**“BABEŞ-BOLYAI” UNIVERSITY OF CLUJ-NAPOCA**

**FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING**

# NEW MODIFIED ELECTRODES WITH ELECTROANALYTICAL APPLICATIONS

## PhD Thesis Abstract

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## Introduction

In the last years, modified electrodes (ME) obtained by deliberate modification of conventional electrode materials have applications in many fields including electrocatalysis, electroanalysis and elecrosynthesis, due to their multiple advantages in comparison to conventional electrodes [1-6]. One of the most important properties of ME is their ability to oxydize / reduce chemical species of interest which take place with high overpotentials on conventional materials. This property confers to the modified electrodes applicability especially in biomedical field, where interferents alter the conventional electrodes response [7-13].

The thesis exploits the advantages of some innovative materials such as zeolites, carbon paste, carbon nanotubes, in combination with different redox mediators such as phenothiazines and phenoxazines, or with new compounds such as crown ethers, for sensitive detection of some chemical species.

The **aims** of the thesis were**:**

**(i)** The obtaining and characterization of new zeolite modified electrodes (ZMEs) for detection of several chemical species ($H_2O_2$, NADH, ascorbic acid etc.)

**(ii)** Preparation of new graphite electrodes modified with redox mediators and carbon nanotubes for amperometric detection of NADH

**(iii)** Preparation and optimization of a new amperometric biosensor for ascorbic acid detection

**(iv)** Obtaining of composite carbon paste electrodes modified with crown ethers for selective detection of potassium ions

**(v)** Elaboration of a modified gold electrode based on gold nanoparticles functionalized with leucine for copper ions detection

The thesis contains two parts: the first one contains a literature review concerning composite electrode materials, redox mediators sensors/biosensors but also electrochemical investigation methods and the second describes the original contributions of the PhD student in the field of obtaining, characterization and applications of several new composite electrode materials with catalytic properties. The original contributions are structured in 5 chapters, each presenting the results recorded for the five different types of composite modified electrodes mentioned above.

# II. ORIGINAL CONTRIBUTIONS

## II.1. Zeolite modified electrodes (ZMEs)

The aims of this chapter were:

- ***Preparation of several composite zeolite-based electrodes by zeolites incorporation in carbon paste.***

Different zeolites were tested:

(i) mesoporous synthetic zeolites (13X and 4A, Aldrich, Germany) modified with Methylene Blue (MB), and/or $Ca^{2+}$ ions ;

(ii) volcanic tuffs containing natural zeolites (Cepari- Vultureni) modified with $Cu^{2+}$

(iii) volcanic tuffs containing natural zeolites modified with $Fe^{3+}$, in different experimental conditions (pH, presence of $Ca^{2+}$ etc.)

- ***Characterization ofZMEs by using physico-chemical and electrochemical methods***
- ***Preparation of some amperometric sensors/biosensors for the detection of $H_2O_2$, NADH and ascorbic acid.***

Some of the results are presented below:

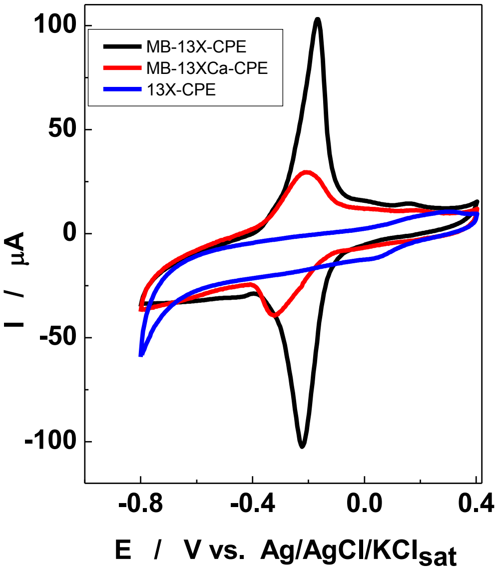


**Figure II.1.8. Cyclic voltammograms for 13X-CPEs (——), MB-13XCPEs (——) and MB-13XCa-CPEs (——).** *Experimental conditions: starting potential, -800 mV vs. Ag|AgCl/KClsat; potential scan rate, 10 mV s-1; supporting electrolyte, 0.1 M tris buffer, pH 7.0*

**Table 6.** Obtained ZMEs

| Nr. crt. | Electrode type | Zeolite | Mediator | Modification methode | Carbon type |
|---|---|---|---|---|---|
| 1 | 13X-CPE | synthetic 13X (Aldrich) | - | - | graphite |
| 2. | MB-13X-CPE | synthetic 13X (Aldrich) | Methylene Blue (MB) | adsorption | graphite |
| 3. | MB-4A-CPE | synthetic 4A (Aldrich) | Methylene Blue (MB) | adsorption | graphite |
| 4 | MB-13XCa -CPE | synthetic 13X (Aldrich) | ions $Ca^{2+}$ | Ionic exchange + adsorption | graphite |
| 5. | M-G-CNT-CPE | natural Mârşid | - | - | graphite + CNT |
| 6 | M-Cu-G-CNT-CPE | natural Mârşid | ions $Cu^{2+}$ | Ionic exchange | graphite + CNT |
| 7 | M-Cu-CPE | natural Mârşid | ions $Cu^{2+}$ | Ionic exchange | graphite |
| 8 | CV-Fe-CPE | natural Cepari-Vultureni | ions $Fe^{2+}$ | Ionic exchange | graphite |

**Table 7. Electrochemical parameters of EMZ**. Experimental conditions: scan rate, 10 mV $s^{-1}$; support electrolytel, phosphate buffer 0,1 M pH 7 ( pH 3 in the case of **CV-Fe-CPE**)

| Electrode | $E_{pa}$* | $E_{pc}$ * | $E^{0'}$ * | $\Delta E_p$ * | $I_{pc}/I_{pa}$ | $\Gamma$ (mol $cm^{-2}$) |
|---|---|---|---|---|---|---|
| **CPEs, MB in solution** | -207 | -240 | -223 | 33 | 0.95 | $1.3 \cdot 10^{-8}$ |
| **MB-4A-CPE** | -124 | -262 | -193 | 138 | 0.97 | $2.3 \cdot 10^{-7}$ |
| **MB-13X-CPE** | -170 | -223 | -197 | 53 | 1.0 | $8.8 \cdot 10^{-8}$ |
| **MB-13XCa-CPE** | -215 | -322 | -269 | 107 | 1.3 | $3.4 \cdot 10^{-8}$ |
| **M-Cu-CPE** | 0 | -0.284 | -142 | 284 | 3.0 | $2.2 \cdot 10^{-7}$ |
| **M-Cu-G-CNT-CPE** | -8 | -318 | -163 | 310 | 1.2 | $2.6 \cdot 10^{-7}$ |
| **CV-Fe-CPE (pH 3)** | 155 | 27 | 91 | 128 | 1.0 | $3.1 \cdot 10^{-9}$ |

* mV *vs*. Ag|AgCl/$KCl_{sat}$

### II.1.2.3. Electrocatalytic applications of ZMEs

#### II.1.2.3.1. $H_2O_2$ electroreduction

*Carbon paste electrodes modified with Methylene Blue*

Modified electrodes with electrocatalytic activity toward $H_2O_2$ reduction were obtained by Methylene Blue adsorption on synthetic zeolites (molecular sieves 13X, Aldrich), followed by their incorporation in carbon paste. The characteristics of the voltammetric response of MB-13X- (ΔEp of 90 mV, respectively and Ipa/Ipc of ~ 1) pointed out to a quasi-reversible, surface confined redox process. In the case of MB-13X-CPEs $E^{0'}$ is pH independent.

The investigated modified electrodes showed moderate catalytic efficiency towards $H_2O_2$ reduction and a relatively low limit of detection (0.13 mM for MB-13X-CPEs, pH 6).

#### II.1.2.3.2. NADH electrooxidation

*Carbon paste electrodes incorporating calcium-exchanged zeolites*

A new composite electrode material consisting of Methylene Blue (as redox mediator), a calcium exchanged synthetic zeolite (as immobilization support) and carbon paste (as conductive material) (MB-13XCa-CPEs) was obtained and characterized. Electrocatalytic oxidation of NADH was studied at this modified electrode and a better electrocatalytic activity in comparison with the electrode incorporating the same zeolite without calcium (MB-13X-CPEs) was put on evidence. The electrochemical properties (formal standard potential, peak currents and peak-to-peak separation) were different in the case of the calcium-exchanged zeolite, probably owing to the formation of a complex between mediator and $Ca^{2+}$. Cyclic voltammetry, rotating disk electrode and amperometry were used to study NADH electrocatalytic oxidation. The kinetic parameters of this electrocatalytic process were evaluated in the presence or absence of $Ca^{2+}$ in zeolite, assuming the formation of an intermediate charge-transfer complex between mediator and NADH. At low overpotentials (0 mV *vs.* Ag|AgCl/KClsat) a higher linear NADH concentration range and a lower detection limit is observed for MB-13XCa-CPEs ($10^{-7}$-$3 \cdot 10^{-5}$ M; 0.8 μM) than for MB-13X-CPEs ($10^{-6}$ - $10^{-4}$ M; 4.3 μM).

The primary result described in this chapter is the significant increase of catalytic efficiency of the NADH electro-oxidation with modified carbon paste electrodes incorporating a calcium-exchanged 13X type zeolite and impregnated with Methylene Blue. In the presence of $Ca^{2+}$ incorporated in zeolite, a closer interaction between mediator and substrate was put on evidence, which results in higher catalytic currents. The measured electrocatalytic rate constants ($k_{\text{obs, [NADH]=0}}$) is 20 times higher for modified electrodes incorporating calcium exchanged zeolite than for electrodes incorporating zeolite without $Ca^{2+}$, leading to a quite important linear range for I = f([NADH]) ($10^{-7}$ - $3 \cdot 10^{-5}$ M). These kinds of modified electrodes can be considered very promising for practical applications.

### II.1.2.3.3.Ascorbic acid electrooxidation

Using an efficient technique for zeolite immobilization in a carbon paste matrix, two new sensors incorporating synthetic mesoporous zeolites (4A and 13X, Aldrich) modified with Methylene Blue were designed and tested for ascorbic acid amperometric detection.

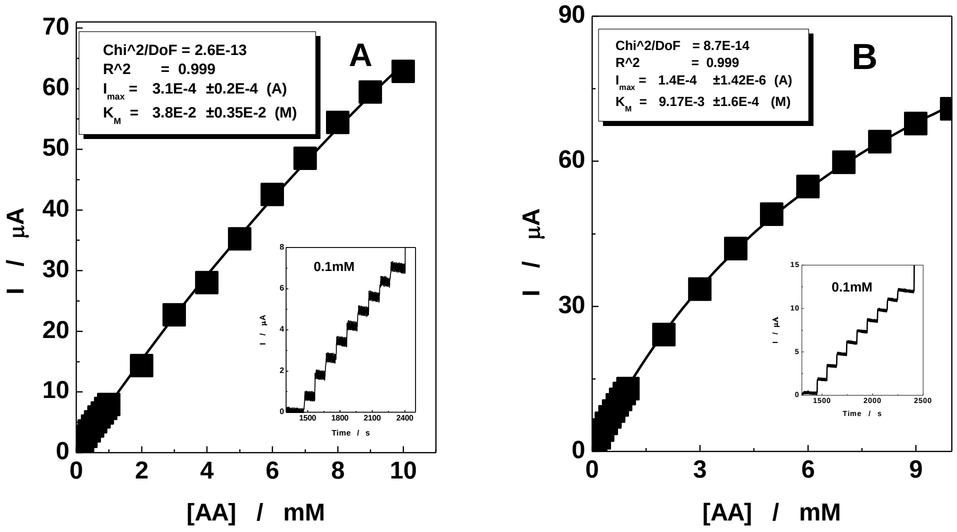


**Figura II.1.49.** Calibration curves at MB-13X-CPEs (A) and MB-4A-CPE (B) and amperometric response to successive increments of 0.1 mM AA (inset). Experimental conditions: applied potential, -50 mV *vs.* Ag|AgCl/$KCl_{sat}$; rotation speed, 500 rpm; supporting electrolyte, 0.1 M phosphate buffer, pH 7.0

Both modified electrodes exhibit a short current time response (5s) and can act as AA sensors. As can be observed, the relationship between the steady-state current and the concentration of AA was linear in the range from $10^{-5}$ M to $10^{-3}$ M AA for MB-13X-CPEs and $10^{-5}$ M to $10^{-4}$ M AA for MB-4A-CPEs. The theoretical detection limits, calculated from the slope of the regression equation and standard deviation of the calibration curves were $4.7 \cdot 10^{-5}$ M for MB-13X-CPEs and $1.7 \cdot 10^{-5}$ M for MB-4A-CPEs

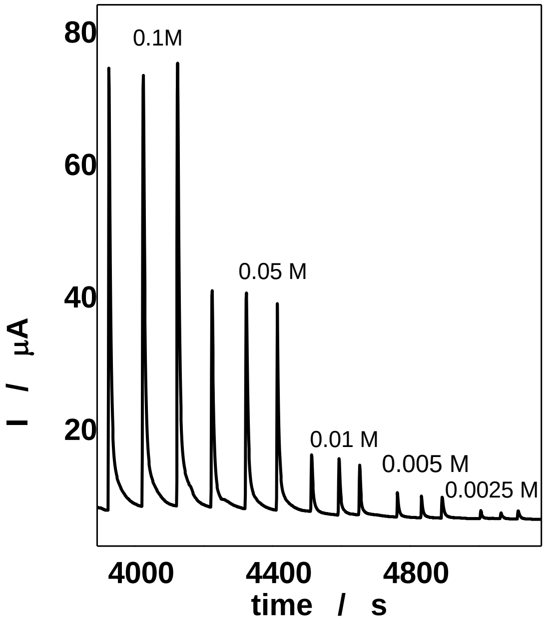


**Figure II.1.55. Amperogram recorded in FIA with the carbon paste electrode incorporating Methylene Blue in the presence of different AA concentrations.** Experimental conditions: working potential, 0 V, debit 1.25 ml/min, electrode-injector distance, 3mm.

## II.2. Graphite Electrode Modified with a New Phenothiazine Derivative and with Carbon Nanotubes

The results detailed above have assessed the ability of coupling CNTs with a new phenothiazine derivative (bis-phenothiazin-3-yl methane, BPhM), as a means of improving performances for NADH electrocatalytic oxidation: promoting low potential and high electrocatalytic rate constants $k_{\mathrm{obs,[NADH]=0}}$.

The electrochemical behavior of a modified electrode obtained by immobilization of single-walled carbon nanotubes onto a graphite electrode modified with a new phenothiazine derivative, bis-phenothiazin-3-yl methane (BPhM), G/BPhM-CNT, has been evaluated and compared with BPhM adsorbed on graphite electrode (G/BPhM).

**Table 25**. Electrochemical parameters of G/BPhM-CNT and G/BPhM electrodes

| **pH** | **Electrode** | **Epa(V)** | **Epc (V)** | **$E^{0'}$ (V)** | **ΔEp (V)** | **Ipa (A)** | **Ipc (A)** | **Ipa/Ipc** |
|---|---|---|---|---|---|---|---|---|
| 1 | **G/BPhM-CNT** | 0.382 | 0.139 | 0.2605 | 0.243 | 3.96e-5 | -7.312e-5 | 0.54 |
| 2 | | 0.354 | 0.090 | 0.222 | 0.264 | 3.911e-5 | -6.644e-5 | 0.58 |
| 3 | | 0.308 | 0.028 | 0.168 | 0.28 | 4.944e-5 | -5.627e-5 | 0.87 |
| 4 | | 0.218 | -0.034 | 0.092 | 0.252 | 3.947e-5 | -3.227e-5 | 1.22 |
| 5 | | 0.160 | -0.096 | 0.032 | 0.256 | 4.913e-5 | -3.540e-5 | 1.38 |
| 6 | | 0.110 | -0.153 | -0.0215 | 0.263 | 5.586e-5 | -3.503e-5 | 1.59 |
| 7 | | 0.040 | -0.227 | -0.0935 | 0.267 | 5.286e-5 | -3.029e-5 | 1.74 |
| 8 | | -0.001 | -0.289 | -0.145 | 0.288 | 4.317e-5 | -2.337e-5 | 1.84 |
| 9 | | -0.071 | -0.380 | -0.2255 | 0.309 | 3.061e-5 | -1.469e-5 | 2.46 |
| 10 | | -0.001 | -0.396 | -0.1985 | 0.395 | 2.176e-5 | -1.088e-5 | 2.0 |
| 7 | G/BPhM | 0.032 | -0.145 | -0.0565 | 0.177 | 3.217e-5 | -2.659e-5 | 1.209 |

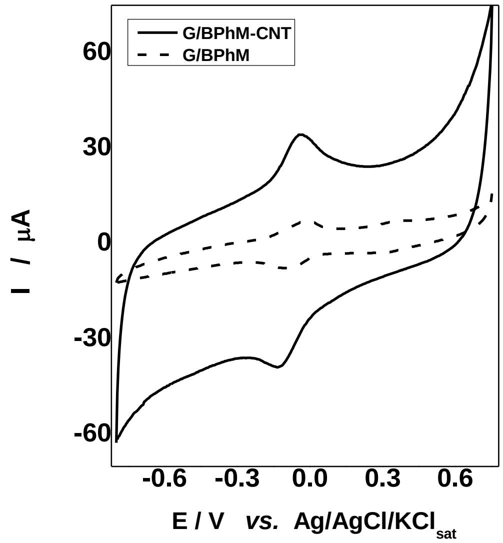


**Figura II.2.2. Cyclic voltammograms for G/BPhM-CNT (—) and G/BPhM (---) modified electrodes.** *Experimental conditions: starting potential, –800 mV vs. Ag|AgCl/KClsat; scan rate, 5 mV s–1; supporting electrolyte, 0.1 mol L–1 phosphate buffer, pH 7.*

**Table 29. Electroanalytical parameters corresponding to obtained modified electrodes**. *Experimental conditions: as in Fig. II.2.9.*

| Electrod | pH | LD/ µM | DL/ mol L$^{-1}$ | S/ mA mol$^{-1}$ | Chi$^2$ | R$^2$ |
|---|---|---|---|---|---|---|
| G/BPhM | 7 | 50 | $5\cdot10^{-4}$-$10^{-2}$ | 0,55±0,002 | $1,3\cdot10^{-14}$ | 0,996 |
| G/BPhM-CNT | 6 | 8 | $10^{-5}$-$10^{-3}$ | 8,3±0.9 | $5,6\cdot10^{-14}$ | 0,998 |
|  | 7 | 10 | $10^{-5}$-$10^{-3}$ | 6,9±0,6 | $36,9\cdot10^{-14}$ | 0,989 |
|  | 8 | 16 | $5\cdot10^{-5}$-$10^{-3}$ | 5,6±0,9 | $4,1\cdot10^{-14}$ | 0,992 |
|  | 9 | 34 | $2\cdot10^{-5}$-$10^{-3}$ | 2,6±07 | $3,0\cdot10^{-14}$ | 0,989 |

The modified graphite electrode based on CNTs was found to be stable and can be used as amperometric sensor with improved characteristics for NADH detection.

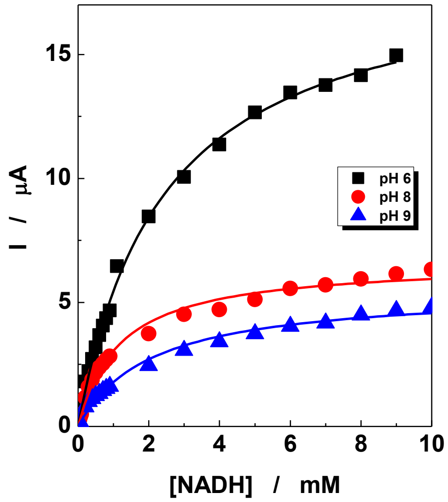


**Figure II.2.9. Calibration curve for NADH oxidation at G/BPhM-CNT electrode at different pH values**. *Experimental conditions: applied potential, +100 mV vs. Ag|AgCl/KClsat; supporting electrolyte, 0.1 mol L$^{-1}$ phosphate buffer; rotation speed, n = 500 min$^{-1}$.*

The G/BPhM-CNT electrode presents improved performances for NADH electrocatalytic oxidation in comparison with G/BPhM electrode, expressed by: (i) a significant increase of electrocatalytic rate constant ($k_{\text{obs},[\text{NADH}] = 0}$) for NADH oxidation (856.32 L mol$^{-1}$ s–$^{1}$ for G/BPhM-CNT and 51.63 L mol$^{-1}$ s–$^{1}$ for G/BPhM, in phosphate buffer, pH 7); (ii) the obtained amperometric sensors for NADH detection present increase sensitivity (*S*= 6.9 mA L mol$^{-1}$ for G/BPhM-CNT and *S*= 0.55 mA L mol$^{-1}$ for G/BPhM, pH 7).

## II.3. Amperometric Biosensor for ascorbic acid determination

A new biosensor design, consisting in ascorbate oxidase (AOx) and bovine serum albumine immobilization on the membrane of an oxygen electrode, followed by cross-linking with glutaraldehyde (GA), was proposed for ascorbic acid (AA) determination. The biosensor was optimized regarding the enzyme loading and GA concentration. The best bioelectroanalytical parameters were obtained when 1.25 % GA and 157 U/cm2 AOx were used: sensitivity of 328 ± 67 μA/M, linear domain up to 0.4 mM and detection limit of 50 μM. The biosensor was used to estimate the AA concentration in synthetic samples and the results were in good agreement with those obtained by using the reference method based on 2,6 dichlorophenolindophenol (relative error ~ 1.1 %).

**Table 30. Kinetic parameters of the amperometric biosensors for AA detection**

*Experimental conditions: supporting electrolyte, 0.1 M phosphate buffer solution (pH 7); stirring rate, 650 rpm.*

| Biosensor | AOx ($U/cm^2$) | GA Conc (%) | Michaelis-Menten fitting | | | Hanes-Woolf linearization | | |
|---|---|---|---|---|---|---|---|---|
| | | | Imax (nA) | $K_M$ $^{app}$ (mM) | $R^2$ | Imax (nA) | $K_M^{app}$ (mM) | R/N |
| 1 | 630 | 2,5 | 65 ± 1,3 | 1,17± 0,08 | 0,991 | 67 ± 2,1 | 1,3 ± 0,2 | 0,9971/ 27 |
| 2 | | 1,25 | 110± 2,3 | 0,85 ± 0,06 | 0,990 | 105 ± 3.1 | 0,63± 0,02 | 0,9990 / 7 |
| 3 | 315 | 2,5 | 139± 3,6 | 0,90 ± 0,08 | 0,983 | 130 ± 4,1 | 0,70 ± 0,01 | 0,9984/ 14 |
| 4 | | 1,25 | 158± 4,8 | 0,65 ± 0,07 | 0,973 | 151 ± 4,7 | 0,46 ± 0,02 | 0,9992/ 10 |
| 5 | 157 | 2,5 | 152± 3,6 | 0,65 ± 0,06 | 0,984 | 167 ± 2,2 | 0,49 ± 0,01 | 0,9895 / 9 |
| 6 | | 1,25 | 181 ± 10 | 0,55 ± 0,08 | 0,963 | 194 ± 3,1 | 0,45 ± 0,08 | 0,9993/ 16 |

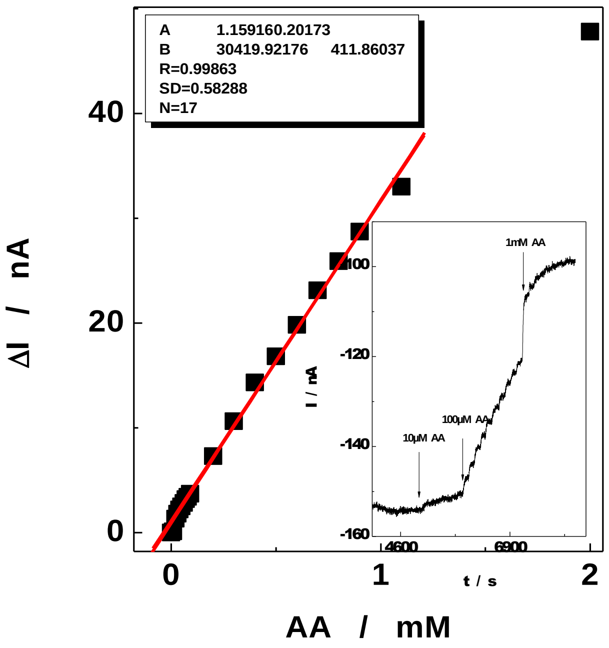


**Figura II.3.4– Calibration curve of the AOx based biosensor and the corresponding amperometric response to successive AA additions (inset).** *Experimental conditions: enzyme loading, 315 U AOx / cm2; 2.5% GA;*

**Table 31. Analytical parameters of the amperometric biosensors for AA detection.**

*Experimental conditions: supporting electrolyte, 0.1 M phosphate buffer solution (pH 7); stirring rate, 650 rpm.*

| Bios. | Conc. AOx (U/cm$^2$) | Conc. GA (%) | LD (M) | DL (M) | Sensibility (µA/M) | | R/N |
|---|---|---|---|---|---|---|---|
| | | | | | M-M | Panta | |
| 1 | 630 | 2,5 | $4 \cdot 10^{-5}$ | $4 \cdot 10^{-5}$- $10^{-3}$ | 55,21±4,90 | 32±0,41 | 0,9981/17 |
| 2 | | 1,25 | $5 \cdot 10^{-5}$ | $5 \cdot 10^{-5}$- $7 \cdot 10^{-4}$ | 129,64±11,92 | 73 ±1,06 | 0,9983/17 |
| 3 | 315 | 2,5 | $5 \cdot 10^{-5}$ | $5 \cdot 10^{-5}$- $6 \cdot 10^{-4}$ | 154,11±17,67 | 95±2,03 | 0,9966/16 |
| 4 | | 1,25 | $5 \cdot 10^{-5}$ | $5 \cdot 10^{-5}$- $5 \cdot 10^{-4}$ | 242,92±33,52 | 133±1,95 | 0,9989/17 |
| 5 | 157 | 2,5 | $5 \cdot 10^{-5}$ | $5 \cdot 10^{-5}$-$5 \cdot 10^{-4}$ | 233,53±27,08 | 150±5,96 | 0,9890/16 |
| 6 | | 1,25 | $5 \cdot 10^{-5}$ | $5 \cdot 10^{-5}$- $4 \cdot 10^{-4}$ | 328,36±66,60 | 203±8,35 | 0,9786/12 |

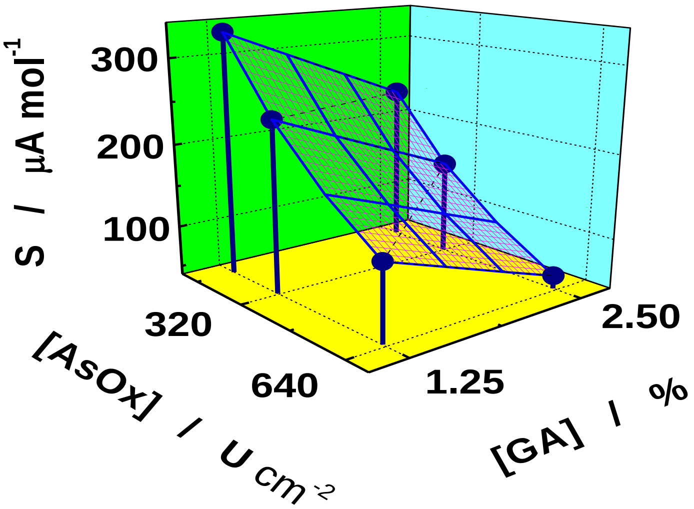


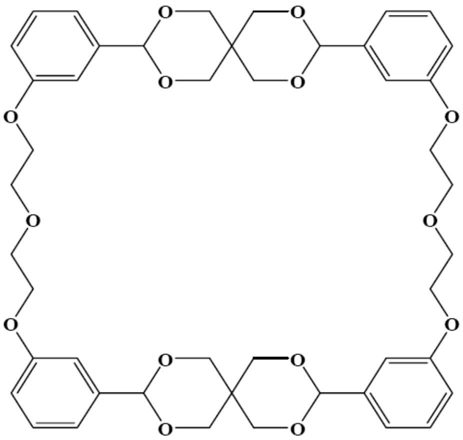
**Figure II.3.5. 3D representation of biosensor sensitivity as function of AOx loading and GA concentration.** *Experimental conditions: supporting electrolyte, 0.1 M phosphate buffer solution (pH 7); stirring rate, 650 rpm.*

**Table 32. Concentrations of** AA from synthetic samples determined with biosensor 3.

| Biosensor 1 | [AA] mg/100 ml determined with the biosensor | [AA] mg/100 ml of synthetic sample |
|---|---|---|
| Measurement A | 34,44 (98.4%) | 35 |
| Measurement B | 34,14 (97.54%) | 35 |

## II.4. Novel composite material based on carbon paste and a crown ether for potassium selective detection

Potassium ion plays a crucial role for the organisms life. Its detection is performed usually by potassium selective electrodes (PSE) having membranes made of a polymer matrix (such as PVC) incorporating macrocyclic compounds as ionophore. We proposed a novel potassium selective material (PSM) based on a new synthesized crown-ether ionophore incorporated in carbon paste conductive matrix (PSM-CP). Electrochemical measurements proved the affinity of PSM-CP for the potassium cathion, which is selectively bound by entrapping in the crown-ether cavity. The results are comparable with those obtained by using a PSE based on PVC-ionophore membrane.



**Scheme 4. Bis(3,9-dibenzo-2,4,8,10- tetraoxo[5.5]undecane)-40-crown-6, (BDBTU40C6).**

Preliminary complexing investigations by spectroscopic methods were performed in order to validate the process have been used to evaluate the binding properties of the ionophore with $K^+$ cations (Figure II.4.2).

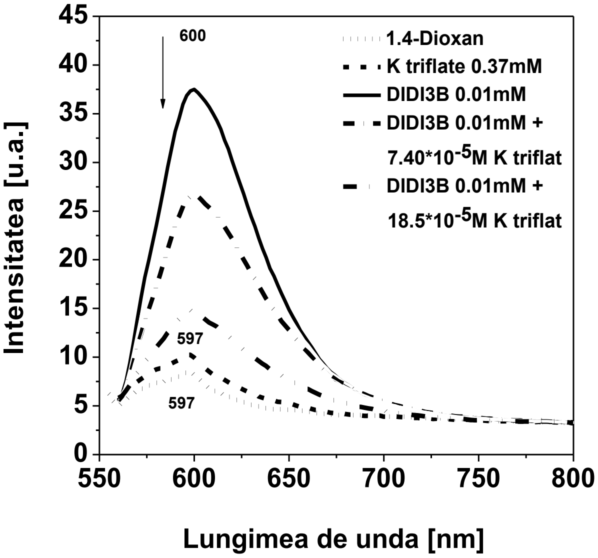


**Figure II.4.2 Decreasing intensities of BDBTU40C6 in 1,4- dioxane solution (0.01 mM) in the presence of potassium triflate in the concentration range of 0.02-0.185 mM, λex = 274 nm, λem = 600 nm.**

**Table 37. Comparison of the electrodes performance in potassium containing solutions.**

| Electrode type | Electrolyte | Slope (mV/decade) | R/N |
|---|---|---|---|
| PSM-CP | TEAC | 14.79±0.26 | 0.9953/29 |
| | $LiClO_4$ | 11.05±1.04 | 0.9867/5 |
| | $Cd(CH_3COO)_2$ | 19.95±0.40 | 0.9990/5 |
| PSM-PVC | $Cd(CH_3COO)_2$ | 19.38±2.01 | 0.9894/5 |
| CPE | $Cd(CH_3COO)_2$ | 6.94±0.23 | 0.9986/5 |

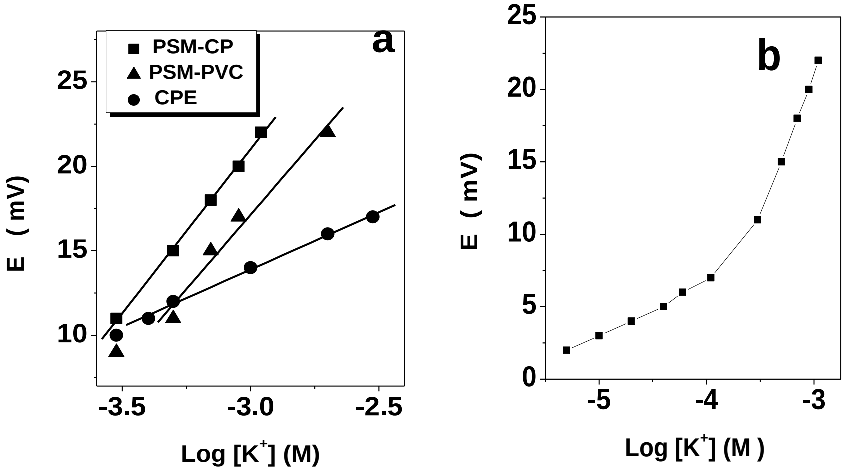
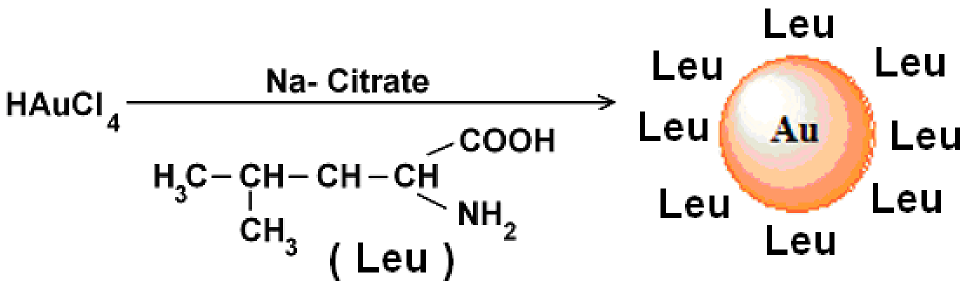


**Figure II.4.4. Linear dependence of the potential on potassium ions concentration for PSM-CP, CPE and PSM-PVC electrodes (a); calibration graph for PSM - CP in KCl solutions (b).**

The new designed carbon paste material based on crown-ether derivative exhibits renewable surface being suitable for rapid and efficient potassium detection in aqueous media. The detection efficiency of this carbon paste selective device for potassium is comparable to that of a classic electrode based on PVC membrane in conditions of the same ionophore usage. The novel potassium sensitive material is simple, versatile and rapid manufactured, solid and less expensive than PSM-PVC.

## II.5. Gold electrodes modified with gold nanoparticles functionalized with L-leucine or with citrate.

A novel modified electrode was designed and fabricated by linking l-Leucine capped gold nanoparticles to 1,3-propanedithiol self-assembled monolayer (SAM), on gold substrate. Using UV-Vis and FT-IR spectroscopy, we have shown that l-Leucine molecules functionalize the gold nanoparticles. Morphological characteristics of capped nanoparticles before and after linking to SAM were evidenced by TEM and tapping TM mode AFM. The properties of the nanostructured assembly were tested for detection of Cu(II) ions, by employing cyclic voltammetry as investigation techniques. A detection limit of 5.4 × 10−7M was obtained in this case. The results were compared with those obtained for bare gold electrode or modified by citrate-capped gold nanoparticles. Additionally, Electrochemical Impedance Spectroscopy was used to further characterize the electrode modified with l-Leucine capped gold nanoparticles. An equivalent electrical circuit was developed to interpret and fit the experimental EIS data. The circuit contains the solution resistance (Rs), the charge-transfer resistance (Rct), the Warburg impedance (ZWt – transmissive boundary) and a constant phase element (CPE) that models the behavior of the double-layer capacitance. The charge-transfer resistance was found to vary with Cu(II) concentration within 10−5 to 10−3M concentration range; a saturation tendency was observed at higher concentrations.



**Scheme 5. Preparation scheme of gold nanoparticles functionalized with L-leucine**

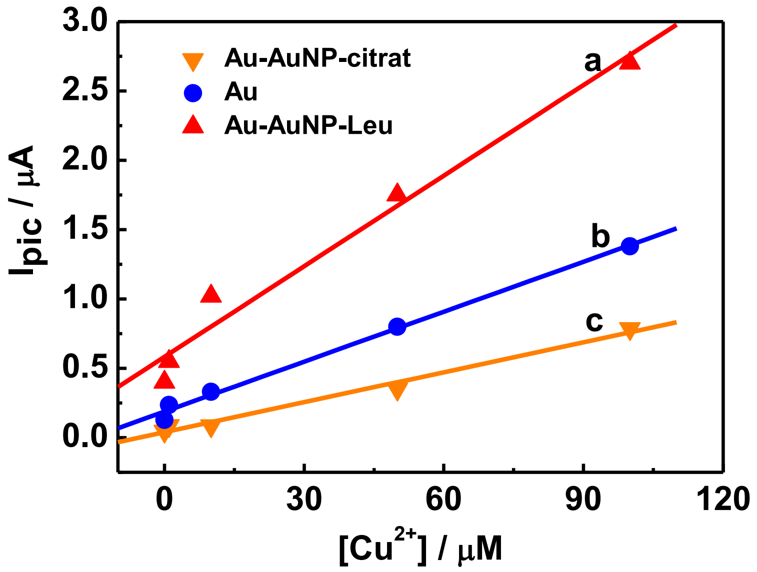


**Figura II.5.5. Calibration curves of the bare/modified gold electrode for Cu(II) ions determination: Au-AuNPs-Leu (a); Au (b); Au-AuNPs-citrate (c).**

The gold electrode modified with L-leucine (AuNPs-Leu) was investigated by Electrochemical Impedance Spectrosopy in a solution containing 1 mM $K_4[Fe(CN)_6]$ (applied potential + 0,25 V vs.Ag/AgCl,KCl).

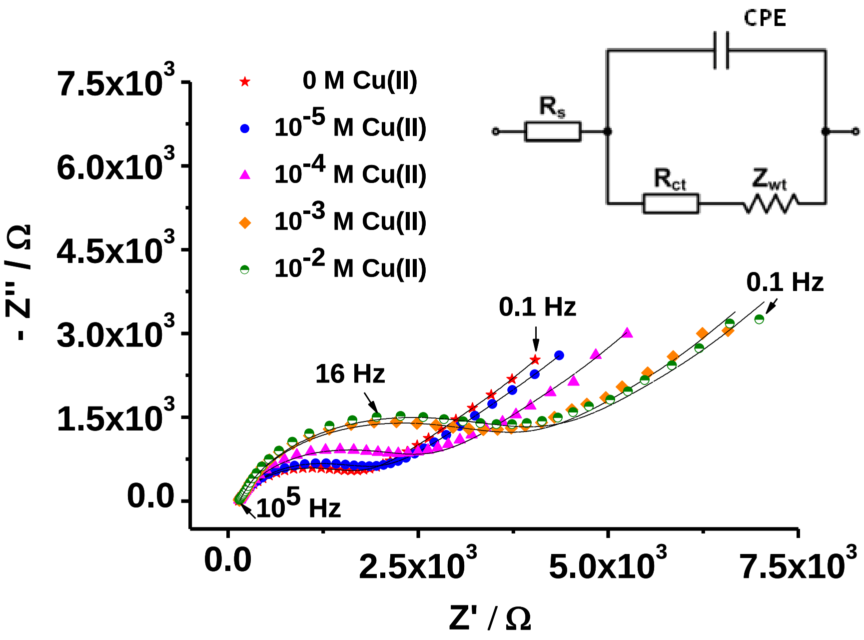


**Figure II.5.7. Nyquist plots obtained after 15 min accumulation in each Cu(II) solution: 10−5 to 10−2 M concentration range; EIS data recorded in 1 mM K4[Fe(CN)6] at +0.25 V *vs.* Ag/AgCl; the continuous lines represent the fit obtained with the equiv-alent electrical circuit (see the inset).**

In conclusion, we have shown for the first time that l-Leucine-capped gold nanoparticles can be successfully used to modify the surface of a gold substrate, using 1,3-propanedithiol as linker molecule. FT-IR measurements have shown that l-Leucine func-tionalizes the nanoparticles via amino group, leaving free the carboxylate moiety which can chelate Cu(II) ions. Therefore, this nanostructured assembly was tested for detection of Cu(II) ions, using cyclic voltammetry as investigation technique. In comparison with bare gold or electrode modified by citrate capped gold nanoparticles, this electrode exhibits the highest sensitivity toward Cu(II) determination. A limit of detection of 5.4 × 10−7M was obtained in this case. In addition, Electrochemical Impedance Spectroscopy was employed to characterize the electrode modified with l-Leucine capped gold nanoparticles. An equivalent electrical circuit was developed based on the solution resistance (Rs), the charge-transfer resistance (Rct), the Warburg impedance (ZWt – transmissive boundary) and a constant phase element (CPE) that models the non-ideal capacitive response of the interface.

# Final conclusions

**1.** Five types of new modified electrodes were obtained, using different preparation methods:

- **Modified electrodes based on natural or synthetic zeolites (EMZ), by their incorporation in carbon paste**:

  (i) synthetic mesoporous zeolites (13X and 4A Aldrich, Germany) modified with Methylene Blue (MB), and/or $Ca^{2+}$ ions;

  (ii) volcanic tuffs containing natural zeolites CV (Cepari- Vultureni) modified with $Cu^{2+}$

  (iii) volcanic tuffs containing a natural zeolite M (Mârşid) modified with $Fe^{3+}$, in different experimental conditions (pH, the presence of $Ca^{2+}$ ions, etc.)

- **Graphite electrodes modified with mediators and carbon nanotubes by adsorption**

  A new type of modified electrode was prepared by immobilization of carbon nanotubes with single walls on a graphite electrode, modified with a new phenothiazine derivative (bis-phenotiazin-3-yl methane).

- **Oxygen electrodes modified with enzymes, for obtaining of biosensors**

  Amperometric biosensors, specific for L-ascorbic acid determination (AA) were realized by immobilization of ascorbate-oxidase (AOx) on the membrane of Clark oxygen electrode, uisng glutaraldehide (GA) and bovin serum albumine (BSA).

- **Carbon paste electrodes modified with supramolecular compounds, which form some complex of „guest-host" type**

  It was obtained a new composite material, **s**ensitive to potassium ions (PSM). The new proposed material is based on a new ionophore, of crown-ether type, incorporated in conductive matrix of carbon paste (PSM-CP), replacing the matrices based on PVC, used frequently in literature.

- **Au electrodes modified with Au nanoparticles functionalized with aminoacid by self-assembling**

2. The so obtained electrode materials were successfully tested for detection of some compounds of biomedical interest, for controlling the environment quality or for biotechnological processes : $H_2O_2$, NADH, AA, $K^+$, $Cu^{2+}$:

- Amperometric sensors based on EMZ were realized for detection of important chemical analytes ($H_2O_2$, NADH and ascorbic acid). The presence of $Ca^{2+}$ ions immobilized in zeolites has the same effect of improving the NADH detection as the $Ca^{2+}$ ions present in solution. The electroanalytical parameters of obtained sensors are comparable with those reported in literature for similar systems. There were obtained electrodes, for which the potential $E^{0'}$ doesn't vary with the pH (MB-13X-CPE and MB-4A-CPE), which is of real use in the case of detection of analytes present in different matrices for which the acidity / basicity varies. The electrocatalytic applications of these new electrodes are based on oxidation/reduction of a large range of analytes ($H_2O_2$, NADH and ascorbic acid), all obtained electrodes presenting very good electrocatalytic characteristics. The new obtained electrodes presented a good electrochemical stability, which reccomends them for obtaining of amperometric sensors. The obtained sensors present good detection limit and sensitivity, useful in applications concerning environmental quality control or biotechnological and biomedical processes.

- Graphite electrodes modified with bis-phenothiazin-3-yl methane (BPhM) and carbon nanotubes (CNT) are stable and can be used as sensors with improved characteristics, for NADH detection. The best performances of the NADH sensors based on G/BPhM-CNT electrodes were obtained at pH 6, at +100 mV, in the linear range $10^{-5}$-$10^{-3}$ M, with the detection limit 8 μM, sensitivity of 8 mA/mol and response time of 10 seconds.

- The biosensors based on ascorbate oxidase can be used for detection of ascorbic acid in aqueous solutions. They present good stability and reproducibility, a good sensitivity for L- ascorbic acid (328.36 μA/M), a good detection limit ($5\cdot10^{-5}$M) and a small response time, in phosphate solution, pH 7, at an applied potential of - 650 mV *vs*. Ag/AgCl. The comparison of obtained results with the amperometric biosensor and standard method (titration with DCPI) shows that the biosensor functions well in synthetic samples of AA and can be reccomended for testing in real samples (wines and fruit juices).

- The new material based on carbon paste modified with crown-ether derivative can be used for rapid and efficient detection of potassium ions in aqueous medium. The efficiency of potassium detection with this new material is comparable with that of a classic electrode, based on PVC membrane, in conditions of using the same ionophore. The electroanalytical parameters of PSM-CP modified sensor are: response time of 10 seconds, linear domain between $10^{-4}$ and $5 \cdot 10^{-2}$ M, and the detection limit of $1.3 \cdot 10^{-4}$ M. The obtained material, sensitive to potassium is solid, simple, rapid and easy to realize and cheaper than PSM-PVC.
- The nanostructurated ansamblies of gold nanoparticles functionalized with L-Leucine, which were attached to a gold electrode surface, using a monolayer of 1,3-propanditiol present good sensitivity towards the determination of Cu (II), (21.78±2.06 mA/M), its detection limit being $5.4 \times 10^{-7}$ M.

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**Keywords**: modified electrodes, biosensor, $H_2O_2$, NADH, ascorbic acid, carbon nanotubes, gold nanoparticles , zeolites, carbon paste, crown ether