 1:10 ratio with 0.1 M phosphate buffer aqueous solution (pH = 7.4). The anodic peak current associated with epinephrine oxidation increases linearly with concentration according to the following equation: I_{pa} (µA) = 0.14 + 0.026 [EP] (µM), R^2= 0.9952. A sensitivity of 0.37 µA × cm^{-2} × $µM^{-1}$ was obtained. Therefore, the developed electrochemical sensor can efficiently determine epinephrine in a real sample, in the presence of an interfering species (uric acid), without a significant matrix effect [8].

Table 2*. Analytical recovery values in urine samples for the PEDOT-PDA/nano-Au electrochemical sensor. Table adapted from reference [8], © Elsevier.*

Urine samples	Added (µM)	Detected (µM)	Recovery (%)	RSD (%)
	20	18.3	91.5	2.4
	40	36.8	92.0	7.4
Sample 1	60	64.4	107.3	2.9
	80	87.2	109.0	4.6
	100	94.7	94.7	2.9
Sample 2	40	42.0	105.0	5.2
Sample 3	20	18.9	94.5	3.3

The analytical recovery values for epinephrine in real urine samples were calculated in order to estimate the accuracy of the detection method. The analytical recovery values for real urine samples are presented in ***Table 17*** [8].

6.2.5. Performance comparison of sensors prepared by simultaneous and sequential analysis

Figure 62 highlights the response of different electrochemical sensors synthesized by the I_{sin}, E_{sin} and CV methods in an aqueous solution containing 10 µM EP and 0.1 M PBS (pH = 7.4). The best analytical performance is ascribed to the PEDOT-PDA/nano-Au sensor developed by the I_{sin} method which exhibits a higher anodic peak current amplitude. The anodic peak current is four times greater than the anodic currents observed for the electrochemical platforms developed using the sinusoidal voltage (E_{sin}) and cyclic voltammetry (CV) methods [8].

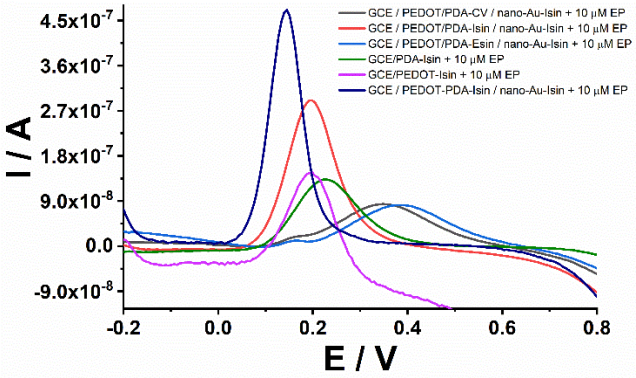


Figure 5*. Comparison of the analytical performance of different electrochemical sensors prepared with I_{sin}, E_{sin} and CV methods in an aqueous solution containing 10 µM EP and 0.1 M PBS (pH=7.4). Image adapted from reference [8], © Elsevier.*

The role of nano-Au in the structure of the PEDOT-PDA/nano-Au electrochemical sensor was investigated in detail. To achieve this objective, the synthesis of nano-Au was performed directly on the surface of the unmodified electrode using the I_{sin} and galvanostatic methods, followed by a comparison with the electrochemical sensor based on electrode modified with the composite material. The synthesis process was carried out over two deposition times, 40 s and 100 s. According to the differential pulse voltammograms shown in ***Figure 63***, the electrodes modified with nano-Au by the I_{sin} and galvanostatic methods exhibited inferior analytical performance compared with the electrode modified with the composite material (PEDOT-PDA/nano-Au) in the presence of 40 µM EP. An additional comparison was performed using an unmodified electrode by evaluating its analytical response toward epinephrine. As expected, the unmodified electrode showed the weakest analytical response in the presence of epinephrine among all synthesized electrochemical platforms. The obtained results highlight the analytical efficiency of the PEDOT-PDA/nano-Au composite material for the determination of epinephrine.

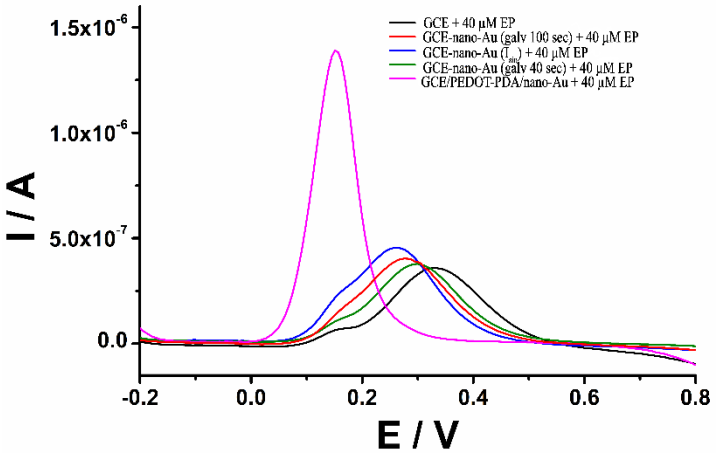


***Figure 6**. Comparison of the analytical performance of the sensor modified with nano-Au (galvanostatic and I_{sin}) and the PEDOT-PDA/nano-Au sensor in an aqueous solution containing 40 µM EP and 0.1 M PBS (pH=7.4). Image adapted from reference [8], © Elsevier.*

Table 18 highlights the main analytical and electrochemical parameters for the electrochemical sensors prepared by simultaneous and sequential synthesis. The electrochemical sensors were implemented for the electroanalysis of epinephrine in synthetic samples (0.1 M PBS, pH = 7.4): anodic peak potential (E_{pa}), anodic peak current (I_{pa}), sensitivity (S), linear response range, limit of detection (LOD), limit of quantification (LOQ), charge transfer resistance (Rct), and the difference between anodic and cathodic peak potentials (ΔE_p).

***Table 3**. Analytical parameters for the electrochemical sensors prepared with the I_{sin} method and applied for the electroanalysis of EP. Table adapted from reference [8], © Elsevier.*

Sensor	GCE/ PEDOT-PDA/nano-Au	GCE/ PEDOT-PDA	GCE/ PEDOT/ PDA/nano-Au	GCE/ PEDOT/ PDA	GCE/ PDA-I_{sin}	GCE/ PEDOT-I_{sin}	GCE
Analytical parameter							
E_{pa} (V)	0.141	0.151	0.217	0.312	0.234	0.205	0.246
I_{pa} (µA)	3.17	2.50	1.26	0.99	0.44	0.74	0.51
S (µA/µM/ cm^2)	0.41	0.28	0.30	0.25	0.18	0.27	0.14
Linear response range (µM)	0.4-100	1-100	0.6-100	4-100	2-100	2-100	6-100
LOD (µM)	0.062	7.87	0.83	9.50	2.92	4.86	6.60
LOQ (µM)	0.21	25.98	2.50	31.35	9.64	16.05	21.78
R_{ct} (Ω)	696	1820	1957	29374	1695	220	1381
ΔE_p (V)	0.162	0.150	0.220	0.156	0.187	0.084	0.174

6.2.6. Performance comparison of the developed sensors with literature data

The analytical performance of the PEDOT-PDA/nano-Au sensor prepared by the I_{sin} method was compared with the analytical performances of sensors reported in the literature for the electroanalysis of epinephrine (***Table 19***). Comparable results can be observed in

terms of key analytical performance criteria, including the limit of detection, linear response range, and sensitivity.

Table 4. *Examples of sensors reported in the literature used for the electroanalysis of EP.*

Sensor type	Detection method	LOD (µM)	Linear response range (µM)	Sensitivity (µA × μM^{-1})	Sample matrix	Ref.
Ag/Cu NC-GCE	DPV	0.43	0.52-1665.02	4.9×10^{-2}	0.25 M H_2SO_4	[9]
Pt_1-$Ru_{7.5}$-Fe_2@N-OMCs/GCE	DPV	0.031	0.07-130	5.385×10^{-1}	0.25 M H_2SO_4	[10]
NC-BM-ZIF	CA	0.01	0.5-1.625	3.79×10^{-1}	0.1 M PBS (pH=7,5)	[11]
PEDOT-PDA/ nano-Au-I_{sin}	**DPV**	**0.062**	**0.4-100**	**2.9×10^{-2}**	**0.1 M PBS (pH=7.4)**	**This study [8].**

PDA-polidopamine; Ag/Cu NC-silver/copper-supported nitrogen-doped carbon; Pt_1-$Ru_{7.5}$-Fe_2-platinum, ruthenium and iron nanoparticles; N-OMCs-nitrogen-doped ordered mesoporous carbon; I_{sin}-sinusoidal current method.

Chapter 7 presents the development, characterization, testing and validation of electrochemical sensors based on electrodes functionalized with an organic polymer (PEDOT) and platinum nanoparticles. The electrochemical sensors based on PEDOT and nano-Pt were obtained by implementing the I_{sin} method. The combination of PEDOT with nano-Pt in a composite material provides synergistic effects that enhance the analytical performance of the sensor used for the detection of the analyte serotonin.

7.3.3. Construction of the calibration curve

The analytical response of the PEDOT/nano-Au sensor was evaluated by cyclic voltammetry for different serotonin concentrations in the range of 2–80 µM. ***Figure 73a*** shows the cyclic voltammograms representing the sensor response in a synthetic sample spiked with different serotonin concentrations, while ***Figure 73b*** illustrates the corresponding calibration curve with the linear dependence of the anodic peak current on serotonin concentration [12]. The stability of the PEDOT-PtNP-based electrochemical sensor was investigated by performing measurements over a period of 21 days, during which the sensor was stored in the laboratory (***Figure 73c***). The sensor response showed a relative decrease compared to the initial value at different concentration levels, as follows: 28.9% at 20 µM, 23.55% at 40 µM, 18.18% at 60 µM, and 15.15% at 80 µM. A more pronounced decrease was observed at low analyte concentrations, whereas at concentrations higher than 40 µM, the decrease in sensor response was significantly lower, which may be considered indicative of acceptable operational stability over the investigated time period. These results demonstrate the good analytical performance of the developed electrochemical sensor for the determination of serotonin [12].

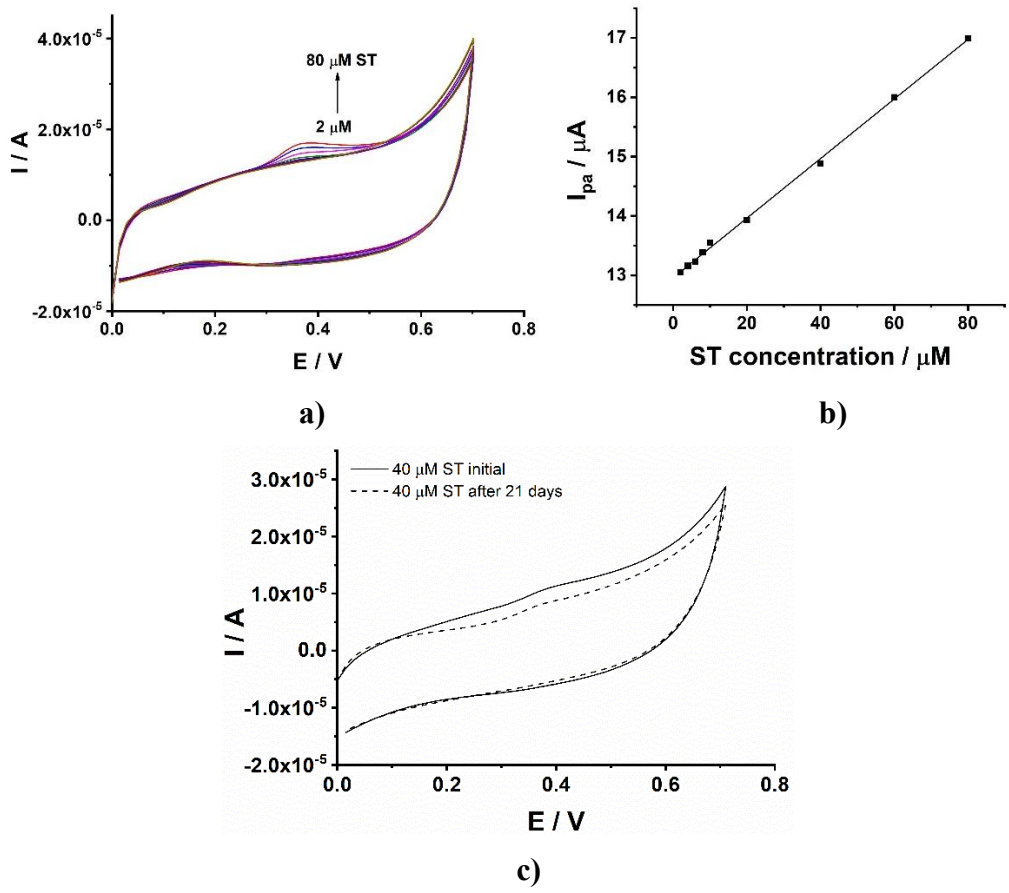


Figure 7. *The analytical response:* ***a)*** *CV for the PEDOT/nano-Pt sensors in an aqueous solution of 0.1 M PBS (pH=7) and 2-80 μM ST; v = 0,05 V/s;* ***b)*** *The calibration curve;* ***c)*** *Sensor response after storage. Images adapted with permission from reference [12].*

The electrochemical sensor exhibited a linear response over the concentration range of 2–80 μM with the following linear regression equation: I_{pa} (μA) = 12.2 + 0.057 [ST] (μM), R^2 = 0.9992. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 1.9 μM and 6.3 μM, respectively. The slope, LOD, and LOQ values of the PEDOT/nano-Pt electrochemical sensor indicate superior analytical performance compared to the PEDOT/nano-Au sensor presented in Chapter 6, both being implemented for serotonin detection. Repeatability and reproducibility were calculated at a concentration of 40 μM, yielding values of 3.6% and 4.7%, respectively [12]. These results demonstrate a very good analytical performance of the developed electrochemical sensor for serotonin determination.

7.5. Comparison of the analytical performance of the developed sensors with literature data

In this section, a comparative evaluation was performed between the electrochemical sensors reported in the literature, developed with conventional methods and the PEDOT/nano-Pt electrochemical sensor developed via the I_{sin} method (***Table 23***). It can be observed that the electrochemical sensor developed and described in this chapter exhibits analytical performance criteria comparable to those of the electrochemical sensors reported in the literature.

Table 5. *Representative examples of sensors reported in the literature for the electroanalysis of ST.*

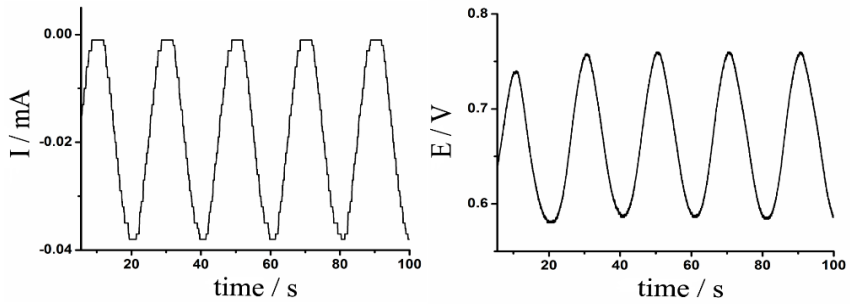
Electrode and selective material	Analyte	Detection methid	LOD (µM)	Linear response range (µM)	Sensitivity ($\mu A \mu M^{-1}$)	Ref.
MIS-coated PtNPs/ GCE	ST	DPV	2×10^{-2}	5×10^{-2}-80	3.90×10^{-3}	[13]
CNTs-Cu_2O-CuO@Pt/ GCE	ST	CA	3×10^{-3}	1×10^{-2}-3×10^{3}	-	[14]
Pt/OPPy/ RGO /GCE	ST	DPV	1.06×10^{-1}	5×10^{-1}-470	1.04×10^{-2}	[15]
PEDOT/ nano-Pt	**ST**	**CV**	**1.9**	**2-80**	**5.70×10^{-2}**	**This study [12]**

MIS-coated PtNPs-platinum nanoparticles coated with molecularly imprinted silica; OPPy-overoxidized polypyrrole; RGO -reduced graphene oxide.

Chapter 8 describes the development of an electrochemical sensor based on PEDOT and nano-Ag and its application for the detection of quercetin in synthetic and real samples. The composite material was synthesized on the surface of the working electrode using an original sinusoidal current–based method (I_{sin}).

8.1. Synthesis, electrochemical and morphological characterization of composite materials

The electrochemical synthesis of nano-Ag on the conventional electrode modified with PEDOT was carried out using the I_{sin} method. ***Figure 75*** presents the graphs of the applied sinusoidal current and the resulted potential during the electrochemical synthesis process. The applied sinusoidal current (***Figure 75a***) generates a potential variation in the range of 0.58–0.75 V (***Figure 75b***).



a) **b)**

Figure 8. *The deposition of nano-Ag on the glassy carbon electrode modified with PEDOT with I_{sin} method:* ***a)*** *The sinusoidal current applied;* ***b)*** *The system response as potential. Image adapted under the CC BY 4.0 licence from reference [1], DOI: 10.3390/bios14070320. Text translated into Romanian language.*

8.2. Assessment of kinetic parameters and analytical performance

8.2.1. Effect of potential scan rate and pH influence

The PEDOT/nano-Ag electrochemical sensor was implemented for the detection of quercetin in 0.1 M acetate buffer solution (pH = 5), exhibiting a linear response over the concentration range of 1–40 µM (***Figures 81a and 81b***). The following linear regression equation was obtained: I_{pa} (µA) = 1.1 + 0.2 [QER] (µM), R^2 = 0.9851. The analytical parameters, including sensitivity, LOD, and LOQ were also determined, yielding values of 2.817 µA × μM^{-1} × cm^{-2}, 2.8 µM, and 9.5 µM, respectively [1].

The stability of the PEDOT/nano-Ag sensor was evaluated over a period of 22 days at two concentration levels (20 and 40 µM). The sensor response showed a relative decrease compared to the initial value of 20.48% for the 20 µM QER concentration and 28.07% for the 40 µM QER concentration. Stability studies performed at lower QER concentrations showed a minimal decrease in the anodic peak current. ***Figure 81c*** illustrates the response of the PEDOT/nano-Ag sensor in the presence of 40 µM QER over 10 consecutive potential cycles. A stabilization of the anodic peak current can be observed after the first three potential scans, demonstrating good electrochemical stability.

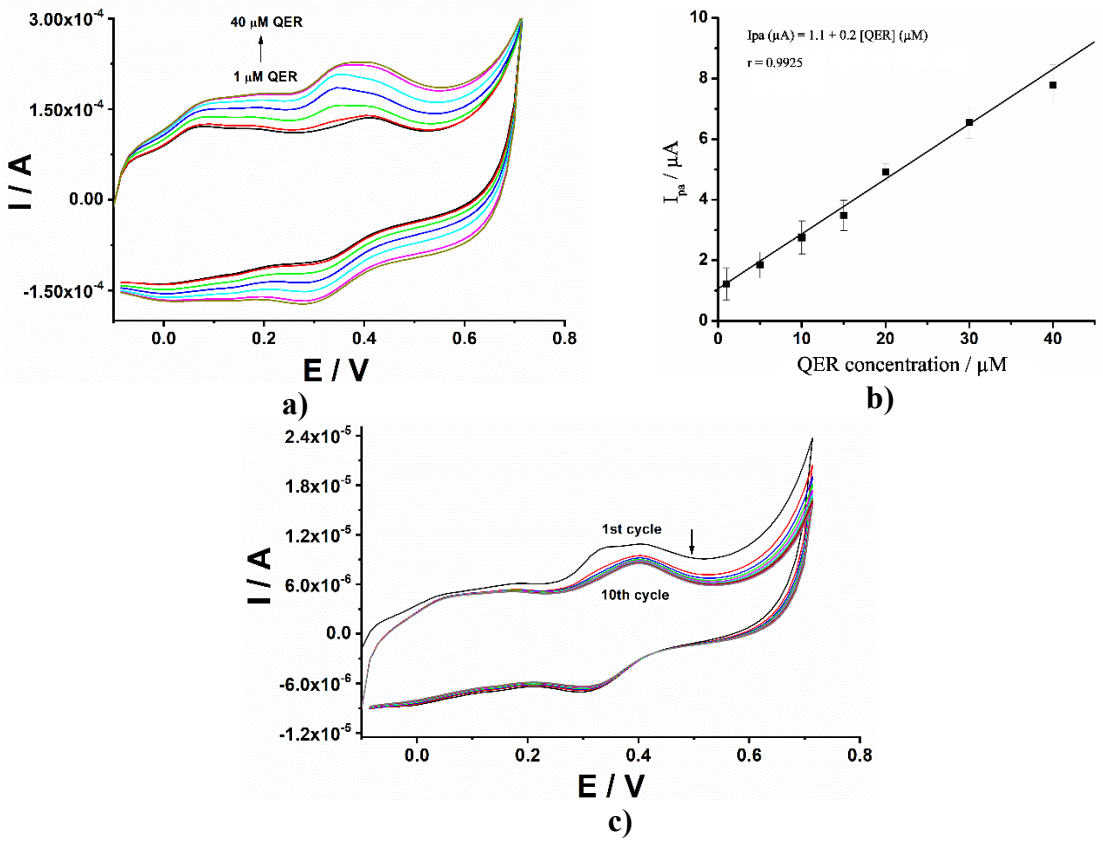


Figure 9. *The analytical response of the PEDOT/nano-Ag sensor:* ***a)*** *CVs recorded in acetate buffer solution (pH=5) and 1-40 µM QER;* ***b)*** *The calibration curve with error bars (n=3);* ***c)*** *Sensor response in 0.1 M acetate buffer containing 40 µM QER during 10 consecutive potential cycles. Images adapted under the CC BY 4.0 licence from reference [1], DOI: 10.3390/bios14070320. Text translated into Romanian language.*

8.3.2. Comparison of analytical performance with literature data

The PEDOT/nano-Ag electrochemical sensor prepared using the original I_{sin} method was analytically evaluated by comparison with other electrochemical sensors reported in the literature for the detection of the same analyte, quercetin (***Table 27***). The results highlight comparable analytical performance for all the mentioned sensors in terms of key analytical parameters, including the limit of detection, linear response range and sensitivity.

Table 6*. Examples of sensors reported in the literature for the electroanalysis of QER.*

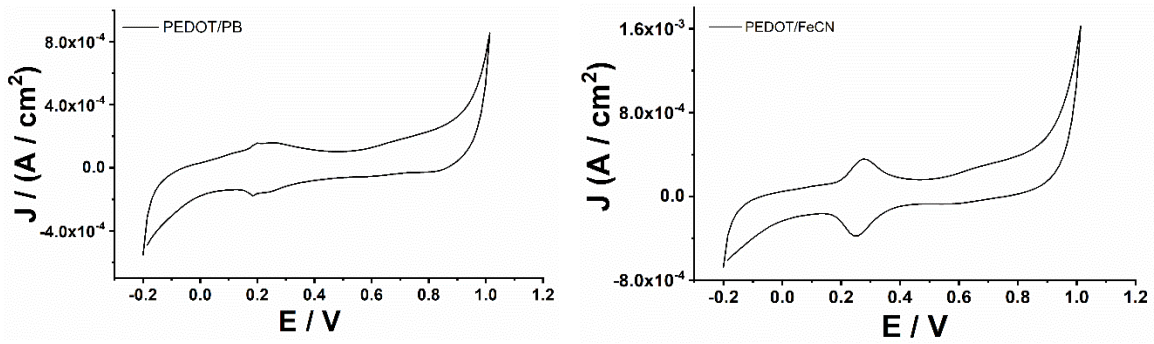
Electrode and selective material	Analyte	Detection method	LOD (µM)	Linear response range (µM)	Sensitivity ($\mu A \mu M^{-1}$)	Ref.
AuNPs/ AgNPs/ CFME	QER	DPV	1.1×10^{-2}	5×10^{-1}-10	1.69×10^{-4}	[16]
AgNPs@ g-CN	QER	DPV	6×10^{-3}	1×10^{-2}-120	3.51×10^{-2}	[17]
AgNPs-AETGO	QER	SWV	3.3×10^{-3}	1×10^{-2}-5	7.79×10^{-2}	[18]
PEDOT/ nano-Ag	**QER**	**CV**	**2.8**	**1-40**	$\mathbf{2 \times 10^{-1}}$	**This study [1]**

CFME-carbon fiber microelectrodes; g-CN-graphitic carbon nitride nanosurfaces; AETGO-2-aminoethanethiol (cysteamine)-functionalized electrochemically reduced graphene oxide sheets.

Chapter 9 describes the development of an electrochemical sensor composed of a conducting polymer (PEDOT) and an inorganic redox mediator (Prussian Blue, PB). The synthesis of the PEDOT-PB composite material was performed using the E_{sin} method on the surface of a glassy carbon electrode (GCE). The developed sensor (PEDOT/PB) was applied for the determination of potassium ions (K^+), an analyte of major importance in the medical field [19].

9.1. Synthesis and electrochemical characterization of modifiers

The electrochemical characterization of the GCE/PEDOT/FeCN and GCE/PEDOT/PB sensors using cyclic voltammetry enables the evaluation of charge transfer at the electrode–electrolyte interface and provides an in-depth understanding of the reaction mechanisms. The voltammetric response of the PEDOT-modified electrode (GCE/PEDOT/FeCN) and the electrode modified with the composite material (GCE/PEDOT/PB) was recorded in an electrolyte solution consisting of 0.1 M KCl and 0.01 M HCl (***Figure 86***). In this way, the redox behavior of the PB material is evaluated [20].



a) b)

Figure 10. *The response in CV for:* ***a)*** *The PEDOT/PB sensor;* ***b)*** *The PEDOT/FeCN sensor in an aqueous solution of 0.1 M KCl and 0.01 M HCl; v = 0.05 V/s. Image adapted with permission from Springer Nature from reference [20], DOI:10.1007/s10008-023-05392-2.*

9.2. Analytical applications. Detection of potassium ions and dopamine

The developed PEDOT/PB electrochemical sensor was validated for the analytical determination of K^+ ions in synthetic samples. The analytical performance parameters, including the linear response range, sensitivity, limit of detection, limit of quantification, and repeatability, were determined and evaluated.

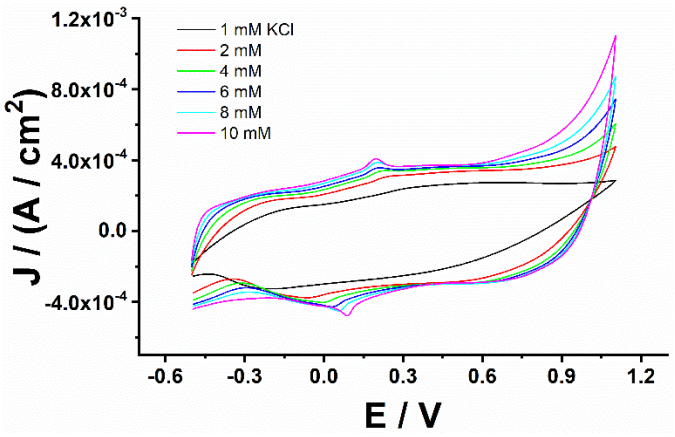


Figure 11. *Analytical applications-The response of PEDOT/PB sensor in the presence of 10^{-3}-10^{-2} M K^+, v = 0.05 V/s. Image adapted with permission from Springer Nature from reference [20], DOI:10.1007/s10008-023-05392-2.*

The analytical applications were performed using cyclic voltammetry over a K^+ concentration range of 10^{-2} M to 10^{-3} M (***Figure 88***). The decrease in K^+ concentration leads to a reduction in both anodic and cathodic peak currents, as well as a shift of the cathodic peak potential toward more negative values [20].

Part C includes general conclusions, original scientific contributions and future directions for development and improvement of analytical performance.

Chapter 10 presents the general conclusions of the doctoral thesis.

The main objective of the doctoral thesis was the development of five studies aimed at designing electrochemical sensors based on modified electrodes with nanocomposite materials, using two original methods based on sinusoidal voltages and sinusoidal currents for the analytical determination of epinephrine, serotonin, quercetin, and potassium ions.

The main conclusions, structured by chapters, are as follows:

The PEDOT/nano-Au electrochemical sensor was developed using the sinusoidal current method through a two-step electrochemical synthesis of the PEDOT polymer matrix and the nano-Au layer. The electrochemical synthesis of the composite material was performed with intermediate synthesis parameters which were subsequently optimized in the final stage. The evaluation of the efficiency of the *in-situ* synthesis process and the electrochemical properties of the synthesized composite material was performed with the electrochemical quartz crystal microbalance, cyclic voltammetry and the electrochemical impedance spectroscopy.

The PEDOT-PDA/nano-Au electrochemical sensor was obtained through the *in-situ* simultaneous synthesis of the hybrid PEDOT-PDA material, followed by the formation of a nano-Au layer using the sinusoidal current technique. The best analytical performance was achieved for the PEDOT–PDA/nano-Au electrochemical sensor: a limit of detection of 62 nM, a limit of quantification of 0.21 μM, a coefficient of determination of 0.9941 and repeatability and reproducibility values below 6% and 7%, respectively. The interference study revealed that uric acid does not significantly interfere with the analyte detection. The platform was successfully applied to the detection of the analyte in urine samples.

The PEDOT/nano-Pt electrochemical sensor was developed by implementing the sinusoidal current method and was successfully applied for the detection of serotonin. The electrochemical sensor exhibited low limits of detection and quantification (1.9 μM and 6.3 μM) with a coefficient of determination of 0.9992. The sensor was successfully employed for the detection of serotonin in urine samples.

The PEDOT/nano-Ag electrochemical sensor was developed using the sinusoidal current method and was successfully employed for the analytical detection of the antioxidant quercetin. The developed electrochemical sensor demonstrated improved analytical performance: a limit of detection of 2.8 μM, a limit of quantification of 9.5 μM and a coefficient of determination of 0.9851. Repeatability and reproducibility exhibited values lower than 3.5% and 6.5%, respectively. Accuracy was expressed as analytical recovery rate in spiked real samples with values ranging from 96% to 108%. The PEDOT/nano-Ag electrochemical sensor was successfully applied for the detection of quercetin in both synthetic samples and complex real samples (grape must).

The PEDOT/PB electrochemical sensor was obtained through the electrochemical synthesis of the PEDOT/PB composite material using the sinusoidal voltage method. The sensor was applied for the analytical determination of potassium ions in synthetic samples, yielding promising results: a limit of detection of 3.1 mM, a limit of quantification of 10.23 mM and a repeatability of 12.8%.

10.2. Main achievements and original contributions

The original scientific contributions of this doctoral thesis are briefly presented below:

- The development and optimization of two original methods for the preparation of electrochemical sensors based on sinusoidal voltages and currents with selected frequencies and amplitudes;
- The electrochemical synthesis and testing of four electrochemical sensors using an original sinusoidal current-based method: PEDOT/nano-Au, PEDOT/nano-Pt, PEDOT/nano-Ag, and PEDOT-PDA/nano-Au;

- The development and testing of a voltametric sensor, PEDOT/PB, via an original sinusoidal voltage-based method based on *in-situ* electrochemical synthesis of an inorganic compound in a polymeric matrix;
- The establishment and optimization of the experimental electrochemical parameters for the implemented synthesis methods;
- The electrochemical and morphological characterization of the modified electrodes;
- The in-situ monitoring of the synthesis processes of composite materials;
- Testing and validation of the electrochemical sensor performance under laboratory conditions;
- The estimation of analytical performance in synthetic and real samples (urine) for the studied analytes.

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12. ANNEXES

ARTICLES INCLUDED IN THE DOCTORAL THESIS

ISI PAPERS LIST

1. **Leau, S.A.**; Lete, C.; Matei, C.; Lupu, S. Electrochemical Sensing Platform Based on Metal Nanoparticles for Epinephrine and Serotonin. *Biosensors (Basel)* **2023**, *13*, doi:10.3390/bios13080781. **Q1, IF (2023) = 4,9**;
2. **Leau, S.A.**; Lete, C.; Marin, M.; del Campo, F.J.; Diaconu, I.; Lupu, S. Electrochemical Sensors Based on Antimony Tin Oxide-Prussian Blue Screen-Printed Electrode and PEDOT-Prussian Blue for Potassium Ion Detection. *Journal of Solid State Electrochemistry* **2023**, *27*, 1755–1766, doi:10.1007/s10008-023-05392-2. **Q3, IF (2023) = 2,6**;
3. **Leau, S.A.**; Diaconu, I.; Lete, C.; Matei, C.; Lupu, S. Electroanalysis of Serotonin at Platinum Nanoparticles Modified Electrode. *Sci. Bull. Series B,* **2024**, *86*. **Q4, IF (2024) = 0,3**;
4. **Leau, S.A.**; Marin, M.; Toader, A.M.; Anastasescu, M.; Matei, C.; Lete, C.; Lupu, S. MeNPs-PEDOT Composite-Based Detection Platforms for Epinephrine and Quercetin. *Biosensors (Basel)* **2024**, *14*, 320, doi:10.3390/bios14070320. **Q1, IF (2024) = 5,6**;
5. **Leau, S.-A.**; Lete, C.; Matei, C.; Lupu, S. Novel Biomimetic Polymer-Based Material Prepared by Alternate Currents Method for Electrochemical Sensing Platform Development. *Journal of Electroanalytical Chemistry* **2026**, *1001*, 119703, doi:10.1016/j.jelechem.2025.119703. **Q1, IF (2024) = 4,1**;

Cumulative IF: 17,5

ATTENDANCE AT NATIONAL/INTERNATIONAL SCIENTIFIC EVENTS

ORAL PRESENTATIONS

1. **Leau, S.-A.**; Lete, C.; Lupu, S. Electrochemical Sensor Based on Poly (3,4-Ethylenedioxythiophene) and Pt Nanoparticles for Biologically Active Compounds Determination. In *Book of Abstracts-The 5th Edition of the International Conference of the Doctoral School at the „Gheorghe Asachi" Technical University of Iasi (TUIASI), 18-20 May 2022, online, oral presentation*; p. 52.
2. **Leau, S.-A.**; Lete, C.; Lupu, S. Development of Sensing Materials Based on PEDOT and Pt NPs for Analytical Applications. In *The 22nd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE 22), 7–9 September 2022, Sinaia, Romania, oral presentation*.
3. **Leau, S.-A.**; Lete, C.; Matei, C.; Lupu, S. PEDOT-AuNPs Nanocomposite Modified Glassy Carbon Electrode for Epinephrine Sensing. In *Book of Abstracts – The 17th Conference of Physical Chemistry (ROMPHYSCHEM), 25–27 September 2023, Bucharest, Romania, oral presentation*; p. 60.
4. Lupu, S.; **Leau, S.-A.**; Lete, C.; Diaconu, I.; Matei, C. Electrochemical Sensing of Neurotransmitters Using a Metal Nanoparticle-Based Composite Platform. In *Book of Abstracts – The 4th International Electronic Conference on Biosensors (IECB 2024), 20–22 May 2024, online, oral presentation*; p. 79.

POSTER PRESENTATIONS

1. **Leau, S.-A.**; Lete, C.; Diaconu, I.; Matei, C.; Lupu, S. Pt-PEDOT Based Electrochemical Sensing Platform for Serotonin Quantification. In *Book of Abstracts – The 17th Conference of Physical Chemistry (ROMPHYSCHEM), 25–27 September 2023, Bucharest, Romania,* p. 68.
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