ROMANIAN ACADEMY
School of Advanced Studies of the Romanian Academy
„Petru Poni" Institute of Macromolecular Chemistry from Iaşi

SUMMARY OF DOCTORAL THESIS

ELECTROCHEMICAL SENSORS WITH BIOMEDICAL APPLICATIONS

PhD SUPERVISOR:

C.S. I Dr. MARIANA PINTEALĂ

PhD STUDENT:

Drd. Oana-Elena RĂCHITĂ

(married CARP)

2023

CUPRINS

I. INTRODUCTION

Electrochemistry is a multidisciplinary science that deals with the study of the relationship between electrical energy and chemical changes. Chemical reactions that involve the input or generation of electric currents are called electrochemical reactions. This complex field has numerous applications in our daily lives. A notable example is in the manufacture of batteries and accumulators, the heart of many modern devices such as phones, laptops and electric cars. Electrochemistry allows the transformation of chemical energy into electrical energy, facilitating the operation of these devices. In addition, electrochemistry has a big impact on the environment, being essential in the treatment, analysis and purification of water, in order to remove pollutants and toxic substances. In the medical field, applications of electrochemistry have an important role in the diagnosis and monitoring of diseases through the use of electrochemical sensors (eg: sensors for glucose or cholesterol).

Electrochemical sensors and biosensors play a crucial role in environmental monitoring, industrial processes, and medical diagnostics, ensuring precise and rapid identification of significant substances. This field has known rapid advancement, since electrochemical sensors through the use of nanotechnology, are able to identify analytes at very low concentration in different samples. These sensors provide notable benefits such as simple and rapid analysis, often eliminating the need for extensive preliminary sample treatment. By 2023, more than 100,000 research reports on electrochemical sensors are registered in the "Web of Science" database, highlighting the growing importance and interest in the scientific community.

Based on these premises, the overal goal of the doctoral thesis was the design, development and optimization of new electrochemical methods and sensors for the determination and quantification of specific analytes, which would offer a reliable alternative to classical methods.

Thesis structure4

The doctoral thesis entitled "**Electrochemical sensors with biomedical applications**" is structured in four distinct parts, summarizing six chapters.

Part I - includes the motivation behind the choice of the research topic, emphasizing its importance, novelty, challenges and state of the art, making a connection between the research

topic and the international and national concerns of the research community. In addition, a concise presentation of the content of the work is provided, highlighting the results obtained.

Part II - focuses on a detailed theoretical study, which discusses the theoretical and conceptual fundamentals essential for understanding the principles of electrochemical cell operating and the main techniques used in electrochemical analysis .

- **Chapter II.1** provides an insight into the structure and components of the electrochemical cell, as well as the electrochemical processes and mechanisms of electronic transfer at the electrode/liquid interface. The electrochemical techniques used in the experimental part: cyclic voltammetry, differential pulse voltammetry and amperometry are presented in detail.

Part III – is structured in three chapters (Chapters III.2-III.4) and includes original contributions regarding the development, optimization and characterization of new electrochemical methods and sensors for the determination and characterization of some biomarkers of interest, namely cholesterol, piperine and nitrite.

- **Chapter III.2** deals with the development and optimization of an innovative electrochemical method for the determination of cholesterol in acidic medium, using the Liebermann-Buchard reaction adapted for electrochemical conditions.

- **Chapter III.3** includes the electrochemical analysis of two natural alkaloids, piperine and capsaicin, using voltammetric techniques in combination with different chemical tests, with the aim of a deeper understanding of their redox behavior and antioxidant activity. The redox behavior of the studied alkaloids was compared with that of some known antioxidants, namely ascorbic acid, protocatechuic acid, syringic acid and tyrosine, using them as a control group. The evaluation of the antioxidant properties using electrochemical techniques was carried out by using four specific tests: (i) DPPH free radical scavenging; (ii) TEMPO scavenging; (iii) Fe^{3+} reducing power; (iv) H_2O_2 scavenging.

- **Chapter III.4** studies the deposition of AuNPs on two-dimensional materials (2DM) used as a substrate: Graphene and MoS_2. The differences obtained in the electrodeposited particles were highlighted and evaluated against the oxidation of nitrite (NO_2^-).

- ***Part IV*** includes the specific and general conclusions drawn from the results obtained during the experimental studies

The results obtained and presented in the doctoral thesis "Electrochemical sensors with biomedical applications" have so far been the subject of two scientific papers published in ISI rated journals and four communications (oral and poster) at international and national scientific events.

III. Personal contributions
III.1. Electrochemical determination of Cholesterol

This chapter contains the development and optimization of a simple and robust electrochemical method for the determination and quantification of serum cholesterol, using the Liebermann-Burchard (LB) reaction in an innovative way for electrochemical conditions and thus offering a reliable alternative to colorimetric methods.

Preliminary Cyclic Voltammetry Tests – Redox behavior of cholesterol

The electrochemical behavior of cholesterol was studied in a mixture of chloroform-tetrabutylammonium perchlorate (TBAP), acetic anhydride and concentrated H_2SO_4, using cyclic voltammetry. The representative current-potential curve for the LB mixture in the presence of cholesterol shows an increase in the anodic current at potentials higher than 1.4 V, indicating the possible oxidation of cholesterol (Figure 1.A.b). As a control experiment, we performed cyclic voltammetry for the mixture of Liebermann-Burchard reagents in the absence of cholesterol, without an increase in the oxidation current being observed (figure 1.A.a).
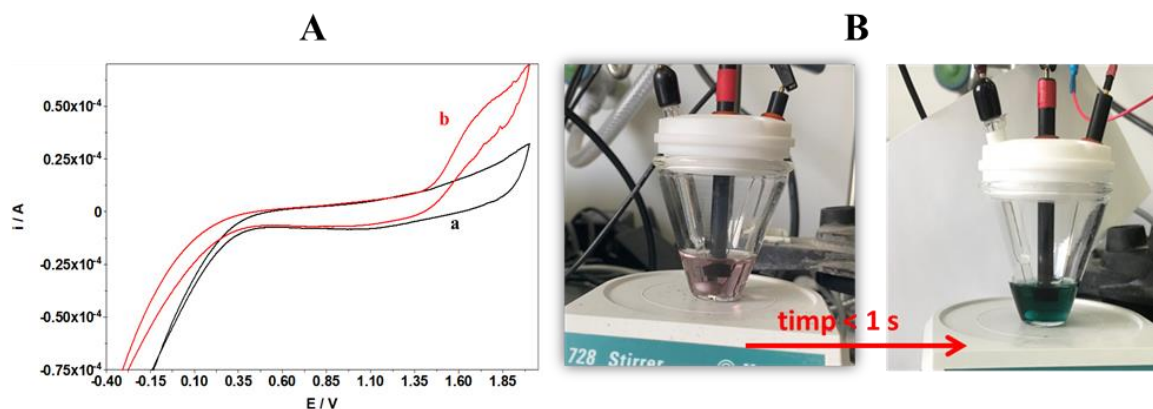
Figure 1. *(A) Cyclic voltammetry of the mixture: 2 mL Chloroform-TBAP, 1 mL acetic anhydride and 40 µL concentrated H_2SO_4: (a) in the absence and (b) presence of 0.64 mM cholesterol, using the GC*

electrode. (B) Color variation of the LB solution with cholesterol in the electrochemical cell when a potential is applied.

Normally, the LB reaction is quite slowly (aprox. 30 min). but when this reaction is placed in the electrochemical environment and coupled with the application of a potential, the reaction is greatly accelerated, and this is easily visible due to the instantly shift of color to green [1,2] (figure 1.B) when the 1.4 V threshold is reached.

Amperometric tests

In order to understand the reaction mechanism, we investigated the amperometric response obtained for the oxidation of cholesterol in the Lieberman–Burchard mixture containing TBAP as electrolyte, under a constant potential of 1.5 V. In the amperometric experiment, the baseline is recorded for the mixture of chloroform-TBAP, acetic anhydride and concentrated H_2SO_4, and when cholesterol is added, the oxidation current increases rapidly (figure 2.A).
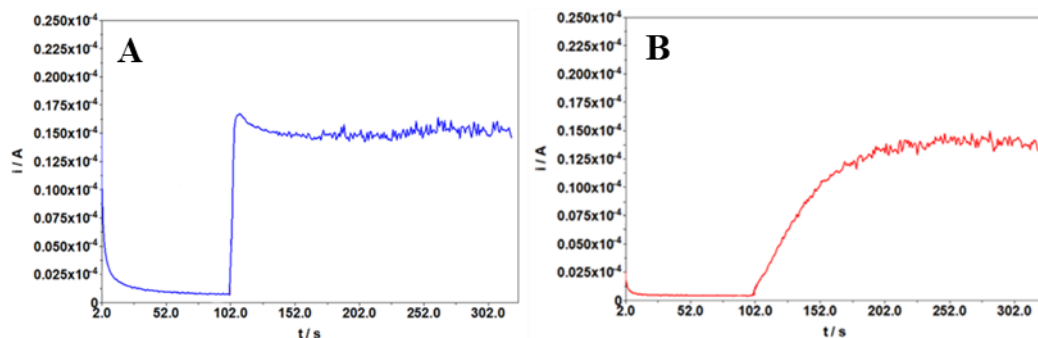
Figure 2. *(A) Amperometric response for 0.64 mM cholesterol added to the mixture of 2 ml chloroform-TBAP, 1 ml acetic anhydride and 40 μL concentrated H_2SO_4); (B). Amperometric response for 40 μL of concentrated H_2SO_4 added to the mixture of 2 mL chloroform-TBAP, 1 mL acetic anhydride, and 0.64 mM cholesterol.*

Since the oxidation of cholesterol under strongly acidic conditions involves more than an electrochemical mechanism, we explored the detection of cholesterol by a reverse approach, adding the reagents in a different order. In this case, the oxidation current increases relatively slowly, reaching a constant response after about 100 s (figure 2.B). As expected, the color change of the solution was slower. The slow evolution of the reaction (the slow increase of the oxidation current and the slow color variation) indicates a difficult electronic transfer thus demonstrating that the electrochemical mechanism is preceded by a chemical one. The chemical

reaction between acetic anhydride and sulfuric acid (R 1) appears to be of critical importance to the electrochemical reaction [3].

$$H_2SO_4 + Ac_2O \rightarrow AcOH + AcO\text{-}SO_2OH \rightarrow HOSO_2\text{-}CH_2\text{-}COOH \quad (R\ 1)$$

Acetylsulfuric acid   Sulfoacetic acid
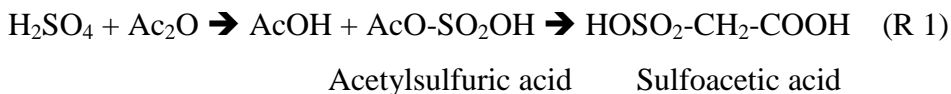
Sulfoacetic acid reacts with cholesterol, this being the first step in cholesterol derivatization (**chemical mechanism**), thus forming cholesta-dienes [1, 4, 5]. The latter are then involved in electronic transfer with the electrode surface under the influence of the applied potential (**electrochemical mechanism**).

Influence of Applied Potential

Six different values of applied potentials 0.8, 1.0, 1.2, 1.4, 1.5 and 1.6 V were tested in the present study for the oxidation of a certain concentration of cholesterol (0,64 mM). The recorded signal increases with the value of the applied potential, and the maximum value of the current was recorded for the potential of 1.6 V. Because the current response was not very stable and the noise was quite significant at 1.6 V potential, we settled on 1.5 V to be used for further experiments.

Influence of Acid Concentration

In this study, we tested the amperometric response obtained for the oxidation of 1 mM cholesterol, varying the volume of concentrated H_2SO_4 in the Lieberman–Burchard reaction mixture. The higher the concentration of the acid involved in the reaction, the higher the response, but addition of 200 μL acid resulted in the instability of response current, probably due to the gradual degradation of products causing passivation of the electrode surface. The results are satisfactory for 100 μL of H_2SO_4 which was used in further experiments.

Calibration curve

In order to determine the sensitivity of the method and the range of linearity of the amperometric response, the oxidation current was studied for different concentrations of cholesterol in the LB mixture, under the previously optimized conditions and shown in figure 3.A. We tested cholesterol standard solutions with concentrations between 0.025 and 7 mM, applying a potential of 1.5 V. The developed method shows the sensitivity of 14.959 μA/mM

calculated from the calibration curve (figure 3.B.) and a detection limit of 19.78 nM cholesterol, with a linear response in the range 0.025-3 mM (R^2=0.99).
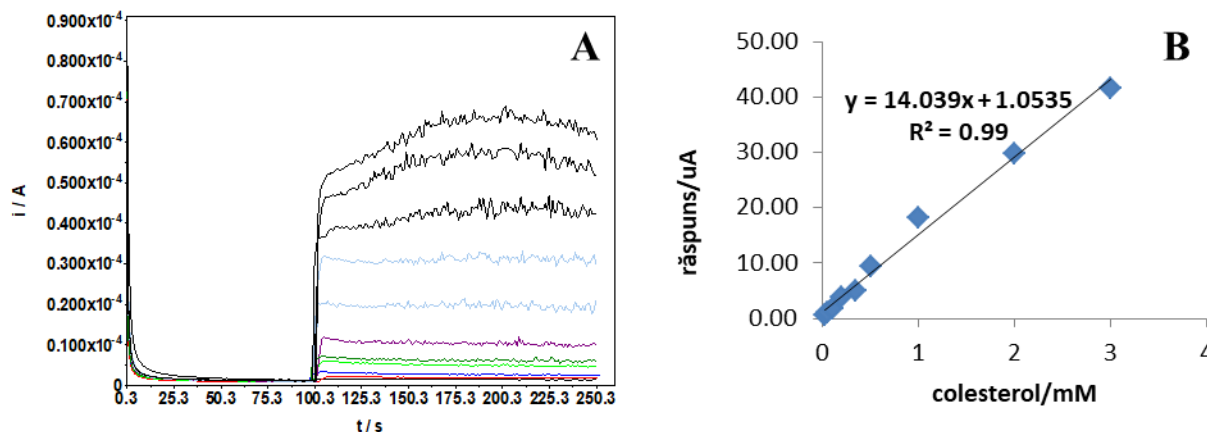
Figura 3. (A) Amperometric response for different concentrations of cholesterol, applying a constant potential of 1.5 V. (B) Calibration curve of amperometric response.

Interferences

To evaluate the selectivity of the method, a number of compounds that can coexist with cholesterol in biological samples, such as ascorbic acid, uric acid, KCl, NaCl and glucose, were tested both individually and together with cholesterol using the working conditions previously optimized. *The compounds tested in this study do not interfere with cholesterol oxidation. Therefore, this method has a high degree of specificity.*

Repeatability of the Method was evaluated by repeating consecutive amperometric measurements of the same cholesterol concentration or repeating them on different days (table 1). *The developed method was shown d to have a suitable level of precision and the analytical performance towards cholesterol, with a good sensitivity of determination.*

Table 1. *Repeatability of cholesterol electrochemical determination.*

	Assays	Mean Response Current (μA)	RSD
Intra-assay precision	triplicate consecutive determinations of the ame concentration	10.56	1.97%
Inter-assay precision	10 determinations from different days	10.37	4.07%

Cholesterol detection in blood samples

Analytical validation of the electrochemical method was accomplished compared with data obtained from an accredited laboratory (Top Medical Grup, Iasi, Romania), from the same subjects involved in the study. The results shown in table 2 demonstrate that cholesterol concentration in serum determined using the optimized electrochemical method agreed well with the data provided by the medical laboratory tests, revealing that the developed method was accurate. Moreover, also from Table 2 it can be seen that the extraction method used is more efficient when a volume of 200 µL serum is used compared to 100 µL serum. Metoda dezvoltată este simplă, rapidă și ar putea fi adaptată pentru a fi utilizată în analizele de rutină. *The developed method was simple, fast and could be adapted to use in routine analyses.*

Table 2. *The results obtained by analyzing the serum samples by the developed electrochemical method and compared with the data provided by the medical laboratory.*

Sample number	Cholesterol concentration (mg/dL)		Difference (A-B)/B*100
	Electrochemical method (A)	**Medical Laboratory (B)**	
1a	140.68	156.1	-9.87
1b	150.13		-3.82
2a	295.69	318.2	-7.07
2b	308.94		-2.91
3a	146.76	160.4	-8.5
3b	153.17		-4.5
4a	204.51	221.3	-7.58
4b	208.64		-5.72
5a	247.06	273.4	-9.63
5b	273.99		0.21
6a	209.07	226.7	-7.77
6b	219.27		-3.27
7a	213.63	223.54	-8.11
7b	221.55		-5.92

a = sample obtained from 100 µL serum
b = sample obtained from 200 µL serum

## III.2. Antioxidants analysis using electrochemical techniques

This chapter presents the electrochemical analysis of the two natural alkaloids, piperine and capsaicin, using voltammetric techniques coupled with different chemical tests to better

understand their redox behavior and antioxidant activity. The redox behavior of the studied alkaloids was compared with that of some known antioxidants, used as control, namely: ascorbic acid, syringic acid, protocatechuic acid and tyrosine.

The electrochemical behavior of piperine and capsaicin compared to that of control antioxidants

Determination of the oxidation potentials of the compounds studied in this thesis (piperine, capsaicin compared to the control antioxidants) is the first step in the evaluation of antioxidant activity of a molecule. A low value of the oxidation potential reflects the propensity of a given molecule to donate electrons and can therefore be considered to exhibit strong antioxidant (antiradical) activity. For many phenolic antioxidants, the reaction involves the transformation of a phenol moiety to a quinone, a typical feature for the oxidation process taking place in the 300–700 mV region. Figures 4. A and B show the voltammograms obtained for the control antioxidants, ascorbic and syringic acid, which are oxidized in a single step forming the corresponding quinones, at relatively low positive potentials in an acidic environment. The potential value is influenced by the number of hydroxyl groups present in their structure [6].

Figure 4.C shows the capsaicin voltammogram for two repetitive cycles. Initially, capsaicin undergoes an irreversible oxidation at 0.45 V, donating two electrons, with the formation of ortho-benzoquinone and methanol as byproducts. During the cathodic scan, the formed benzoquinone is reduced to ortho-hydroxyphenol (catechol) generating the reduction peak at 0.2 V [7]. During the second cycle, catechol is re-oxidized to ortho-benzoquinone, generating a second anodic peak at 0.26 V, simultaneously with the decrease of the one at 0.45 V.
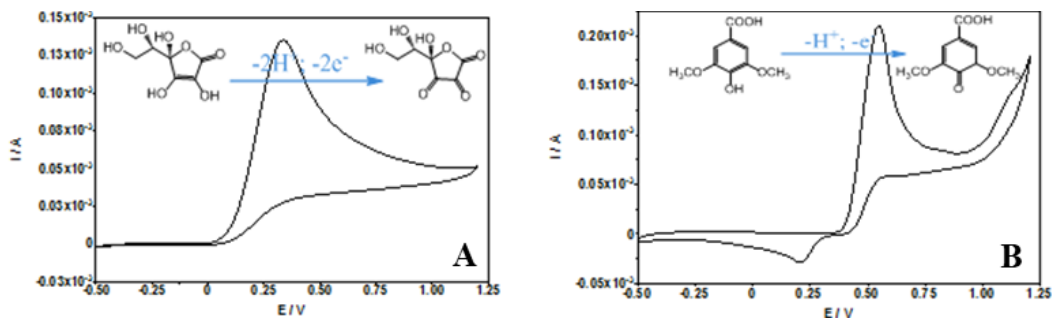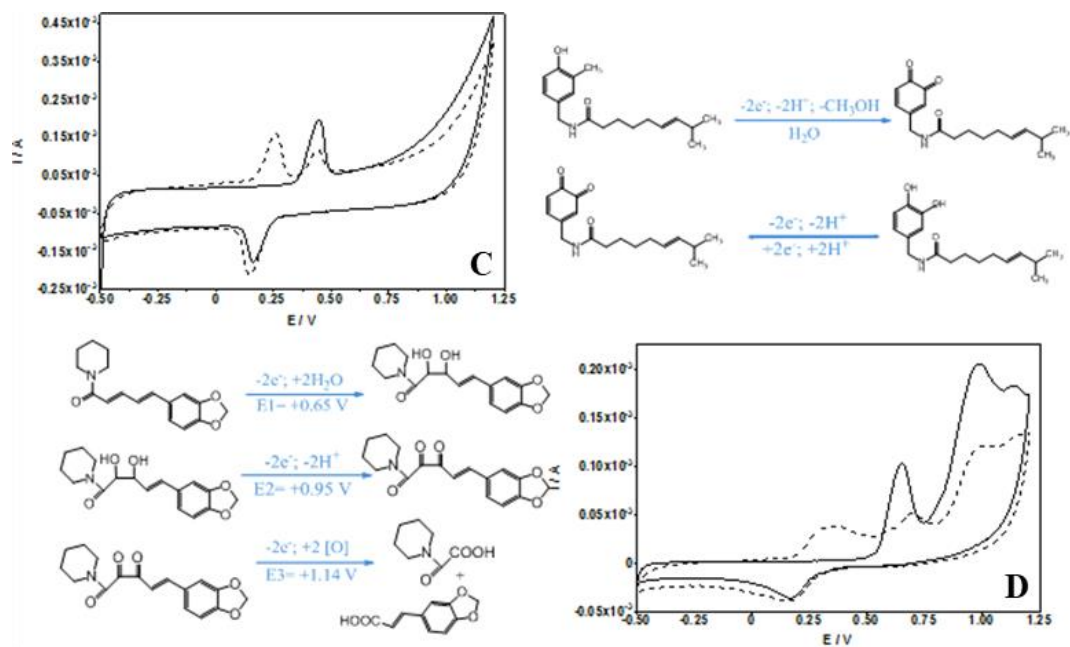
Figure 4. Cyclic voltammograms of 0.5 mM (A) ascorbic acid, (B) syringic acid, (C) capsaicin, and (D) piperine in 0.1 M HClO₄, and the oxidation reactions.

The electrooxidation of piperine involves a three-step electronic transfer process. Initially, at 0.65 V, the formation of piperine diol is initiated, succeeded by its subsequent oxidation into a dionic compound at 0.95 V. Next, the oxidation of the resulted diionic compound leads to the breaking of the bond between two carbon atoms with the formation of two carboxylic acids (1.14 V) [157]. Sweeping the potential in the opposite direction, a reduction reaction takes place, followed by dimerization and passivation of the electrode surface, as suggested by the sharp decrease of the peaks at repeated scans (cycle two - dotted line) (figure 4.D).

The relatively low oxidation potential of ascorbic acid demonstrates that this is the most easily oxidized compound among the six studied compounds and should have a significant antioxidative activity compared to the others. At the opposite pole is tyrosine with the highest oxidation potential value and the lowest number of –OH groups. However, the number of phenolic –OH groups present in the structure of a molecule is not always the only factor that determines its antioxidant activity. The positions of phenolic –OH groups, the presence of other functional groups such as double bonds and their conjugation also play an important role in the antioxidant activity.

The *in vitro* antioxidant ability of piperine and capsaicin was evaluated by four tests widely employed in assessing the antioxidant activity of bioactive compounds, namely: DPPH•, TEMPO, hydroxyl radicals scavenging and Fe^{3+} reducing power assays. Ascorbic, syringic, protocatechuic acids along with tyrosine have been used for comparison as control antioxidants.

Electrochemical study of 1,1-diphenyl-2-picryl-hydrazyl (DPPH•) scavenging activity

The remaining redox activity of the DPPH• radical following the interaction with an antioxidant [8] is determined electrochemically after their incubation for 45 minutes.

Figure 5.A reports the electrochemical study of initial electrooxidation of DPPH• (black line) and piperine (blue line) compared to remaining peaks after their incubation in $HClO_4$ (red line). The decrease of all the peaks after mixture incubation indicates the partial consumption of DPPH•.
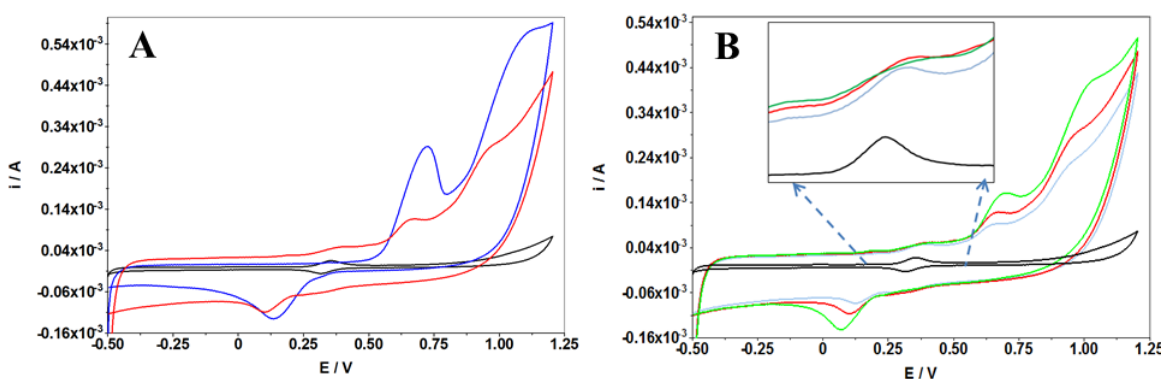
Figure 5. *(A) Cyclic voltammetry of 0.5 mM DPPH• (black), 0.5 mM piperine (blue) and the mixture: 0.5 mM DPPH• + 0.5 mM piperine (red). (B) Cyclic voltammetry of 0.5 mM DPPH• (black) and of the mixture: 0.5 mM DPPH• + 0.25 mM piperine (blue); 0.5 mM DPPH• + 0.5 mM piperine (red) and 0.5 mM DPPH• + 1 mM piperine (green).*

Figure 5.B supports this finding by showing how the oxidation peak of DPPH• decreases for higher concentrations of piperine, as the radical is progressively involved in the reaction with the piperine.

The DPPH method was also used to evaluate the antioxidant activity of capsaicin. Figure 6.A shows the well-defined peaks of DPPH• (black line) and capsaicin (blue line) compared to the response obtained for their mixture after incubation for 45 minutes (red line). Capsaicin shows very well defined redox peaks, which are significantly reduced after incubation with

DPPH•, accompanied by the complete disappearance of DPPH• peaks, as a results of the radical-scavenging process.
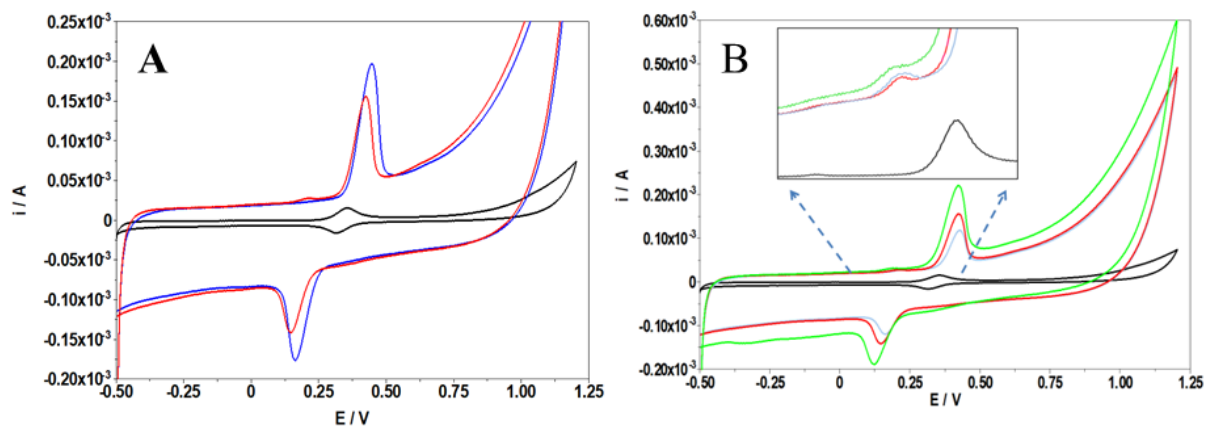

Figure 6. *(A) Cyclic voltammetry of 0.5 mM DPPH• (black), 0.5 mM capsaicin (blue) and the mixture: 0.5 mM DPPH• + 0.5 mM capsaicin (red. (B) Cyclic voltammetry of 0.5 mM DPPH• (black) and the mixture: 0.5 mM DPPH• + 0.25 mM capsaicin (blue); 0.5 mM DPPH• + 0.5 mM capsaicin (red) and 0.5 mM DPPH• + 1 mM capsaicin (green).*

As in the case of piperine, the variation of the DPPH• peak was studied for several concentrations of capsaicin. In the image inserted in figure 6.B, it can be seen how DPPH• oxidation takes place at lower potential values, and the height of the peak is influenced by the concentration of capsaicin.

To confirm the effect of the studied antioxidants (piperine and capsaicin) on DPPH•, the same test was performed under similar conditions using the control antioxidants (ascorbic acid, protocatechuic acid – figure 7. A and B). It was found that in all the cases the oxidation peak of DPPH• decreases or even disappears after incubation with the antioxidant, and the DPPH● radical scavenging activity is related to the nature and structure of phenolics compounds.
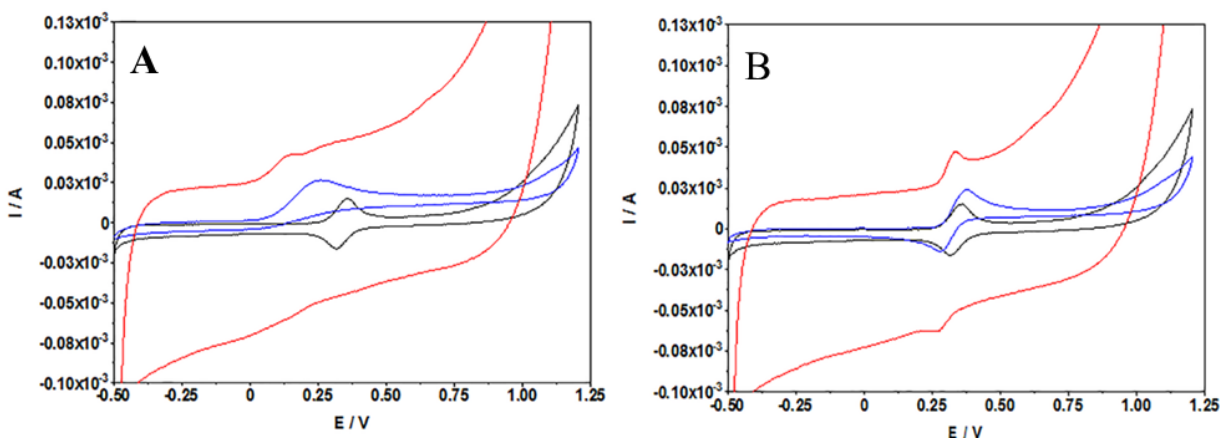
Figura 7. Cyclic voltammetry of 0.5 mM DPPH• (black), 0.5 mM antioxidant (blue) and the mixture 0.5 mM DPPH• + 0.5 mM antioxidant (red; figure (A) for ascorbic acid and (B) for protocatechuic acid, in 0.1 M HClO$_4$.

In this experiment, it was shown that even molecules that do not contain hydroxyl groups (piperine) possess the ability to neutralize free radicals, depending on their structure and the arrangement of double bonds [9].

Electrochemical study of 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) scavenging activity

The electrochemical investigation into the ability of piperine and the control antioxidants to neutralize the TEMPO radical was studied in 0.1 M HClO$_4$ aqueous solutions, using the differential pulse voltammetry (DPV) technique.
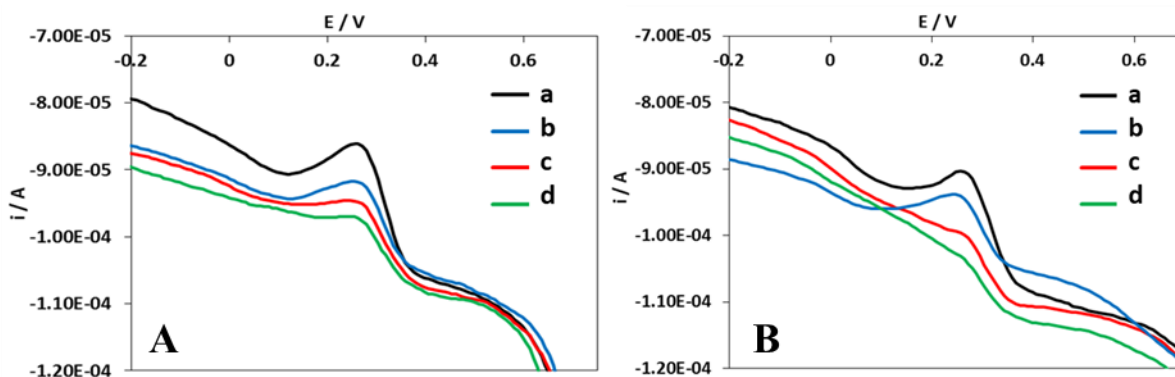
Figure 8. (A) DPV for 2 mM TEMPO after incubation with 0 mM (a), 0.25 mM (b), 0.5 mM (c) and 1 mM piperine (d); (B) DPV for 2 mM TEMPO after incubation with 0 mM (a), 0.25 mM (b), 0.5 mM (c) and 1 mM ascorbic acid (d).

With the DPV technique, the antioxidant effectiveness of piperine can be evaluated by its ability to scavenge the free radical species and figure 8.A shows the DPV of 2 mM TEMPO (line a) and after incubation with different concentrations of piperine (lines b, c and d). The anodic peak of TEMPO decreases when increasing the concentration of piperine [10-12]. Similar effect was obtained with the control antioxidants when incubated with TEMPO and figure 8.B shows the comparable case of ascorbic acid: the higher the concentration of ascorbic acid, the smaller the TEMPO peak.

Measurements with TEMPO revealed that piperine might possess the same antioxidant properties similar to the control studied antioxidants. The results proved consistent with those provided by DPPH method.

Electrochemical reduction of the ferric ion (Fe^{3+})

Based on the electrochemical behavior of the studied compounds, this chapter proposes to monitor the interactions between the Fe^{3+} ion and an antioxidant using cyclic voltammetry, in order to follow the possible changes of the redox peaks specific to the Fe^{3+}/Fe^{2+} transition [13].

Figure 9.A shows the individual voltammograms of each reactant: $K_3Fe(CN)_6$ - black line and piperine - blue line, compared to the response obtained for their mixture - red line. When the mixture is evaluated, a decrease of redox peaks typical for Fe^{3+}/Fe^{2+} process is noticed, due to the interaction with piperine and thus suggesting the reducing capacity of piperine. Meantime, piperine is partially oxidized during the interaction with Fe^{3+}, and consequently, its electrochemical oxidation is slower as indicated by the shift of anodic peaks to higher potentials.
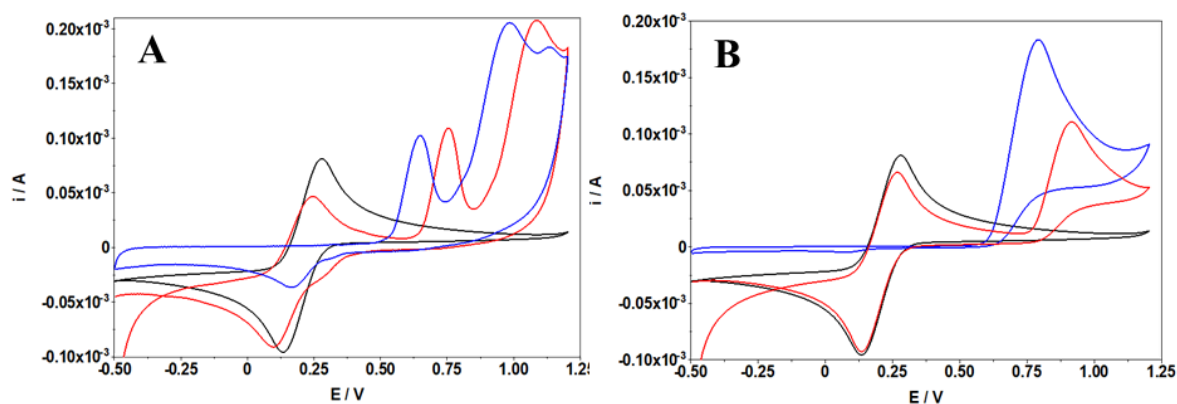

Figure 9. *(A) Cyclic voltammetry of 0.5 mM $K_3Fe(CN)_6$ (black), 0.5 mM piperine (blue) and the mixture of 0.5 mM $K_3Fe(CN)_6$ + 0.5 mM piperine (red);. (B) Cyclic voltammetry of 0.5 mM $K_3Fe(CN)_6$ (black), 0.5 mM tyrosine (blue) and mixture 0.5 mM $K_3Fe(CN)_6$ + 0.5 mM tyrosine (red);*

The same behavior on Fe^{3+}/Fe^{2+} transfer was also observed for the tested control antioxidants. The case of tyrosine is shown for comparison (figure 9.B). *The effect of tyrosine is similar to that of piperine, demonstrating its ability to reduce the Fe^{3+} ion, respectively their antioxidant activity.*

Electrochemical reduction of hydrogen peroxide

Anodic oxidation of hydrogen peroxide is widely used for studying the reactivity of molecules, evaluating the antioxidant capacity of different compounds, investigating oxidative effects on biomolecules, etc. [14-16]. Figure 10.A shows the CV curves obtained for an initial concentration of the OH• radical (line a) and after the addition of different concentrations of piperine (lines b, c, d and e). The addition of the antioxidant causes decrese of the H_2O_2 anodic peak, suggesting a decrease of its concentration due to the interaction with the antioxidant and the decrease is proportional with the antioxidant concentration.

Figure 10.B shows the curves of the OH• radical after the addition of different concentrations of capsaicin. The obtained results indicate the same consumption of H_2O_2 by capsaicin. The same effect was also observed for syringic acid used as a control (figure 10.C), demonstrating the antioxidant capacity of the studied compounds.
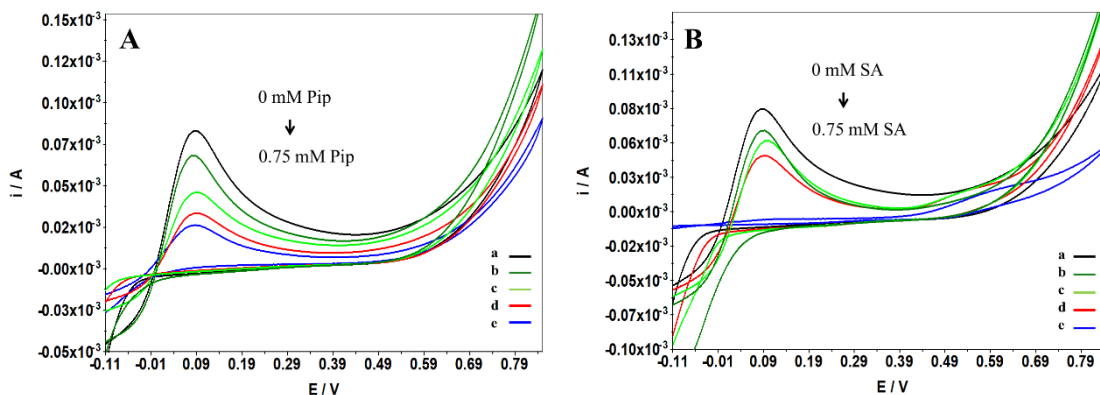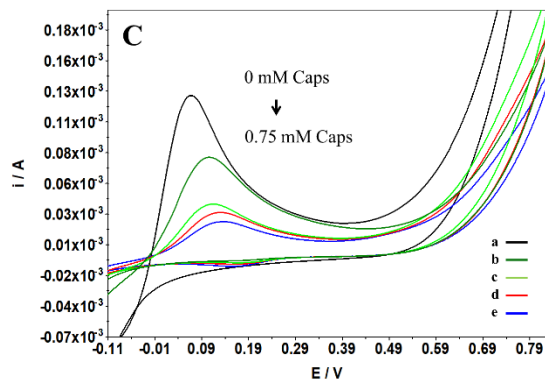
Figure 10. *Cyclic voltammetry for 5 mM H$_2$O$_2$ (a) and different concentrations of (A) piperine, (B) capsaicin and (C) syringic acid: 0.125 mM (b), 0.25 mM (c), 0.375 mM (d) and 0.75 mM (e), in 0.1 M KCl.*

III.3. Comparative electrodeposition of gold nanoparticles on two-dimensional materials. Applications for nitrite detection.

This chapter presents a comparative analysis of Au nanoparticles electrodeposited on different 2D materials used as substrates (Gr and MoS$_2$) or co-deposited with the metallic 2D material MoS$_2$. The electrocatalytic activity of the gold nanoparticles obtained in these configurations was evaluated against the oxidation of nitrite (NO$_2$$^-$) in order to obtain a sensitive electrochemical sensor for the determination of this analyte.

Electrodeposition of AuNPs on different 2D materials

AuNPs electrodeposition on different 2D materials was performed by Cyclic Voltammetry (figure 11). This involves the formation of nuclei through the direct reduction of metal cations at the electrode's surface from an electrolyte solution by applying an appropriate potential and the growth of particles on the surface of the formed nuclei, controlling the time and the applied potential. In the inset figure from figure 11, we can see the reduction peaks characteristic of the reduction process of gold ions (Au^{3+}) to metallic gold (Au^0) according to reaction R1 [17, 18]. These cathodic peaks appear in the potential range between 0.39 V - 0.47 V, depending on the nature of the material used as a substrate on the electrode.

$$AuCl_4 \text{ (aq)} + 3e^- \rightarrow Au(s) + 4\,Cl^- \hspace{3cm} (R1)$$

Figure 11. *Cyclic voltammetry for 10 mM HAuCl$_4$ on different substrates: unmodified SPE (yellow line), Gr/SPE (black line), MoS$_2$/SPE (blue line), co-deposition of HAuCl$_4$ 10 mM +MoS$_2$ 2 mg/mL (red line).*

In the case of AuNPs co-deposition with MoS$_2$, the presence of MoS$_2$ lamellae in the electrolyte solution provides a larger contact surface with Au^{3+} ions, facilitating the nucleation process [19, 20]. The modified electrodes resulting from the three configurations were denoted: AuNPs/Gr/SPE, AuNPs/MoS$_2$/SPE and MoS$_2$-AuNPs/SPE.

Morphological characterization of the modified electrodes

Figure 12 compares the SEM image of the unmodified SPE gold electrode (figure 12.A) and the image of the electrode with AuNPs electrodeposited directly on it (figure 12.B), used as a control.

Figure 12. *SEM images for A) bare SPE and B) AuNPs/SPE electrode.*

Figure 13.A, B and C highlight the presence of lamellar layered structures of MoS$_2$ and respectively, of graphene, with varying sizes from tens of nanometers to several micrometers,

which are stacked together increasing the porosity of the surfaces. These layered structures provide a larger surface area with more favorable sites for the nucleation and growth of gold nanoparticles.

Figure 13. SEM images for the electrode A) AuNPs/Gr/SPE, B) AuNPs/MoS$_2$/SPE and C) MoS$_2$-AuNPs/SPE.

The SEM analysis highlights the efficiency of the electrodeposition process and the preference of gold nucleation and growth for certain attachment sites.

Electrochemical characterization of AuNPs electrodeposited on different substrates

The electrochemical activity of AuNPs electrodeposited in the three configurations denoted: AuNPs/Gr/SPE, AuNPs/MoS$_2$/SPE and MoS$_2$-AuNPs/SPE, was evaluated using cyclic voltammetry in 0.1 M H$_2$SO$_4$ electrolyte and compared with the activity of AuNPs electrodeposited directly on the unmodified gold electrode (SPE), used as a control (figure 14). The voltammograms show the anodic peaks specific to the oxidation process of electrodeposited gold nanoparticles according to reactions (R 2) and (R 3) ref [21-23]:

$$\text{Au-(H}_2\text{O)}_{ads} \longrightarrow \text{Au-OH} + \text{H}^+ + \text{e}^- \qquad \text{(R 2)}$$

$$\text{Au-OH} \longrightarrow \text{Au=O} + \text{H}^+ + \text{e}^- \qquad \text{(R 3)}$$
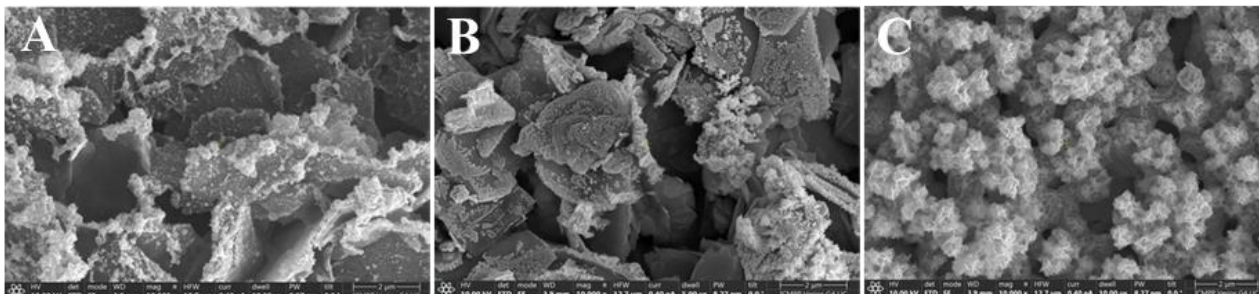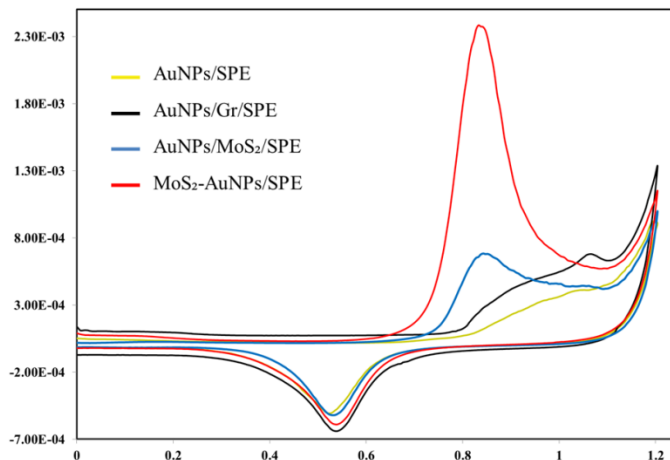
Figure 14. *The voltammetric response of AuNPs/SPE (yellow line), AuNPs/Gr/SPE (black line), AuNPs/MoS$_2$/SPE (blue line) and MoS$_2$-AuNPs/SPE (red line) in 0.1 M H$_2$SO$_4$.*

The differences in the height of the anodic peaks indicate an enhanced electronic transfer in the case of gold nanoparticles co-deposited with MoS$_2$ (MoS$_2$-AuNPs/SPE) compared to gold nanoparticles electrodeposited on Gr or MoS$_2$, due to a larger number of gold particles co-deposited with MoS$_2$ on the electrode surface, a result also confirmed by the SEM images.

Oxidation of nitrites using the electrodes resulting from the electrodeposition of AuNPs on different substrates

The electrocatalytic activity of the chemically modified electrodes obtained (AuNPs/Gr/SPE, AuNPs/MoS$_2$/SPE and MoS$_2$-AuNPs/SPE) was studied by cyclic voltammetry against nitrite oxidation. Figure 15 shows the recorded voltammograms for 0.2 mM NaNO$_2$ solution in 0.1 M H$_2$SO$_4$ using the three configurations for modified electrode and compared to the response obtained on the 2D material-free gold particle electrode (AuNPs/SPE) used as control.
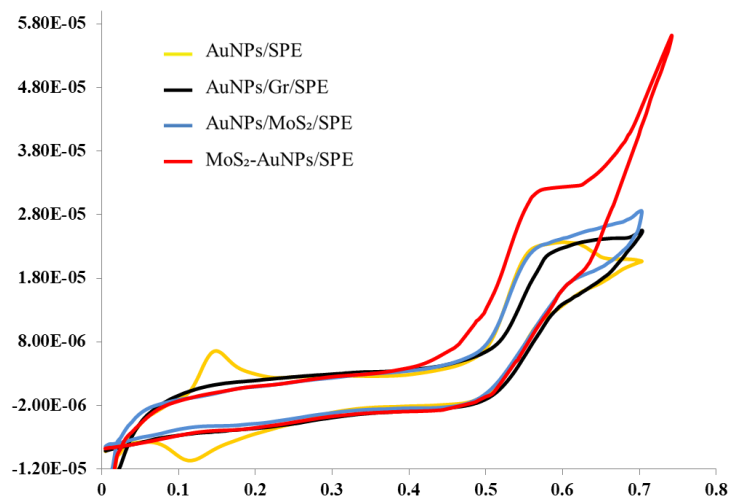
Figure 15. *The voltammetric response for 0.2 mM NaNO$_2$ at chemically modified electrodes: AuNPs/SPE (yellow line), AuNPs/Gr/SPE (black line), AuNPs/MoS$_2$/SPE (blue line) and MoS$_2$-AuNPs/SPE (red line) in 0.1 M H$_2$SO$_4$.*

The electrocatalytic activity of gold nanoparticles co-deposited with MoS$_2$ is superior compared to that of AuNPs electrodeposited either on the unmodified electrode or on the 2D materials (Gr and MoS$_2$), which is in agreement with previous data demosntrationg a higher number of AuNP when codeposited with MoS$_2$. Therefore, only the MoS$_2$-AuNPs/SPE configuration was further used to optimize the electrocatalytic detection of nitrite in order to develop a sensitive electrochemical sensor.

Optimization of nitrite detection - Influence of the applied potential

The influence of the applied potential was studied on MoS$_2$-AuNPs/SPE in order to improve the performance of the sensor, aiming to achieve a great sensitivity in the determination of nitrite. To investigate this relationship, we conducted a study where four different values of the applied potential (0.5; 0.6; 0.7 and 0.8 V) have been tested and the obtained oxidation current for a certain nitrite concentration was recorded. According to the obtained results, the signal recorded for 0.1 mM nitrite increases proportionally with the increase of the applied potential value until reaching the threshold of 0.7 V, after which at a higher potential (0.8 V) the recorded signal decreases. Therefore the potential of 0.7 V was used as the optimum applied potential in the following experiments.

Optimization of nitrate detection - Influence of acid concentration

Using the optimal applied potential of 0.7 V, we investigated the influence of H_2SO_4 concentration on the electrochemical oxidation reaction of nitrite (NO_2^-) on the MoS_2-AuNPs/SPE electrode. The amperometric response obtained for the oxidation of 0.1 mM nitrite was recorded using different concentrations of the acid used as electrolyte. It was observed that the analytical signal increases with the concentration of the electrolyte up to 0.25 M. Therefore, this concentration of sulfuric acid was used in the following experiments for the electrochemical determination of nitrite.

Calibration curve

To evaluate the analytical performance of electrodes chemically modified with AuNPs and 2D materials for the determination of nitrite, their calibration was performed by amperometry, measuring the analytical signal (current) for successive additions of nitrite (NO_2^-) in a 0.25 M H_2SO_4 solution, applying a constant potential of 0.7 V, under continuous stirring. Figure 16.A shows the nitrite oxidation current recorded over time for the MoS_2-AuNPs/SPE electrode (curve b) and compared with the signal recorded for AuNPs/SPE (curve a) used as a control. In both cases, for each addition of nitrite in the electrochemical cell, the oxidation current increases rapidly in 2-3 seconds until it reaches a stable value.
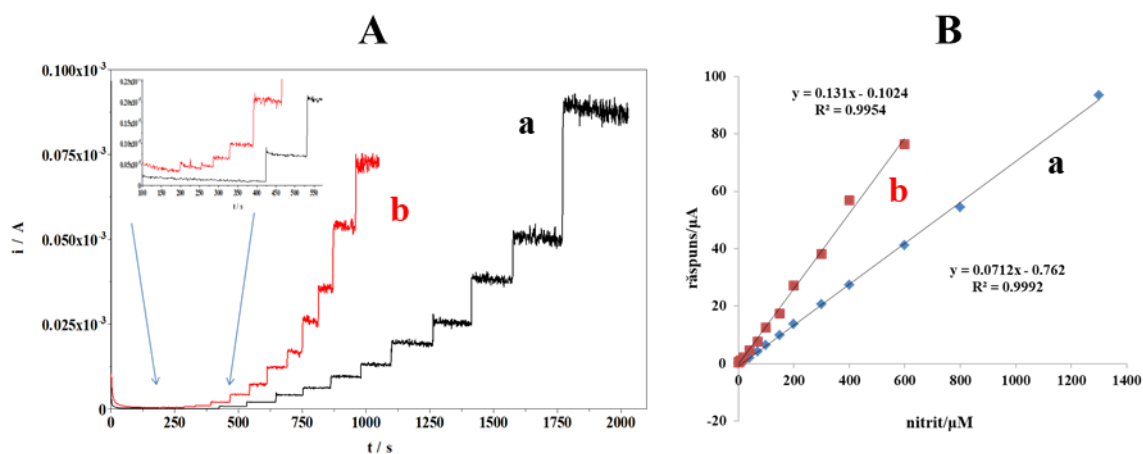
Figure 16. (*A*) *Amperometric response for successive additions of nitrite, applied potential 0.7 V. (B) Calibration curve: a) AuNPs/SPE si b) MoS₂-AuNPs/SPE.*

Table 3. *Analytical parameters for the determination of nitrite extracted from the calibration curves.*

The chemically modified electrode	Linear Range (µM)	Sensitivity (µA/µM)	Limit of detection (µM)
AuNPs/SPE	20-1300	0,071	0,34
MoS$_2$-AuNPs/SPE	0,5-600	0,131	0,16

Interferences

In order to evaluate the selectivity of the MoS$_2$-AuNPs/SPE electrode to nitrite, several substances that commonly coexist with nitrite ion (NO$_2^-$) in real samples and could possibly be oxidized at the applied potential for nitrite detection have been tested, such as KCl, MgCO$_3$, CaCl$_2$, NaNO$_3$, MgSO$_4$, glucose, and NaCl. It was experimentally demonstrated that the changes caused by the addition of these interferents are negligible, indicating a highly selective detection of NaNO$_2$ using the optimized MoS$_2$-AuNPs/SPE electrodes.

IV. GENERAL CONCLUSIONS

The results obtained in the framework of doctoral thesis "Electrochemical sensors with biomedical applications" lead to a series of general conclusions, summing up data from each chapter of the personal contributions, as follows:

Chapter III.1 Electrochemical determination of Cholesterol

In this chapter, electrochemical techniques combined with the Liebermann-Buchard reaction were explored as an innovative and simple approach for the quantitative determination of cholesterol, with high sensitivity and selectivity. The developed electrochemical method represented a reliable and robust alternative to conventional colorimetric or chromatographic methods, which are more expensive and require specialized personnel.

To enhance the efficiency of cholesterol oxidation through electrochemical methods, a comprehensive assessment of reaction mechanisms, optimal applied potential, acid volume, and possible interferences was conducted within the conditions of Liebermann-Burchard mixture. The developed method is sensitive and selective, demonstrating that other biological compounds do not interfere in the cholesterol response and shows good reproducibility. The optimized method was successfully applied in determining the cholesterol content in real samples, and validated with results obtained by a certified spectrophotometric method.

Chapter III.2 Analysis of antioxidants using electrochemical techniques

In this chapter, the applicability of electrochemical techniques to evaluate the antioxidant properties of piperine and capsaicin was explored. The study showed that electrochemical methods, in combination with free radical neutralization tests, can be used to evaluate the redox behavior and possible antioxidant properties of these compounds. Comparing the redox behavior of piperine and capsaicin with that of some known control antioxidants, the obtained results attest the fact that both piperine and capsaicin can function as antioxidants.

The experiments carried out in order to investigate the possible antioxidant properties of the studied compounds, led to a series of conclusions that highlight several determining factors for the antioxidant activity: (i) the configuration and the total number of hydroxyl groups; (ii) redox potential and electron transfer capacity; (iii) the presence or absence of an unsaturated bond. The obtained results also revealed that the number of phenolic -OH groups present in the

structure of the molecule is not the only factor influencing the antioxidant activity. Piperine, although does not have -OH groups in its structure, shows a similar activity to the antioxidants already known and used as references in this study. The position of the phenolic -OH groups and the presence of other functional groups, such as double bonds and conjugations, also play a significant role in determining antioxidant activity.

Chapter IV.3 Comparative electrodeposition of gold nanoparticles on two-dimensional materials. Applications for nitrite detection.

In this chapter, a comparative analysis of gold nanoparticles electrodeposited on two different 2D materials (Gr and MoS_2) was carried out using two working protocols. Through the electrodeposition of AuNPs on Gr and MoS_2 and co-deposition of AuNPs with the 2D metallic material MoS_2, obtaining three electrode configurations denoted: AuNPs/Gr/SPE, AuNPs/MoS_2/SPE and MoS_2-AuNPs/SPE.

Electrodeposition of gold nanoparticles in the 3 configurations was successfully achieved using cyclic voltammetry. Al the resulting voltammograms show the reduction peaks characteristic for the nucleation process = the transformation of gold ions (Au^{3+}) into metallic gold (Au^0). Moreover the differences between the potential of these peaks highlighted the influence given by the nature of the material deposited as a substrate on the electrode.

The morphological and electrochemical analysis demonstrated the efficiency of the electrodeposition process and the preference of AuNPs for certain attachment sites depending on the nature of the material used as a substrate and the deposition method used.

The resulting chemically modified electrodes were tested for nitrite oxidation. The oxidation peak of nitrite appears at a low potential, approximately 0.5 V. The electrocatalytic activity of gold nanoparticles co-deposited with MoS_2 is superior compared to that of gold nanoparticles deposited either on the bare electrode or on 2D materials (Gr and MoS_2) used as substrate. As a result, only this configuration (MoS_2-AuNPs /SPE) was optimized regarding the development of a sensitive and selective electrochemical sensor for the determination of nitrite.

The developed electrochemical sensor is sensitive enough to determine small concentrations of nitrite and selective enough, demonstrating that other biological compounds do not interfere in the nitrite response.

RESULTS DISSEMINATION

Articles published in ISI-rated scientific journals, results that made the subject of the doctoral thesis:

1. **Carp, O. E**., Moraru, A., Pinteala, M., & Arvinte, A. Electrochemical behaviour of piperine. Comparison with control antioxidants. *Food Chemistry* 339, 128110 (**2021**). (IF 9.231)

2. **Carp, O. E**., Pinteala, M., & Arvinte, A. Innovative Non-Enzymatic Electrochemical Quantification of Cholesterol. *Sensors* 22(3), 828 (2022). (IF 3.874)

Articles published in ISI listed journals (results not included in the thesis)

1. Sardaru, M. C., Morariu, S., **Carp, O. E**., Ursu, E. L., Rotaru, A., & Barboiu, M. Dynameric G-quadruplex–dextran hydrogels for cell growth applications. *Chemical Communications 59*(21), 3134-3137 (2023). (IF 6.065)

2. Sardaru, M. C., **Carp, O**., Ursu, E. L., Craciun, A. M., Cojocaru, C., Silion, M., & Rotaru, A. Cyclodextrin encapsulated pH sensitive dyes as fluorescent cellular probes: self-aggregation and in vitro assessments. *Molecules* 25(19), 4397 (2020). (IF 4.927)

Team member in research projects

- ➢ (**DynaCoPlat**), PN-III-P1-1.1-TE-2016-1180.
- ➢ (**TM-Vector**), PN-III-P4-ID-PCE-2020-1523, Nr. contract: PCE161/2021.

Research stages

Scientific Services Company Otava Ltd, Kiev, Ukraine for one month between 12.09.2020-11.10.2020, within the Project H2020-MSCA-RISE-2019, NoBiasFluors No 872331.

Communications at scientific events

Oral communications

1. Evaluation of antioxidant properties using Electrochemistry combined with in vitro peroxidation and reducing assays; **Oana Elena Carp**, Mariana Pinteala, Adina Arvinte; in 6th International Biomaterials and Biosensors Congress (BIOMATSEN), Muğla, Turkey.

2.   Electrochemical assays applied for evaluation of antioxidant properties; **Oana Elena Carp**, Mariana Pinteala, Adina Arvinte; XXVII Session of Scientific Communications of the Institute of Macromolecular Chemistry "Petru Poni" Iaşi, PROGRESS IN THE SCIENCE OF ORGANIC AND MACROMOLECULAR COMPOUNDS, organized within the Iași Academic Days.

Poster

1.   Cyclodextrin inclusion complex of indolizinyl-pyridinium salt as fluorescent probes for cell components staining; **Oana Carp**, Monica Sardaru, Ramona Danac, Corneliu Cojocaru, Alexandru Rotaru; International conference Materials, Methods & Technologies, September 2020, Burgas, Bulgaria,

2.   Electrochemical Study of Piperine and Comparison to Reference Antioxidants, **Oana Elena Rachita**, Aurelian Moraru, Mariana Pinteala, Adina Arvinte; International conference of Euroanalusis, XX – Edition, Istanbul, Turkey 2019

BIBLIOGRAPHICAL REFERENCES

1. Burke, R.; Diamondstone, B.; Velapoldi, R.; Menis, O., Mechanisms of the Liebermann-Burchard and Zak color reactions for cholesterol. *Clinical chemistry* **1974,** 20, (7), 794-801.

2. Adu, J. K.; Amengor, C. D.; Kabiri, N.; Orman, E.; Patamia, S. A. G.; Okrah, B. K., Validation of a Simple and Robust Liebermann–Burchard Colorimetric Method for the Assay of Cholesterol in Selected Milk Products in Ghana. *International journal of food science* **2019,** 2019.

3. Tanghe, L. J.; Brewer, R. J., Equilibrium between sulfuric and acetylsulfuric acids in acetic acid-acetic anhydride. *Analytical Chemistry* **1968,** 40, (2), 350-353.

4. Zuman, P., A review of reactions of some sterols in strongly acidic media. *Microchemical journal* **1991,** 43, (1), 10-34.

5. NIIYA, T.; GOTO, Y.; ONO, Y.; UEDA, Y., Study on the Correspondence of Color Change with Polyenyl Cation Formation of Cholesterol in Strong Acids. *Chemical and Pharmaceutical Bulletin* **1980,** 28, (6), 1747-1761.

6. Ziyatdinova, G. K.; Budnikov, H. C., Evaluation of the antioxidant properties of spices by cyclic voltammetry. *Journal of Analytical Chemistry* **2014,** 69, (10), 990-997.

7. Yardım, Y.; Şentürk, Z., Electrochemical evaluation and adsorptive stripping voltammetric determination of capsaicin or dihydrocapsaicin on a disposable pencil graphite electrode. *Talanta* **2013,** 112, 11-19.

8. Bukman, L.; Martins, A. C.; Barizão, É. O.; Visentainer, J. V.; Almeida, V. d. C., DPPH assay adapted to the FIA system for the determination of the antioxidant capacity of wines: optimization of the conditions using the response surface methodology. *Food Analytical Methods* **2013,** 6, 1424-1432.

9. Brand-Williams, W.; Cuvelier, M. E.; Berset, C., Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* **1995,** 28, (1), 25-30.

10. Nutting, J. E.; Rafiee, M.; Stahl, S. S., Tetramethylpiperidine N-Oxyl (TEMPO), Phthalimide N-Oxyl (PINO), and Related N-Oxyl Species: Electrochemical Properties and Their Use in Electrocatalytic Reactions. *Chemical Reviews* **2018,** 118, (9), 4834-4885.

11. Tsunaga, M.; Iwakura, C.; Tamura, H., Electrode reactions of nitroxide radicals at platinum in acetonitrile. *Electrochimica Acta* **1973,** 18, (3), 241-245.

12. Barhdadi, R.; Comminges, C.; Doherty, A.; Nédélec, J.; O'Toole, S.; Troupel, M., The electrochemistry of TEMPO-mediated oxidation of alcohols in ionic liquid. *Journal of applied electrochemistry* **2007,** 37, 723-728.

13. Gülçin, İ., Fe3+–Fe2+ Transformation Method: An Important Antioxidant Assay. In *Advanced Protocols in Oxidative Stress III*, Armstrong, D., Ed. Springer New York: New York, NY, 2015; pp 233-246.

14. Alam, M. W.; Najeeb, J.; Naeem, S.; Usman, S. M.; Nahvi, I.; Alismail, F.; Abuzir, A.; Farhan, M.; Nawaz, A., Electrochemical Methodologies for Investigating the Antioxidant Potential of Plant and Fruit Extracts: A Review. *Antioxidants* **2022,** 11, (6), 1205.

15. Ivanova, A.; Gerasimova, E.; Gazizullina, E., Study of Antioxidant Properties of Agents from the Perspective of Their Action Mechanisms. **2020,** 25, (18).

16. Brainina, K. Z.; Shpigun, L. K., State-of-the-art electrochemistry for the assessment of oxidative stress and integral antioxidant activity of biological environments. *Electrochemical Science Advances* **2022**, e2100219.

17. Hezard, T.; Fajerwerg, K.; Evrard, D.; Collière, V.; Behra, P.; Gros, P., Gold nanoparticles electrodeposited on glassy carbon using cyclic voltammetry: Application to Hg (II) trace analysis. *Journal of Electroanalytical Chemistry* **2012,** 664, 46-52.

18. O'Mullane, A. P.; Ippolito, S. J.; Sabri, Y. M.; Bansal, V.; Bhargava, S. K., Premonolayer Oxidation of Nanostructured Gold: An Important Factor Influencing Electrocatalytic Activity. *Langmuir* **2009,** 25, (6), 3845-3852.

19. Govinda Rao, B.; Matte, H. R.; Rao, C., Decoration of few-layer graphene-like MoS 2 and MoSe 2 by noble metal nanoparticles. *Journal of Cluster Science* **2012,** 23, 929-937.

20. Chu, X.; Yao, G.; Wee, A. T. S.; Wang, X.-S., Size-tunable Au nanoparticles on MoS2 (0001). *Nanotechnology* **2012,** 23, (37), 375603.

21. Wang, Y.; Laborda, E.; Crossley, A.; Compton, R. G., Surface oxidation of gold nanoparticles supported on a glassy carbon electrode in sulphuric acid medium: contrasts with the behaviour of 'macro'gold. *Physical Chemistry Chemical Physics* **2013,** 15, (9), 3133-3136.

22. Angerstein-Kozlowska, H.; Conway, B.; Hamelin, A.; Stoicoviciu, L., Elementary steps of electrochemical oxidation of single-crystal planes of Au—I. Chemical basis of processes

involving geometry of anions and the electrode surfaces. *Electrochimica Acta* **1986,** 31, (8), 1051-1061.

23. Saldan, I.; Dobrovetska, O.; Sus, L.; Makota, O.; Pereviznyk, O.; Kuntyi, O.; Reshetnyak, O., Electrochemical synthesis and properties of gold nanomaterials. *Journal of Solid State Electrochemistry* **2018,** 22, 637-656.