Babes-Bolyai University
Faculty of Chemistry and Chemical Engineering

PhD Thesis Summary

Modified electrodes based on noble metal nanoparticles capped with organic molecules

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INTRODUCTION

Biomolecules are building blocks of living matter; therefore the development of detection methods for biomolecules is important, especially for clinical applications. Among the methods used for analytical purposes, the electrochemical detection methods are widely used due to their selectivity, sensitivity, simplicity, rapidity and relatively low cost.

On the other hand, recently much attention is paid to the preparation of functionalized nanoparticles having specific physical and chemical properties which can be used for the preparation of analytical systems, capable to detect chemical species of biomedical interest.

In this context, was investigated the detection of three biomolecules: uric acid, ascorbic acid and dopamine using electrochemical methods. Also, the conversion of ammonium ion involved in the reductive amination of α-ketoisocaproic acid to L-leucine was performed. Thus, modified gold electrodes with organic molecules (thiocytosine/ guanine) and gold nanoparticles, and a modified biosensor based on gold nanoparticles and leucine dehydrogenase were prepared. Modified electrodes with gold nanoparticles and thiocytosine/ guanine were tested as sensors for uric acid detection in the presence of ascorbic acid interference, respectively for dopamine detection in the presence of its interferences uric acid and ascorbic acid. The modified biosensor was used as a substrate for chemically bonded enzyme needed for the electrochemical amination reaction for L-leucine synthesis.

The novelty aspects introduced by this thesis are:

- ✓ The use of guanine and thiocytosine molecules for gold nanoparticles self assembling on the electrodes surface; thiocytosine molecules were used before only for monolayers preparation;
- ✓ The use of multilayer modified electrodes for uric acid and ascorbic acid detection;
- ✓ The use, in premiere, of leucine dehydrogenase for modified electrodes based on thiocytosine and gold nanoparticles preparation;
- ✓ Tracking the conversion of ammonium ion involved in the reductive amination of α-ketoisocaproic acid to L-leucine using a modified electrode;
- ✓ NADH electrochemical regeneration using the modified biosensor.

The PhD thesis is structured on 4 chapters.

The first chapter presents a brief review of the scientific literature on chemically modified electrodes based on gold nanoparticles. A brief description of gold nanoparticles synthesis methods and their applications in modified electrodes preparation is presented. Also, in this chapter are presented some aspects about amino acid dehydrogenases and their use for modified biosensors preparation.

In **chapter 2** are described the experimental methods for the synthesis (using 3 methods) and characterization (UV-Vis and TEM) of gold nanoparticles used for surface modification of gold electrodes. Also, is presented the preparation, morphological characterization using atomic force microscopy and electrochemically chracaterization by cyclic voltammetry and electrochemical impedance spectroscopy of modified electrodes.

Chapter 3 presents the results of a research focused on two directions: (1) detection of uric acid in the presence of ascorbic acid interference; (2) detection of dopamine in the presence of ascorbic acid and uric acid interferences. Modified gold electrodes were prepared with thiocytosine/ guanine and gold nanoparticles. Modified electrodes exhibit electrochemical activity toward the oxidation of uric acid in the presence of ascorbic acid interference, respectively for the oxidation of dopamine in the presence of uric acid and ascorbic interference.

In **chapter 4** was prepared a modified biosensor with gold nanoparticles and leucine dehydrogenase by self-assembly of gold nanoparticles with thiocytosine, followed by enzyme immobilization. Biosensor was used both as an indirect ammonium sensor and as a substrate for chemically bonded enzyme needed for the electrochemical amination reaction for L-leucine synthesis. Also, the biosensor was used for NADH electrochemical regeneration (*in situ*).

In the Summary, numbering of chapters/subchapters, figures and tables is that from the thesis.

Key words: modified electrodes, gold nanoparticles, thiocytosine, guanine, leucine dehydrogenase, uric acid, ascorbic acid, dopamine.

ORIGINAL CONTRIBUTIONS

THESIS OBJECTIVES

The **main objective** of this thesis is preparation, characterization and testing of new modified electrodes with gold nanoparticles and organic molecules in order to use them as sensors for different organic and inorganic molecules detection.

To achieve this general objective were established several **specific objectives**:

- ✓ Gold nanoparticles synthesis by Turkevich method, Turkevich-Frens method and a method based on metallic salt reduction using a mixture of sodium citrate and sodium borohydride;
- ✓ Gold nanoparticles characterization by transmission electron microscopy (TEM) and UV-Vis spectroscopy;
- ✓ The preparation of modified electrodes with thiocytosine/ guanine and gold nanoparticles, respectively of a modified electrode (biosensor) with thiocytosine, gold nanoparticles and leucine dehydrogenase;
- ✓ Modified electrodes characterization by atomic force microscopy (AFM) and specular reflectance infrared Fourier transform spectroscopy (FTIR-RAS);
- ✓ Testing of modified electrodes with organic molecules (thiocytosine/ guanine) and gold nanoparticles as sensors for uric acid detection in the presence of its interference ascorbic acid, respectively for dopamine detection in the presence of its interferences uric acid and ascorbic acid;
- ✓ Testing of modified biosensor with thiocytosine, gold nanoparticles and leucine dehydrogenase as indirect sensor for detection of ammonium ion involved in the reductive amination of α-ketoisocaproic acid to L-leucine.

CHAPTER 2

GENERAL EXPERIMENTAL METHODS

2.2. Gold nanoparticles synthesis

Gold nanoparticles (AuNPs) were synthesized using three methods: Turkevich method (AuNPs-T), Turkevich-Frens method, a modification of Turkevich method (AuNPs-F) and a method based on metallic salt reduction using a mixture of sodium citrate and sodium borohydride (AuNPs-B). In this way, where obtained AuNPs with diameters between 30-40 nm using Turkevich method, respectively 40-45 nm using Turkevich-Frens method and 35-40 nm using the method with sodium citrate and sodium borohydride.

The prepared gold nanoparticles were characterized by transmission electron microscopy (TEM) and UV-Vis spectroscopy.

2.3. Gold nanoparticles characterization

2.3.1. Transmission electron microscopy (TEM)

The gold nanoparticles (AuNPs-T, AuNPs-F) size and shape were examined by TEM. In **figure 2.1.** are presented TEM images of AuNPs-T (**figure 2.1.a**) and AuNPs-F (**figure 2.1.b**). TEM images reveal the formation of spherical (AuNPs-T) or ovoid (AuNPs-F) structures with size between 30 – 40 nm (AuNPs-T) and 40 – 45 nm (AuNPs-F). All samples were diluted in double distilled water (1:4 volume ratio AuNPs:H_2O).

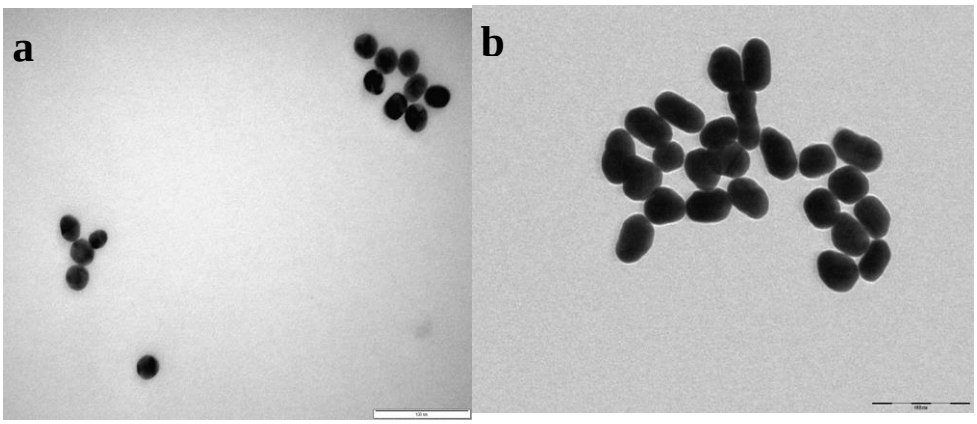

Figure 2.1 TEM images of gold nanoparticles: **a** –AuNPs-T; **b** –AuNPs-F

2.3.2. UV-Vis spectroscopy

Gold nanoparticles prepared by reduction of gold salt with sodium citrate are negatively charged. **Figure 2.2** presents the ligand influence on the color of gold nanoparticles.

Figure 2.2 Ligand influence on the color of gold nanoparticles

Figure 2.3 presents UV-Vis spectra of gold nanoparticles prepared by tetrachloroauric acid reduction with sodium citrate. For AuNPs-T the maximum absorbtion is at 525 nm, while for AuNPs-F is at 535 nm.

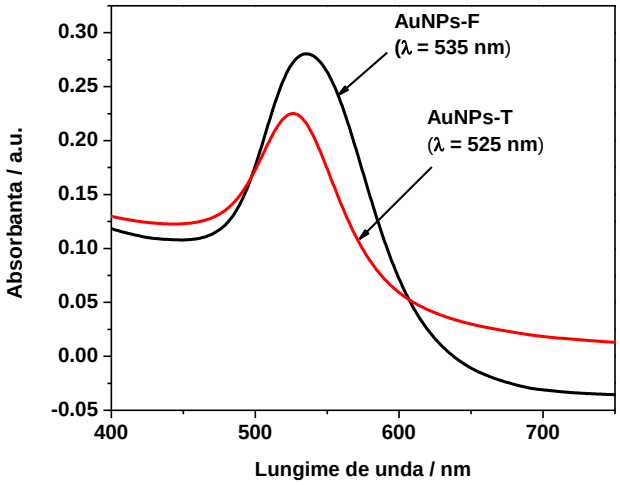

Figure 2.3 UV-Vis spectra of AuNPs-T and AuNPs-F (1:4 volume ratio AuNPs:H_2O)

Figure 2.6 presents the UV-Vis spectra of gold nanoparticles obtained by citrate and borohidryde reduction method (AuNPs-B) and AuNPs-B functionalized with thiocytosine. The absorbtion peak for functionalized gold nanoparticles is shifted to higher wavelengths, from 530 nm to 595 nm.

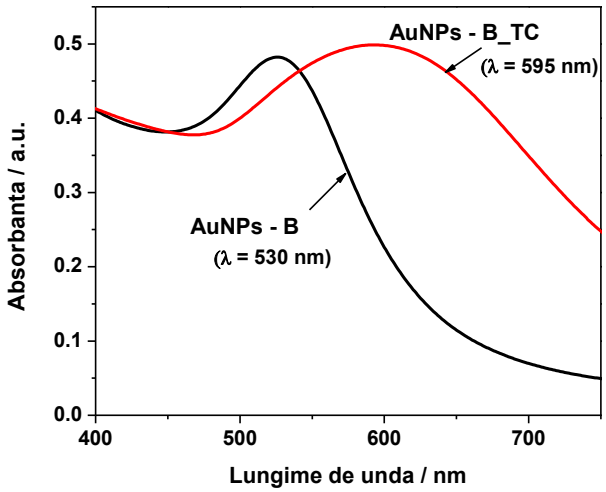

Figure 2.6 UV-Vis spectra of AuNPs-B and AuNPs-B functionalized with thiocytosine (AuNPs-B_T), 1:0.3 AuNPs-B:TC

2.4. Preparation of modified electrodes with organic molecules and gold nanoparticles

The modified electrodes with various layers were prepared in a closed Teflon cell. The gold disk electrode was fixed in the cell and every layer was obtained, in the presence, respectively, of: 2 mL alcoholic solution 10^{-3} M of thiocytosine or guanine for the first layer, 2 mL colloidal AuNPs for the second layer. Every solution was maintained 24 h on the disk electrodes.

2.5. Modified electrodes characterization

2.1. Cyclic voltammetry (CV)

The electrochemical behavior of Au disk, Au/TC, Au/GU, Au/TC/AuNPs-T and Au/GU/AuNPs-T electrodes was investigated by means of cyclic voltammetry. In **figure 2.10** are shown the typical voltammograms for Au disk, Au/TC, Au/GU, Au/TC/AuNPs-T and Au/GU/AuNPs-T electrodes recorded in Zobell solution (0.003M $K_3Fe(CN)_6$ şi 0.003M $K_4Fe(CN)_6$ în 0.1M KCl).

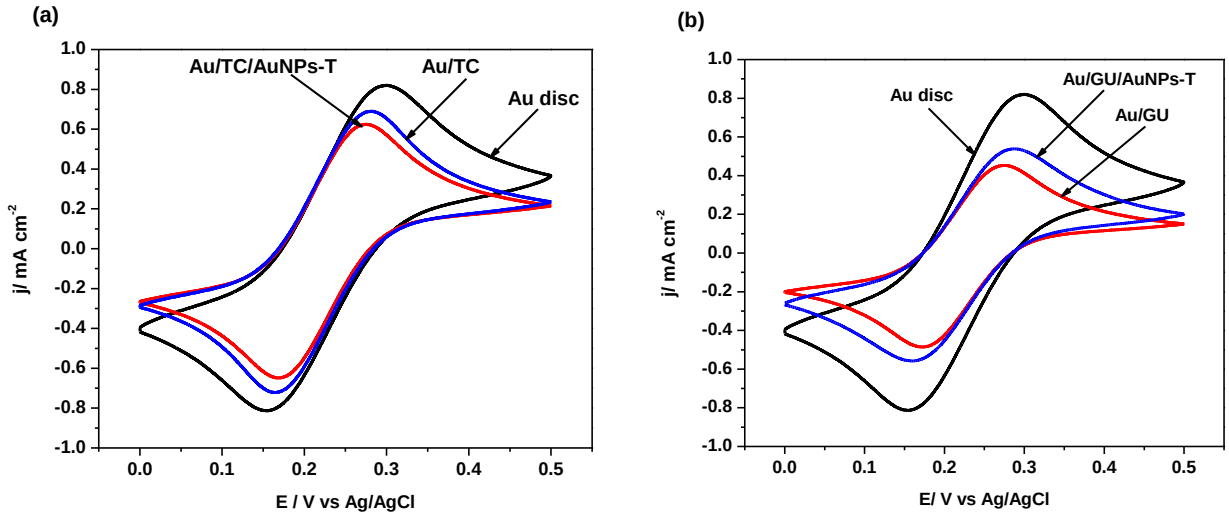

Figure 2.10 Cyclic voltammograms for **(a)** Au disk, Au/TC, Au/TC/AuNPs-T electrodes and **(b)** Au disk, Au/GU, Au/GU/AuNPs-T in Zobell solution, scan rate 50 mV/s

These results suggest that all layers deposited onto the surface of disk electrodes do not completely block it having a slight influence in decreasing the contact between the solution and the gold surface.

2.5.2. Electrochemical impedance spectroscopy (EIS)

Electrochemical impedance spectroscopy is a powerful tool used for studying electrochemical systems as well as the properties of modified electrodes surfaces. Modified electrodes with thiocytosine/ guanine (Au/TC, Au/GU) and modified electrodes based on gold nanoparticles (Au/TC/AuNPs, Au/GU/AuNPs) were investigated by means of electrochemical impedance spectroscopy. Data fitting to equivalent circuit was performed using Nova 1.8 software (EcoChemie Netherlands).

In **figure 2.15** (**a** and **b**) are shown the Nyquist plots of Au disk, Au/TC, Au/TC/AuNPs, Au/GU and Au/GU/AuNPs electrodes. One can see that after each modification step the Nyquist plot has changed, indicating that the immobilization of thiocytosine/ guanine and of gold nanoparticles took place on the electrode surface.

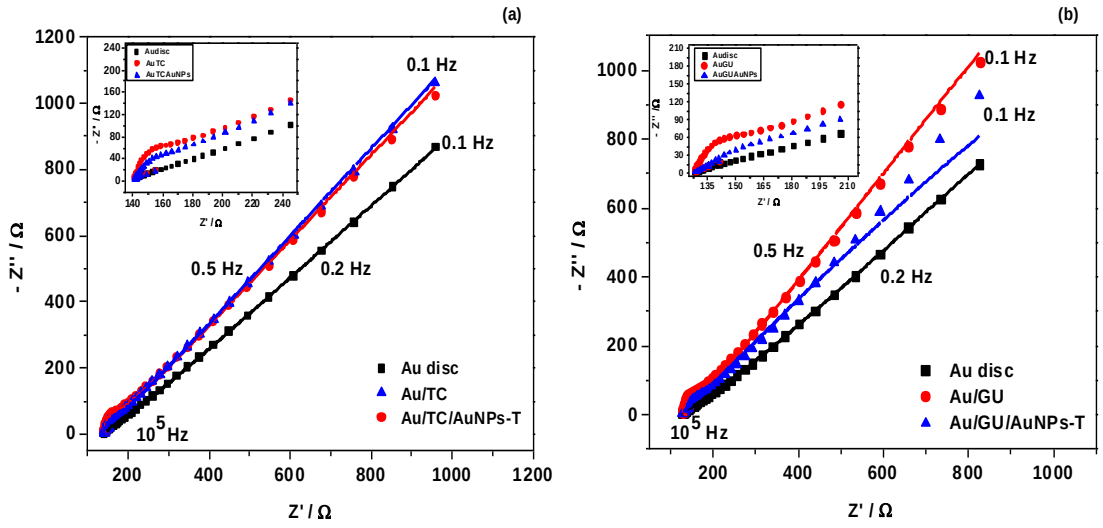

Figure 2.15 (a, b). Nyquist plots for: Au disk, Au/TC, Au/TC/AuNPs, Au/GU, Au/GU/AuNPs electrodes. The experiments were recorded in Zobell solution (0.003 M $K_3Fe(CN)_6$ and 0.003 M $K_4Fe(CN)_6$ in 0.1 M KCl), at an applied potential of +0.3 V *vs* Ag/AgCl; the continuous lines represent the fit based on the equivalent circuit

2.5.3. Atomic force microscopy (AFM)

Modified electrodes with thiocytosine/ guanine and gold nanoparticles were morphological characterized using atomic force microscopy.

In **figure 2.17** (**a**) are shown 2D images of gold electrode modified with thiocytosine and gold nanoparticles synthesized using Turkevich-Frens method (Au/TC/AuNPs-F). Ovoid structures with about 60-70 nm average thickness are observed (3D image - **b**).

Au/TC/AuNPs - F

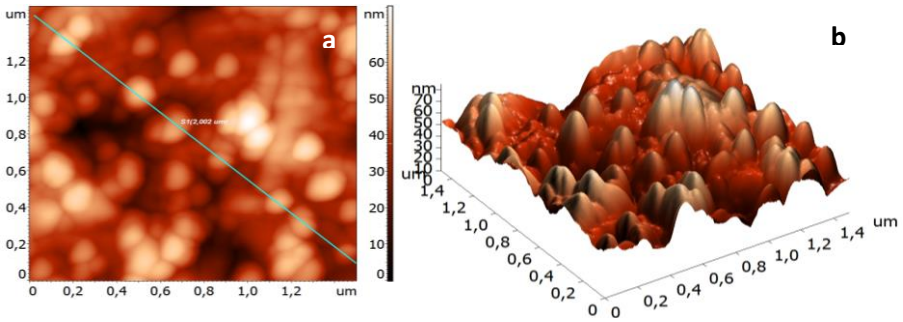

Figure 2.17 AFM images of Au/TC/AuNPs-F electrode: (**a**) – 2D height image; (**b**) – 3D image

In the case of modified electrode with guanine and gold nanoparticles (**figure 2.18**), pyramid structures (**a**) with an average layer thickness of 60-80 nm are observed (**b**).

Au/GU/AuNPs - F

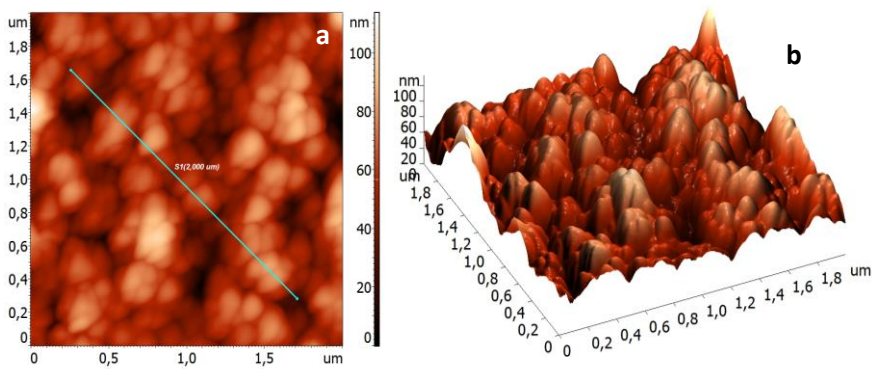

Figure 2.18 AFM images of Au/GU/AuNPs-F electrode: (**a**) – 2D height image; (**b**) – 3D image

AFM images recorded for modified electrodes with thiocytosine and gold nanoparticles synthesized by reduction of metallic salt with citrate and borohydride (Au/TC/AuNPs-B) are presented in **figure 2.19.** Surface analysis reveals that the layer thickness average is about 114 nm.

Au/TC/AuNPs - B

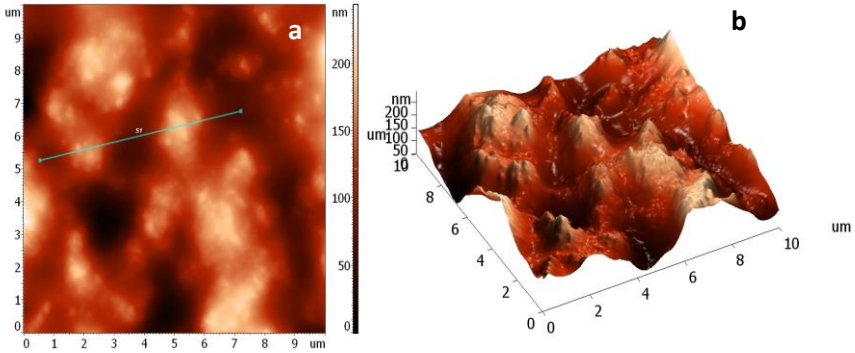

Figure 2.19 AFM images for Au/TC/AuNPs-B electrode: (**a**) – 2D height image; (**b**) – 3D image

CHAPTER 3

MODIFIED ELECTRODES WITH THIOCYTOSINE/ GUANINE AND GOLD NANOPARTICLES FOR URIC ACID AND DOPAMINE DETECTION IN THE PRESENCE OF ASCORBIC ACID INTEREFERENT

3.2. Modified electrodes with thiocytosine/ guanine and gold nanoparticles for uric acid detection in the presence of its intereferent ascorbic acid

Modified electrodes with different layers (Au/TC, Au/GU, Au/TC/AuNPs-T, Au/GU/AuNPs-T, Au/TC/AuNPs-T/TC, Au/GU/AuNPs-T/GU) were prepared and characterized. A schematic illustration for modified electrodes preparation is presented in **figure 3.1**.

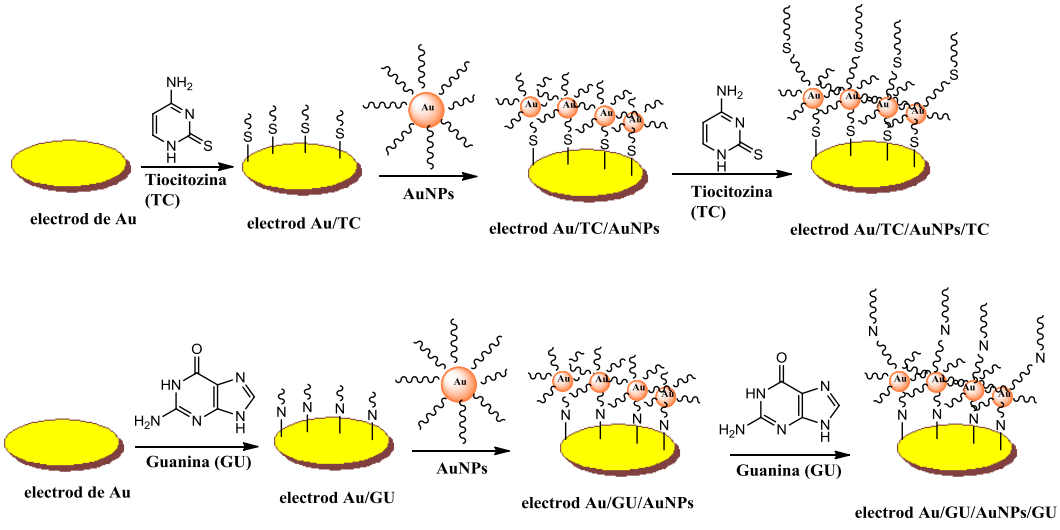

Figure 3.1 Schematic illustration of modified electrodes preparation

3.2.3. Electrochemical studies

The electrochemical behavior of modified electrodes with different layers was investigated by means of cyclic voltammetry and electrochemical impedance spectroscopy.

The modified electrodes were tested as sensors for uric acid (UA) and ascorbic acid (AA) detection in solutions containing either only one of the molecules, either both molecules in mixture.

The measurements were performed in pH 7.2 PBS solutions containing various concentrations of uric and ascorbic acid. Since the normal concentration for a healthy person of UA in serum is 2–5 mg/100 ml and 1.4–4.4 mM in urine, our measurements are in the range of 10^{-5} –10^{-2} M, which include these values existing in human fluids.

3.2.3.1. Cyclic voltammetry

The voltammetric response of bare (Au disk) and modified electrodes with different layers (Au/TC, Au/GU, Au/TC/AuNPs, Au/GU/AuNPs, Au/TC/AuNPs/TC, Au/GU/AuNPs/GU) was tested in solutions containing various uric and ascorbic concentrations.

Among all modified electrodes, the best to determine AA and UA are: Au/TC and Au/GU. AuNPs brings a separation of peaks, but not as good as in the cases of first layer. It follows that the AuNPs layer also may be well used to detect the two analytes. The second layer of TC/GU deposited on AuNPs does not bring any improvements comparatively with the AuNPs layer.

In **figure 3.5** are presented the cyclic voltammograms recorded for Au/TC electrode in solutions containing ascorbic acid (**figure 3.5.a**) or uric acid (**figure 3.5b**). One can see that the peak separation is good (0.2 V *vs.* Ag/AgCl) due to the fact that the potential corresponding to AA oxidation is negatively shifted (from 0.5 V to 0.35 V).

A similar behavior is observed in case of guanine modified gold electrodes (Au/GU). **Figure 3.6** presents the cyclic voltammograms recorded for Au/GU electrode in solutions containing ascorbic acid (**figure 3.6a**) or uric acid (**figure 3.6b**). The peak separation is good (0.2 V *vs.* Ag/AgCl), but compared with Au/TC electrode, in this case, the current density is corresponding to the anodic voltammetric peaks is higher.

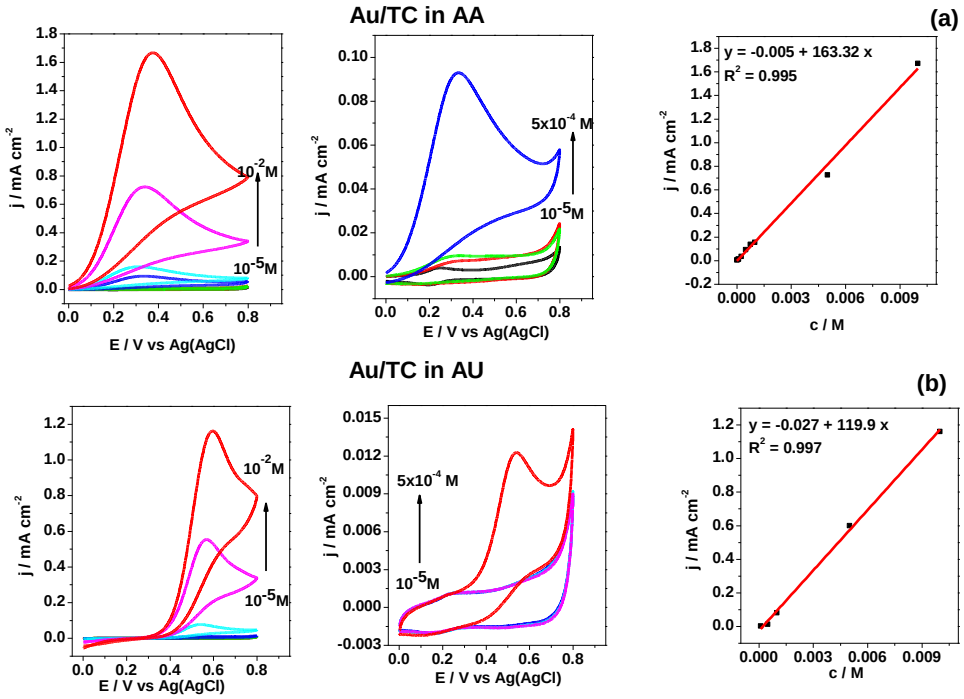

Figure 3.5 Cyclic voltammograms recorded for **Au/TC** electrode in solutions containing ascorbic acid (**a**) or uric acid (**b**) and the corresponding calibration plots; scan rate 50 mV/s

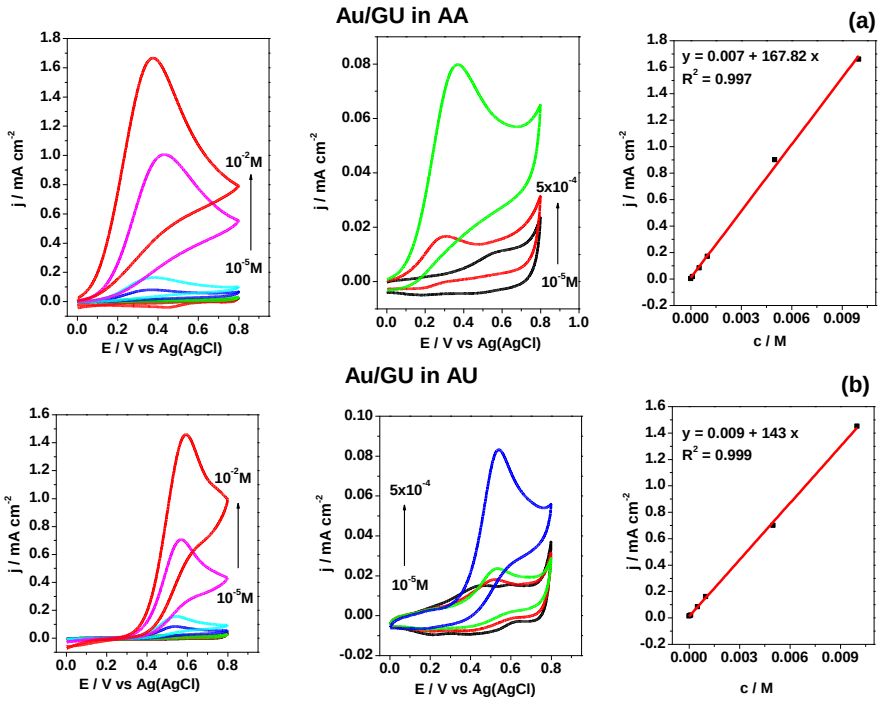

Figure 3.6 Cyclic voltammograms recorded for **Au/GU** electrode in solutions containing ascorbic acid (**a**) or uric acid (**b**) and the corresponding calibration plots; scan rate 50 mV/s

From the calibration plots (**figure 3.5** and **3.6**) it can be seen that gold electrodes modified with monolayer (Au/TC and Au/GU) respond linearly in the concentration range 10^{-5} – 10^{-2} M both in solutions containing ascorbic acid or uric acid.

Further was tested the electrochemical behavior of Au disk, Au/TC and Au/GU electrodes in solutions containing mixture of ascorbic and uric acid.

In **figure 3.11** is presented the electrochemical response of gold disk electrodes (Au disk) in solutions containing mixture of ascorbic and uric acid.

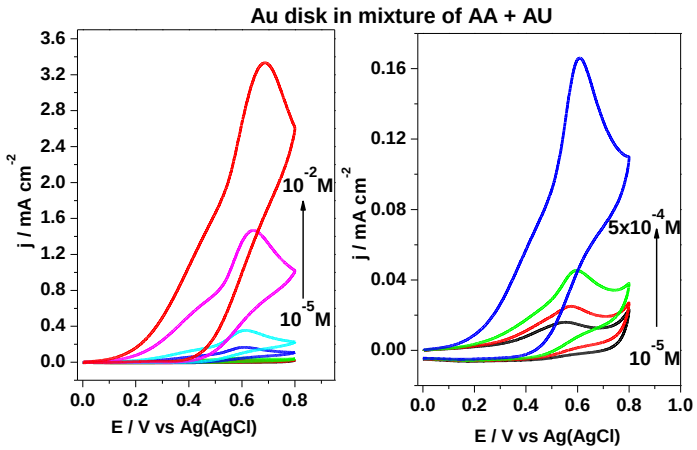

Figure 3.11 Cyclic voltammograms recorded for **Au disk** electrodes in mixture of ascorbic and uric acid; scan rate 50 mV/s

Using bare gold electrodes only one oxidation peak is obtained at ~0.65 V *vs.* Ag/AgCl, which includes both oxidation peak of AA and AU.

Figure 3.12 presents the electrochemical response of **Au/TC** (**figure 3.12a**) and **Au/GU** (**figure 3.12b**) electrodes in solutions containing AA and AU in mixture. For both modified electrodes is observed a very good peak separation: 0.270 V for **Au/TC** and 0.240 V for **Au/GU**.

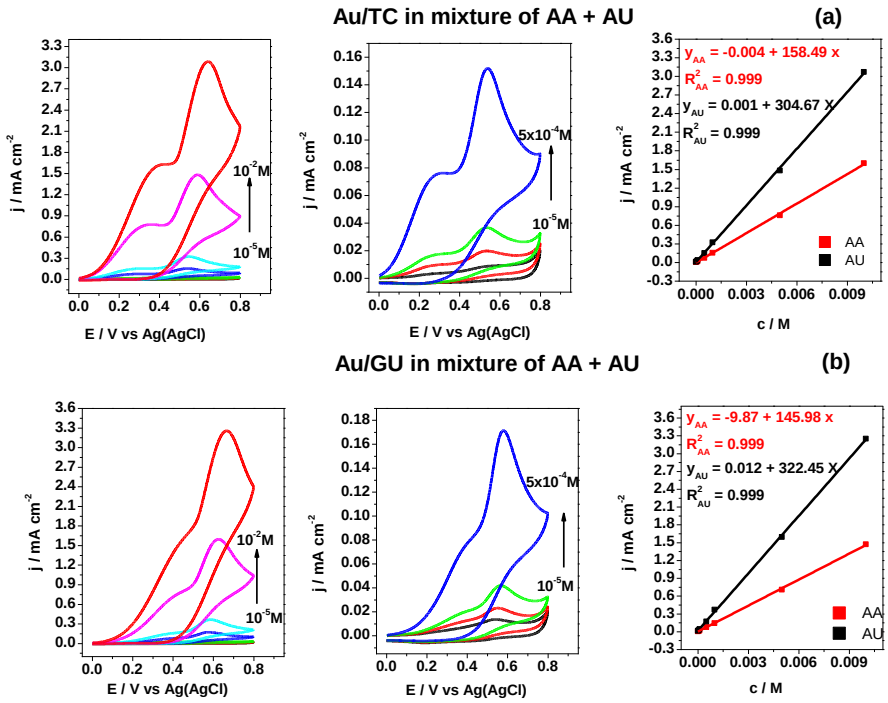

Figure 3.12 Cyclic voltammograms recorded for **Au/TC** (**a**) and **Au/GU** (**b**) electrodes in solutions containing ascorbic and uric acid in mixture; the corresponding calibration plots; scan rate 50 mV/s

3.2.3.2. Electrochemical impedance spectroscopy (EIS)

The impedimetric behavior of bare gold electrodes (Au disk) and modified gold electrodes with thiocytosine/ guanine (Au/TC, Au/GU), in solutions containing ascorbic acid and uric acid, was investigated. The measurements were performed at +0.55 V *vs.* Ag/AgCl in ascorbic acid solutions and at +0.55 V *vs.* Ag/AgCl in uric acid solutions. Data fitting to equivalent circuit was performed using Nova 1.8 software (EcoChemie Netherlands).

In **figure 3.13** are shown the Nyquist plots of Au disk electrode. EIS spectra were recorded in PBS solution containing various concentrations of ascorbic acid/ uric acid (10^{-4} – 10^{-3}M). One can see that Au disk has same behavior in solutions containing ascorbic acid or uric acid, without significant differences in terms of solution resistance and charge transfer resistance.

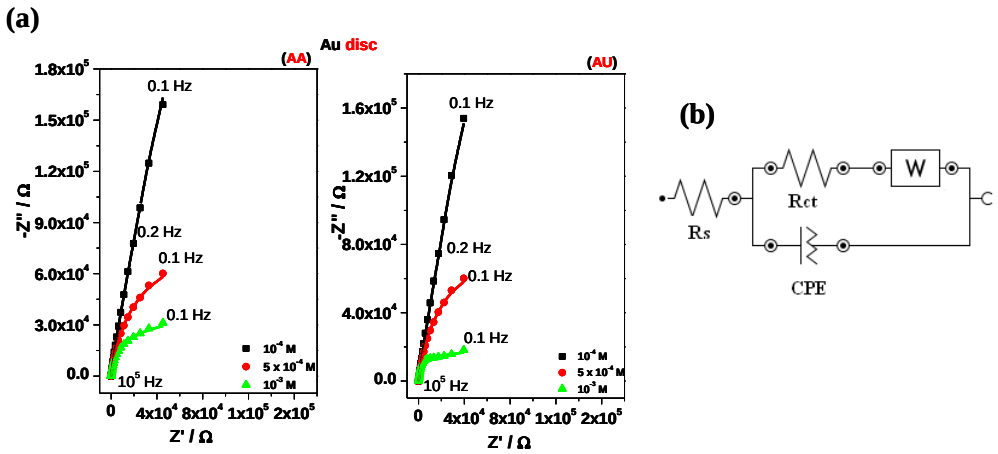

Figure 3.13 (a) Nyquist plots for Au disk electrode in PBS solution containing various ascorbic acid/ uric acid concentrations; the continuous lines represent the fit based on the equivalent circuit **(b)**

Next, modified electrodes with thiocytosine/ guanine (Au/TC, Au/GU) were investigated by means of EIS. Nyquist plots (**figure 3.14 a** and **b**) show a variation of R_{ct} with concetration increase.

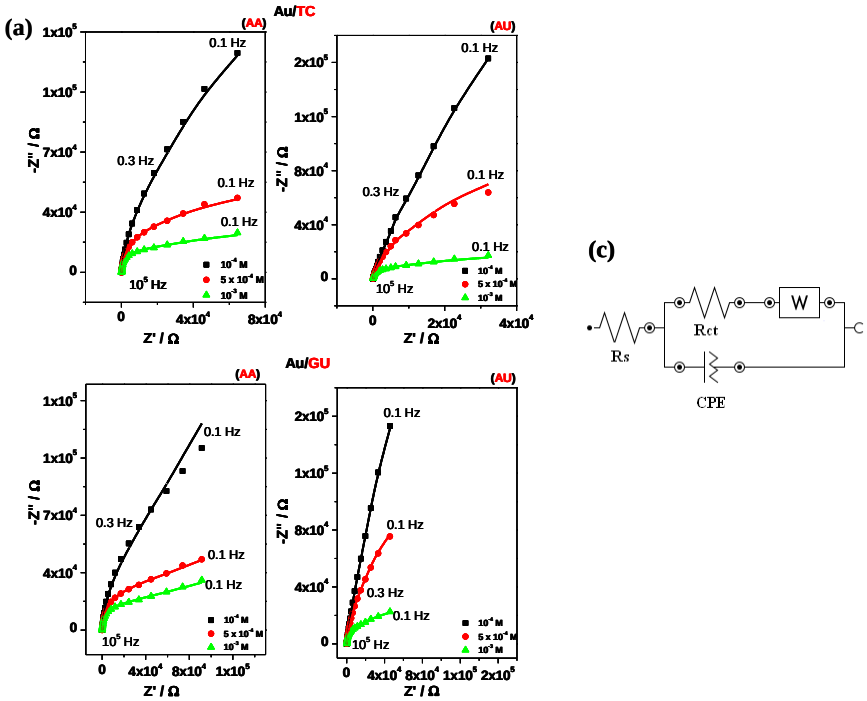

Figure 3.14 Nyquist plots for: **a** – Au/TC electrode and **b** – Au/GU electrode in PBS solution containing various ascorbic acid/ uric acid concentrations; the continuous lines represent the fit based on the equivalent circuit **(b)**

With increasing concentration there is a decrease of R_{ct}; the R_{ct} decrease can be attributed to the fact that at high concentrations of uric acid or ascorbic acid the number of molecules which are oxidized at electrode surface is higher.

3.3. Modified electrodes with thiocytosine and gold nanoparticles for dopamine detection in the presence of its interferents uric acid and ascorbic acid

3.3.1. Electrochemical studies – differential pulse voltammetry

Modified electrodes with thiocytosine (Au/TC) and thiocytosine – gold nanoparticles (Au/TC/AuNPs-B) were tested as sensors for dopamine (DA) detection in the presence of its interferences ascorbic acid (AA) and uric acid (AU).

It is well known that one of the major problems in the determination of DA is its interference with AA, due to the fact that DA and AA oxidation occur at same potential. Moreover, AA concentration in biological fluids is found to be much higher (normal values: AA – serum 60-80 μM, urine 95-270 μM, DA – serum <0.0001 μM, urine 0.34-3.139 μM/24h).

In **figure 3.15** are presented differential pulse voltammograms recorded for bare gold electrode (Au disk). One can see that gold electrode response to dopamine oxidation is good (**figure 3.15a**) and responds linear in the concentration range $2.5x10^{-6}$ – $2.6x10^{-5}$ M (inset). But, using this bare gold electrode is not possible to detect dopamine in a mixure (DA and AA) (**figure 3.15b**).

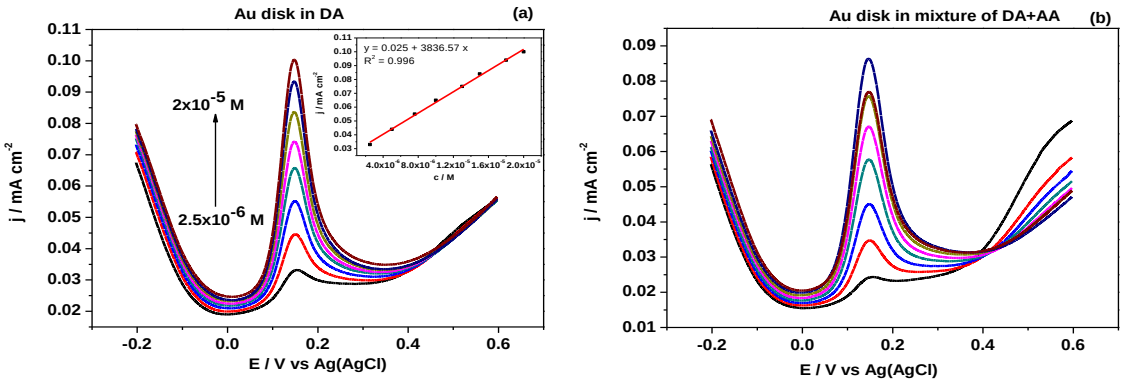

Figure 3.15 (a) DPVs recorded for Au disk electrode in pH 7.2 PBS containing DA: $2.5x10^{-6}$ M – $2x10^{-5}$ M, **inset**: linear response in concentration range $2.5x10^{-6}$ M – $2x10^{-5}$ M; **(b)** DPVs recorde for Au disk electrode recorde in solutions containing DA (successive addition of 2.5 μM) and AA (successive addition of 6.5 μM)

Figure 3.16 presents the electrochemical response of thiocytosine modified electrodes (Au/TC) in solutions containing DA and AA. Using these modified electrodes did not bring significant improvements regarding selective detection of dopamine in a mixture DA – AA, however one can see a split of voltammetric peak.

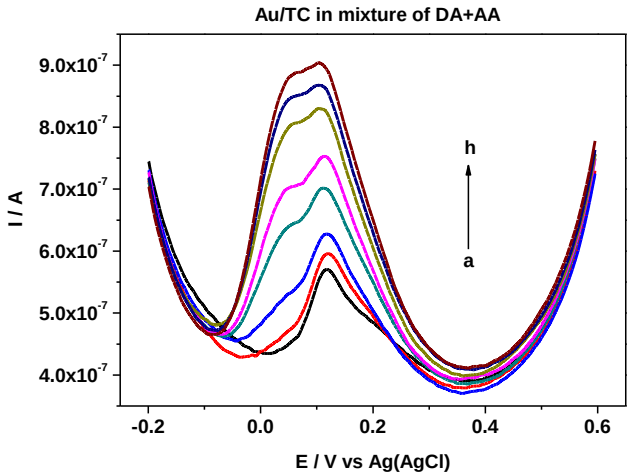

Figure 3.16 DPVs recorded for Au/TC electrode in pH 7.2 PBS containing DA (5 μM) and AA – successive addition: a) 0, b) 5, c) 5, d-h) 10 μM

Modified gold electrodes with thiocytosine and gold nanoparticles (synthesized by metallic salt reduction using a mixture of sodium citrate and sodium borohydride) – **Au/TC/AuNPs-B** were used to modify the oxidation potential of these biomolecules, in order to detect DA and AA simultaneous. The electrochemical response of Au/TC/AuNPs-B electrode in pH 7.2 PBS containing various dopamine concentrations is presented in **figure 3.18**.

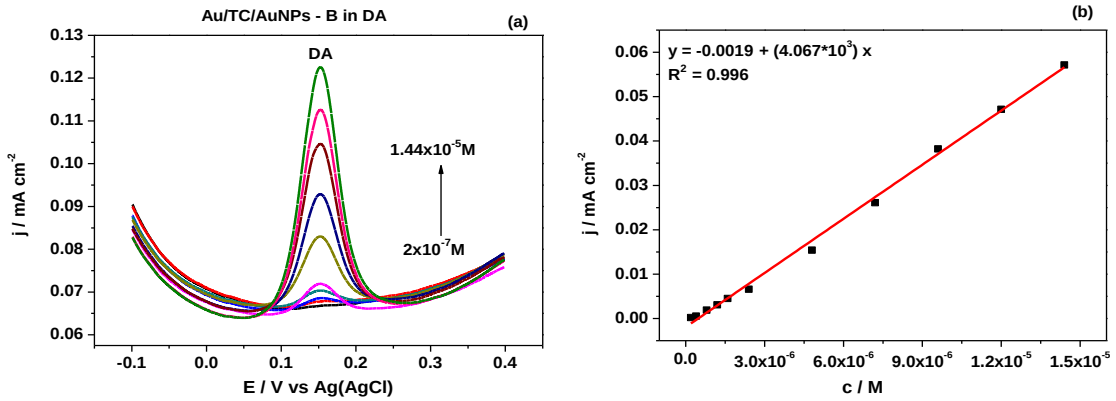

Figure 3.18 (a) DPVs recorded for Au/TC/AuNPs-B electrode in pH 7.2 PBS containing DA: $2x10^{-7}$ – $1.44x10^{-5}$ M; **(b)** linear response of the modified electrode in the concentration range: $2x10^{-7}$ – $1.44x10^{-5}$ M

The gold nanoparticles modified electrode (Au/TC/AuNPs-B) was next tested for dopamine detection in the presence of AA (**figure 3.19a**). One can see a positive shift of the peak potential corresponding to AA oxidation. In solutions containing DA, AA and AU only two peaks are obtained: one corresponding to dopamine oxidation (+0.15 V *vs.* Ag/AgCl) and the other corresponding to ascorbate and uric acid oxidation (+0.35 V *vs.* Ag/AgCl). One can see that dopamine oxidation peak is not influenced by the presence of interferences, even if they are found in high concentration. A limitation of Au/TC/AuNPs-B electrode use is that it cannot discern between AA and AU oxidation potential (**figure 3.19b**).

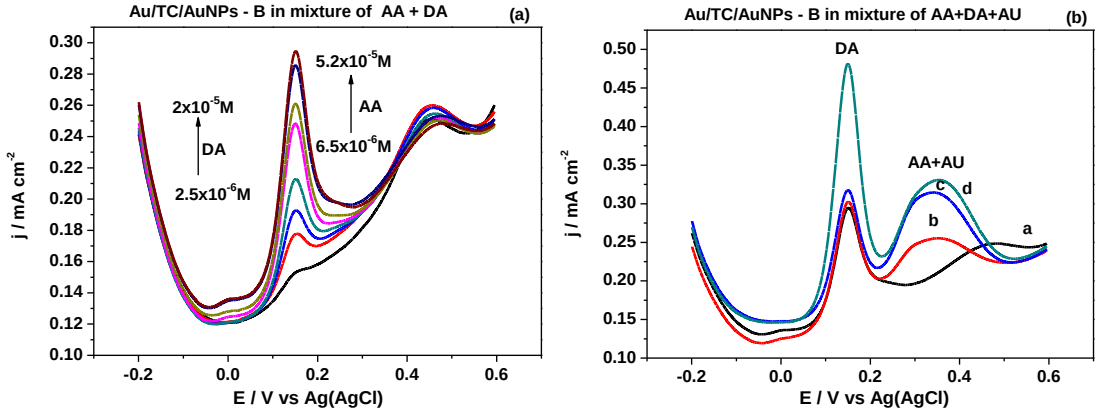

Figure 3.19 (a) DPVs recorded for Au/TC/AuNPs-B electrode in solutions containing DA (successive addition of 2.5 µM) and AA (successive addition of 6.5 µM); **(b)** DPVs recorded for Au/TC/AuNPs-B electrode in solutions containing DA: a-c) 20 µM, d) 50 µM; AA: a-d) 52 µM and AU: a) 0, b) 50, c-d) 100 µM

CHAPTER 4

MODIFIED BIOSENSOR WITH LEUCINE DEHYDROGENASE FOR DETERMINATION OF AMMONIUM ION INVOLVED IN THE REDUCTIVE AMINATION OF α-KETOISOCAPROIC ACID

4.2 Preparation of modified biosensor with leucine dehydrogenase

A new modified biosensor based on gold nanoparticles and leucine dehydrogenase was developed. The illustration of modified biosensor preparation is presented in **figure 4.1.**

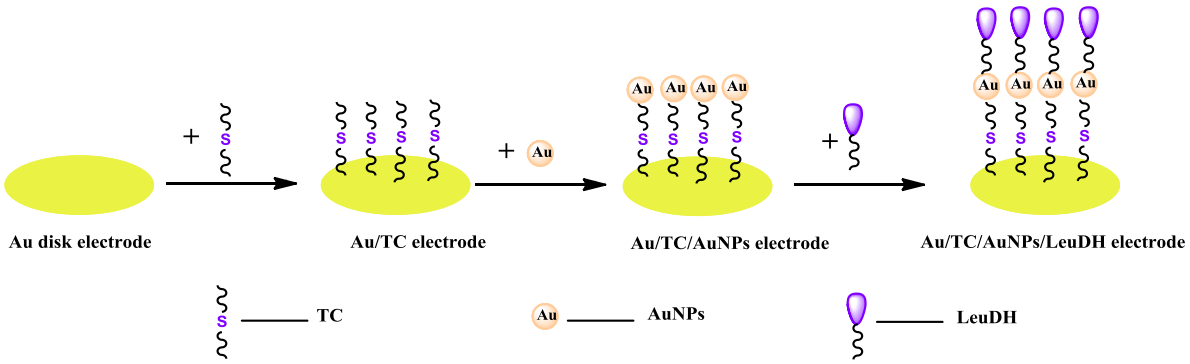

Figure 4.1 Illustrative representation of leucine dehydrogenase biosensor

4.4. Electrochemical studies

The modified biosensor response was investigated by chronoamperometry for indirect quantification of ammonium ion (NH_4^+) involved in the reductive amination of α-ketoisocaproic acid to L-leucine. This reaction takes place in the presence of ammonium chloride, as nitrogen source, and nicotinamide adenine dinucleotide (NADH), as cofactor. The developed leucine dehydrogemase biosensor acted both as a substrate for chemically bonded enzyme needed for the electrochemical amination reaction for L-leucine synthesis and as indirect sensor to monitor the ammonia conversion. In the same time the modified electrode was used and for the electrochemical NADH regeneration.

4.4.1. Cyclic voltammetry

In the presence of ammonium ion, LeuDH catalyzes the conversion of α-ketoisocaproic acid to L-leucine. During the enzymatic reaction, NADH is oxidized to NAD^+ and generates two electrons which lead to the increase of the current proportional with the concentration of ammonium ion. Thus, the electrocatalytic oxidation of NADH could be used for the indirect quantification of ammonium ion concentration.

To establish the optimum working potential two experiments were performed with the Au/TC/AuNPs/LeuDH modified biosensor (**figure 4.7a** and **b**).

In the first experiment, the modified electrode was immersed in pH 8 PBS that contained 60 mM α-ketoisocaproic acid and 7.2 mM NADH and CVs were recorded before (**Fig. 4.7a**-black line) and after the addition of 60 mM ammonium ion (**Fig. 4.7a**-red line). The oxidation peak at around +0.5 V may be attributed to the oxidation of NADH, which slightly increased after the addition of the ammonium ion.

In the second experiment, the modified electrode was immersed in pH 8 PBS that contained 60 mM α-ketoisocaproic acid and 60 mM ammonium ion and CVs were recorded before (**Fig. 4.7b**-black line) and after the addition of 7.2 mM NADH (**Fig. 4.7b**-red line). In this case, one can see a considerably increase of NADH oxidation peak (around +0.8 V).

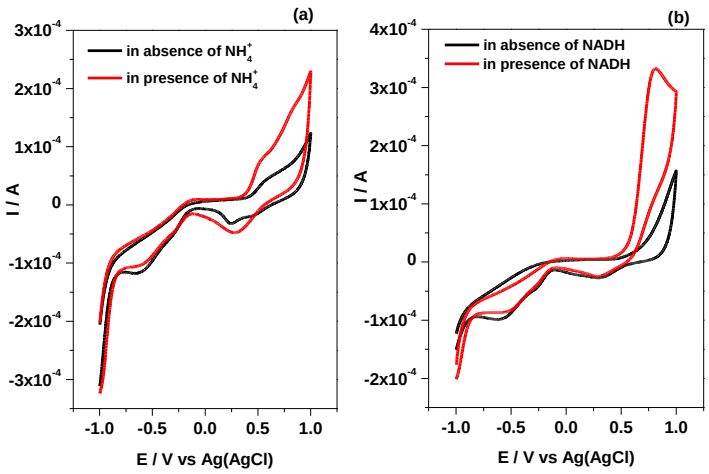

Figure 4.7 Cyclic voltammograms of Au/TC/AuNPs/LeuDH modified electrode, in the absence and presence of 60mM NH_4^+ ion (a) and in the absence and presence of 7.2 mM NADH (b) at pH 8; 50 mV/s scan rate

From the above results it was concluded that the optimum working potential for Au/TC/AuNPs-F/LeuDH biosensor is +0.8 V *vs.* Ag/AgCl.

4.4.2. Chronoamperometry

Further measurements were performed using chronoamperometry at an applied potential of +0.8 V *vs.* Ag/AgCl.

In **figure 4.8** is presented the chronoamperogram recorded at Au/TC/AuNPs-F/LeuDH biosensor for successive additions of 7.2 mM NADH to the reaction mixture containing 60 mM α-ketoisocaproic acid and 60 mM ammonium ion (**Fig. 4.8a**). Calibration plots are presented for both NADH successive additions (**Fig. 4.8b**) and indirect ammonium ion concentration (**Fig. 4.8c**).

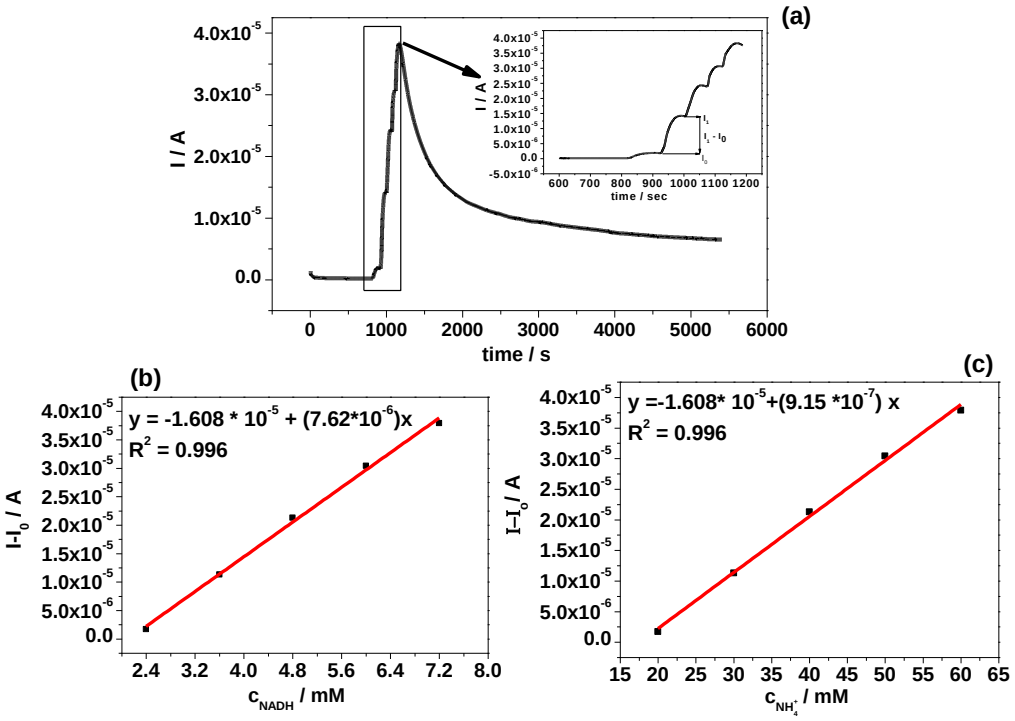

Figure 4.8. (a) Chronoamperogram obtained at Au/ TC/ AuNPs-F/ LeuDH modified electrode for successive addition of 1.2 mM NADH; (b) linear response in the 2.4 – 7.2 mM NADH concentration range; (c) indirect calibration plot of ammonium ion in the 20 – 60 mM concentration range; I_0 – is background current

The sensitivity of the leucine dehydrogenase modified electrode was calculated using calibration plot (**Fig. 4.8c**) and it was found to be 0.91×10^{-3} A/ M.

4.4.3. In situ regeneration of NADH using Au/TC/AuNPs-F/ modified biosensor

NADH *in situ* regeneration was performed using chronoamperometry without changing the electrodes configuration and the electrolyte. The immobilized leucine dehydrogenase facilitates the electrons transfer and the addition of one atome of hydrogen to NAD^+. The experiments were carried out at -0.6 V *vs.* Ag/AgCl for 1200 seconds.

In **figure 4.12** are presented the chronoamperograms obtained for Au/TC/AuNPs/LeuDH modified electrode in the presence of 7.2 mM NADH (black line) and after NADH regeneration (red line).

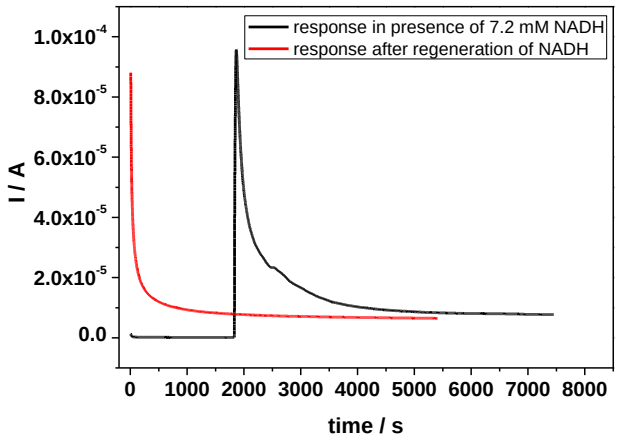

Figure 4.12 Chronoamperograms obtained at Au/TC/AuNPs-F/LeuDH modified electrode in the presence of 7.2 mM NADH (black line) and after NADH regeneration (red line)

After the NADH regeneration a new chronoamperogram was recorded and the conversion of ammonium ion was improved with 20 %. As a result, after two cycle of recorded chronoamperometry the total conversion of ammonium ion is 80 percent. This experiment can be repeated several times in order to obtain a better ammonium ion conversion.

4.4.5. Method validation using Berthelot reaction

The Berthelot spectrophotometric method was used to confirm the ammonium ion determination, with the enzyme modified electrode. This method is based on the indophenol blue formation which takes place in two steps: first, mono-chloroamines are formed by the addition of hypobromide to the ammonium samples followed by the indophenol blue preparation from the reaction of mono-chloramines with phenol. In the absorption spectrum this product has a maximum absorption peak at 625 nm.

Table 2 Comparative results for ammonium ion determination using chronoamperometric and Berthelot methods

Experiment	Ammonium ion concentration [mM]		Error [%]
	Chrononamperometric method	Berthelot method	
1	24.50	24.00	+2.08
2	28.90	28.00	+3.21
3	29.00	30.00	-3.33

In **Table 2** are presented comparatively the data obtained with the modified electrode and Berthelot method. One can see that there are not significant differences between the results obtained with these two methods.

CONCLUSIONS AND PERSPECTIVES

GENERAL CONCLUSIONS

This thesis was focused on two directions: **(1)** development of new modified electrodes with organic molecules and gold nanoparticles for biomolecules detection and **(2)** development of a new biosensor based on gold nanoparticles and leucine dehydrogenase for indirect detection ammonium ion involved in the reductive amination reaction of the α-cetoizocaproic acid to L-leucine.

The main results achieved during the thesis are:

- ✓ Gold nanoparticles (AuNPs) were synthesized using three methods: Turkevich method (**AuNPs-T**), Turkevich-Frens method, a modification of Turkevich method (**AuNPs-F**) and method based on metallic salt reduction using a mixture of sodium citrate and sodium borohydride (**AuNPs-B**);
- ✓ The prepared gold nanoparticles were characterized by UV-Vis spectroscopy and TEM;
- ✓ Were prepared modified gold electrodes with:
  - organic molecules: **Au/TC, Au/GU**;
  - organic molecules and gold nanoparticles: **Au/TC/AuNPs-T, Au/GU/AuNPs-T, Au/TC/AuNPs-F, Au/TC/AuNPs-B**;
  - multilayers: **Au/TC/AuNPs-T/TC; Au/GU/AuNPs-T/GU**.
- ✓ The prepared electrodes were characterized by atomic force microscopy (AFM) and specular reflectance infrared with Fourier transform spectroscopy (FTIR-RAS);

- ✓ The modified electrodes (**Au/TC, Au/GU, Au/TC/AuNPs-T, Au/GU/AuNPs-T, Au/TC/AuNPs-T/TC; Au/GU/AuNPs-T/GU**) were tested as sensors for uric detection in the presence of its interference ascorbic acid;
- ✓ Among all modified electrodes, the best to determine uric acid are: **Au/TC, Au/GU** and satisfactory results were obtained with **Au/TC/AuNPs-T, Au/GU/AuNPs-T**;
- ✓ **Au/TC/AuNPs-B** modified electrodes exhibit electrochemical activity towards the oxidation of dopamine, not only in the absence of interferences but also in their presence. However this electrode cannot discern between AA and AU oxidation potential;
- ✓ The modified electrodes have good reproducibility, stability and sensitivity;
- ✓ A new amperometric biosensor based on gold nanoparticles and leucine dehydrogenase was developed: **Au/TC/AuNPs-F/LeuDH**;
- ✓ The biosensor acted both as an indirect ammonia sensor and as a substrate for chemically bonded enzyme needed for the electrochemical amination reaction for L-leucine synthesis;
- ✓ Using **Au/TC/AuNPs-F/LeuDH** biosensor the reductive amination was performed with only one enzyme (leucine dehydrogenase) and in the same time NADH was *in situ* regenerated;
- ✓ As compared to the Berthelot spectrophotometrically method, the developed biosensor demonstrated its suitability for rapid analysis of ammonium ion.
- ✓ The biosensor exhibits good analytical performance, reproducibility and stability for ammonium ion analysis.

PERSPECTIVES

The future work will be focused on the following topics:

- o The improvement of the experimental conditions for simultaneous detection of dopamine, uric acid and ascorbic acid using **Au/TC/AuNPs-B** modified electrode. Optimization of experimental conditions could lead to a shift of oxidation potential of the analyzed species, and as a result, a voltammetric peak separation.

- o The developed biosensor will be <u>improved</u> and used for the biosynthesis of ^{15}N labelled L-leucine. ^{15}N labelled L-leucine will be synthesized by the reductive amination of α-ketoisocaproic in the presence of LeuDH, immobilized on the electrode surface, NADH as cofactor and $^{15}NH_4Cl$, as source of isotope ^{15}N.

SCIENTIFIC ACTIVITY

Papers published in ISI journals

1. **Adriana Vulcu**, Camelia Berghian Grosan, Maria Chiriac, Valer Almasan, "*Biosynthesis of ^{15}N Labelled L-Glutamic Acid: Experimental and Computational Studies"*, **Revue Roumaine de Chimie**, **2011**, 56(6), 667-674.
2. Camelia Berghian Grosan, Codruta Varodi, **Adriana Vulcu**, Liliana Olenic, Stela Pruneanu, Valer Almasan, *Structural and Electrochemical Characterization of Novel Leucine-Gold Nanoparticles Modified Electrode*, **Electrochim. Acta**, **2012**, 63, 146-152.
3. **Adriana Vulcu**, Camelia Grosan, Liana Maria Muresan, Stela Pruneanu, Liliana Olenic, *"Modified Gold Electrodes Based On Thiocytosine/Guaninegold Nanoparticles For Uric And Ascorbic Acid Determination"*, **Electrochimica Acta**, **2013**, 88, 839-846.
4. Maria Crisan, Luminita David, Bianca Moldovan, **Adriana Vulcu**, Simina Dreve, Maria Perde-Schrepler, Corina Tatomir, Adriana Gabriela Filip, Pompei Bolfa, Marcela Achim, Ioana Chiorean, Irina Kacso, Camelia Berghian Grosan, Liliana Olenic, *New Nnanomaterials for the Iimprovement of Psoriatic Lesions*, **J. Mater. Chem. B**, **2013**, 1, 3152-3158.
5. Luminita David, Bianca Moldovan, **Adriana Vulcu**, Liliana Olenic, Maria Perde-Schrepler, Eva Fischer-Fodor, Adrian Florea, Maria Crisan, Ioana Chiorean, Simona Clichici Gabriela Adriana Filip, *Green synthesis, characterization and anti-inflammatory activity of silver nanoparticles using European black elderberry fruits extract*, **Colloids and Surfaces B**, **2014**, 122, 767-777.
6. Camelia Berghian-Grosan, Liliana Olenic, Gabriel Katona, Maria Perde-Schrepler, **Adriana Vulcu**, *L-Leucine for gold nanoparticles synthesis and their cytotoxic effects evaluation*, **Amino Acids, 2014**, 46, 2545-2552.

Papers published in ISI conference proceedings

1. **Adriana Vulcu**, Stela Pruneanu, Camelia Berghian-Grosan, Liliana Olenic, Liana Maria Muresan, Lucian Barbu-Tudoran, *Impedimetric Investigation of Gold Nanoparticles – Guanine Modified Electrode*, **AIP Conference Proceedings**, 1565, 2013, 273-277.

Scientific conferences

1. **Adriana Vulcu**, Camelia Berghian Grosan, Maria Chiriac, Valer Almasan, **Biosynthesis of ^{15}N Labelled L-Glutamic Acid: Experimental and Computational Studies**, RomCat 2010, 23-26 June, **2010**, Iasi, Romania
2. **Adriana Vulcu**, Camelia Grosan, Liliana Olenic, Ioan Bratu, Diana Bogdan, Liana Muresan, Lucian Barbu-Tudoran, "**Determination of Uric Acid using Gold Nanoparticles Modified Interfaces"**, 5th International Conference "Biomaterials, Tissue Engineering & Medical Devices", 29 August – 1 September **2012**, Constanta, Romania
3. **Adriana Vulcu**, Stela Pruneanu, Camelia Berghian-Grosan, Liliana Olenic, Liana Maria Muresan, Lucian Barbu-Tudoran, **Impedimetric Investigation of Gold Nanoparticles – Guanine Modified Electrode**, PIM 2013, 25 – 27 September **2013**, Cluj-Napoca, Romania

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