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SENSORS BASED ON AZULENE MODIFIED ELECTRODES FOR
TESTING METALS IN WATERS

SENZORI PENTRU METALE BAZAȚI PE ELECTROZI MODIFICAȚI CU
AZULENE PENTRU TESTAREA APELOR

Author: Chem. Georgiana-Luiza ARNOLD

PhD COMMISSION

Președinte	Prof. Em. Dr. Ing. Teodor VIȘAN	from	University Politehnica of Bucharest
Conducător de doctorat	Prof. Dr. Ing. Eleonora-Mihaela UNGUREANU	from	University Politehnica of Bucharest
Referent	Prof. Dr. Elena DIACU	from	University Politehnica of Bucharest
Referent	Prof. Dr. Habil. Dr. Raluca-Ioana van STADEN	from	National Institute of Research for Electrochemistry and Condensed Matter, Timisoara
Referent	Prof. Dr. Farm. Robert Valentin SĂNDULESCU	from	Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca

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INTRODUCTION

Chemicals are part of our daily life. All living and inanimate matter is made up of chemicals and virtually every manufactured product involves the use of chemicals. Many chemicals can, when properly used, significantly contribute to the improvement of our quality of life, such as calcium, magnesium, sodium, potassium, etc, when used properly. But other chemicals are highly hazardous and can negatively affect our health and environment when improperly managed, such as cadmium, copper, mercury and lead [1].

According to law no. 311 of 28 June 2004, the allowable value for tap water samples for cadmium is $5.0 \mu\text{g} / \text{L}$, for copper $0.1 \text{ mg} / \text{L}$ for mercury $1.0 \mu\text{g} / \text{L}$ and for lead $10.0 \mu\text{g} / \text{L}$ [2]. Decision no. 352 of 21 April 2005 amending and supplementing the Government Decision no. 188/2002 approving some rules regarding the discharge conditions in the aquatic environment of the wastewater admit maximum values for these heavy metals: cadmium - $0.3 \text{ mg} / \text{dm}^3$, copper - $0.2 \text{ mg} / \text{dm}^3$, mercury - $0.05 \text{ mg} / \text{dm}^3$ and lead - $0.5 \text{ mg} / \text{dm}^3$ [3].

Cadmium (Cd) is a toxic metal with a half-life of 10-30 years, being classified as a human carcinogen 1 [4, 5]. Human exposure occurs mainly from consumption of contaminated food. In an area of Japan where soil has been contaminated with cadmium, Itai-itai disease used to be widespread and is still seen in women over 50 years of age. It is characterized by osteomalacia, osteoporosis, painful bone fractures and kidney dysfunction. The results of the studies in Japan suggest the link between the cadmium causing the disease and the cadmium present in the rice. Cadmium is also associated with breast cancer, chronic renal disease [8, 9], prostate cancer [10].

Exposure to cadmium has been associated with the increase in the suPAR plasma (urokinase soluble plasminogen receptor activator), independent of smoking and cardiovascular disease. SuPAR is a biomarker for activation of the inflammatory and immune

systems. SuPAR levels are positively correlated with pro-inflammatory biomarkers, such as tumor necrosis factor- α , leukocyte counts, and C-reactive protein (CRP). These results imply that cadmium is a probable sample for raising levels of this inflammatory marker [11, 12]. Workers engaged in tobacco factories have kidney problems being [13, 14]. No treatment against cadmium intoxication is known, but recent studies in rats have shown that *Tinospora Cordifolia* extract attenuates biochemical and histological changes induced by cadmium. The study suggests that *Tinospora Cordifolia* is a strong cardioprotective agent against Cd-induced toxicity [15].

Copper (Cu) has long been known to have antimicrobial activity [16]. In Parkinson's disease local dysregulation of iron metabolism in the substantia nigra (SN) seems to be related to neurodegeneration with an increase in SN iron concentration, accompanied by decreased SN Cu and ceruloplasmin concentrations and increased free Cu concentrations and decreased ferroxidase activity in the cerebrospinal fluid. [17, 18]. SN is a component of cerebral peduncles (along with many ribbed nerves) being a region of neurons containing closed melanin, which is involved in inhibiting the movement; Degradation of this substance leads to the loss of a control engine, known as Parkinson's disease.

In the early 1990s, many people with Alzheimer's disease (the most common form of dementia) were registered in highly developed countries. One of the causes involved was the change of water pipes with copper pipes, which led to water contamination. To avoid such contamination, an inverse osmosis device can be fitted to lower the copper concentration to a value below the maximum allowable limit [19, 20]. In patients identified with Skoghol's disease, the iron and copper concentrations in the cerebrospinal fluid are approximately four times higher than in the control [21]. Menkes syndrome [22] and Wilson's disease are caused by copper poisoning. For Wilson's disease, zinc therapy and penicillamine treatment, or multivitamin and mineral supplements are recommended [23-26].

Mercury (Hg) is considered by the World Health Organization (WHO) as one of the ten most troublesome chemical to public health Mercury was quantified in samples of spleen, bile, brain, liver, lung, kidney and cardiac blood [27]. One source of mercury contamination may be plastics waste [28, 29], the nervous system is the primary target for mercury poisoning, gold miners are most likely to mercury poisoning [30, 31]. British literature presents the first case of mercury intoxication in a child [32]. Autism is a developmental disorder caused by mercury [33-35]. A nonmetal used as a mercury protection agent is selenium [30, 36]. In order to cure a disease induced pulmonary inhalation of mercury was used with 2,3-dimercapto therapy-1-propanesulfonic acid or meso-2,3-Dimercaptosuccinic, which are chelating agents [37-39].

Lead (Pb) is a toxic metal that leads to Parkinson's disease, urological (kidney) disease [40-42]. The treatment used is the one with zinc supplements, meso-2,3-dimercaptosuccinic acid, 2,3-dimercapto-propanesulfonic acid, dimercaptopropanol [38, 43-46].

Several methods used for heavy metal determination are spectrometric methods (graphite furnace atomic absorption spectroscopy, flame atomic absorption spectrometry, inductively coupled plasma mass spectrometry, atomic fluorescence spectrometer, inductively coupled plasma atomic emission spectroscopy) or chromatographic (gas chromatography, high-performance liquid chromatography, etc.). These methods are classic but require more complex equipment, are expensive to maintain and require well-trained personnel [47-53].

Electrochemical methods have been developed in recent years using metal detection sensors [54, 55], modified electrodes, or screen-printed electrodes. The glassy carbon electrode is the most used, and can be modified with bismuth films [56,57], reduced-magnetite oxide [58], bismuth films and poly (violet pyrocatechol), multi-wall carbon nanotubes [59], DNA modified gold electrode [60, 61], etc.. The screen-printed electrodes used for detecting metals can be modified with glutathione [62], gold [63], or porous bismuth [64], etc.

In the Laboratory of Electrochemical Processes in Organic Solvents (PESO) from the Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry, Faculty of Applied Chemistry and Materials Science, POLITEHNICA University of Bucharest (UPB), methods for detection of heavy metals based on azulene-modified complex electrodes were initiated and tested [65, 66]. This PhD thesis that was developed in this laboratory is part of these concerns regarding ligand testing for complex electrodes.

The thesis brings original contributions related to the electrochemical characterization of new azulene derivatives synthesized recently in Institute of Organic Chemistry C.D.Nenitescu, Romanian Academy in the research team coordinated by Dr. Alexandru C. Răzuș and Dr. Liviu Bîrzan. A large number of new ligands have been tested and new electrochemical sensors have been proposed for heavy metal detection.

The PhD thesis presents the electrochemical studies of these new azulene derivatives (shown in Table 1) used in the preparation of modified electrodes with applications for the detection of heavy metals (cadmium, copper, mercury and lead) in water. Based on the results obtained, new electrochemical sensors based on modified electrodes as well as new stochastic sensors have been proposed with very low detection limits, difficult to reach due to the low limits allowed by legislation for these pollutants in water. This objective was achieved by discovering a ligand with favorable properties (**L2352**) which was tested on real samples, and the validation step of the method to be patented was also carried out.

The thesis is structured in two parts and contains 12 chapters: PART I – LITERATURE REVIEW (5 chapters) and PART II - ORIGINAL CONTRIBUTIONS (7 chapters).

LITERATURE REVIEW contain 5 chapters:

Chapter 1. Wastewater treatment and the current water sources for the purpose of drinking water

Chapter 2. Electrochemical methods for heavy metals determination from water

Chapter 3. Methods used for the characterization of azulenic derivatives

Chapter 4. General principles in stochastic method

Chapter 5. Performance analytics parameters for the optimization of a method for determining metals in water

ORIGINAL RESEARCH contains 7 chapters:

Chapter 6. Experimental details on the carried out studies

Chapter 7. Electrochemical characterization of several azulene derivatives

Chapter 8. Modified electrodes based on azulene derivatives

Chapter 9. Films characterization based on polyazulene

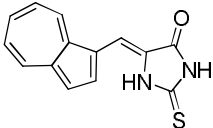
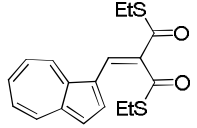
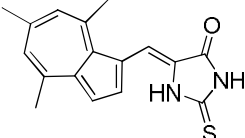
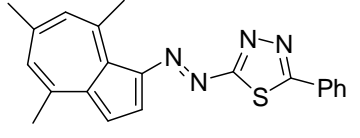
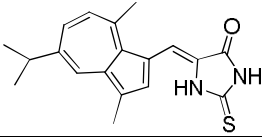
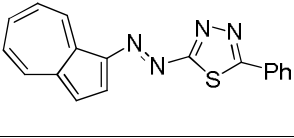
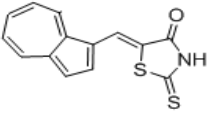
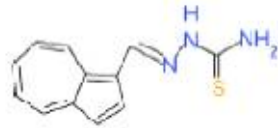
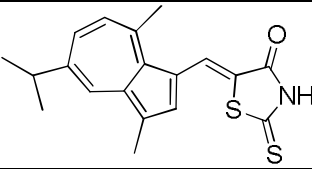
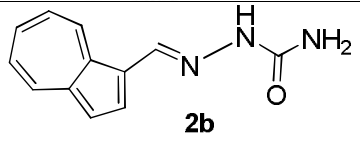
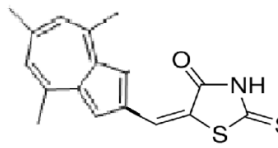
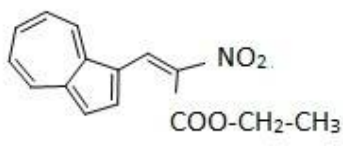
Chapter 10. Heavy metals recognition using modified electrodes based on polyazulene

Chapter 11. Optimization of analytical method for the determination of lead from tap water using modified electrodes based on poly(**L2352**);

Chapter 12. Stochastic microsensors based on **L2352** azulene.

The PhD thesis ends with the conclusions chapter, in which all results are summarized and reevaluated in the general context; the original contributions are underlined and an outlook of the work is proposed.

Table 1. Structures of azulene derivatives

Compound	Structure	Compound	Structure
L2353		L 2370-2	
M539		L 2372	
M541		L 2363	
L 2349		L 2352	
M 540		L 2366-2	
M 538		L 2184-2	

II. ORIGINAL RESEARCH

CHAPTER 6.

EXPERIMENTAL DETAILS ON THE CARRIED OUT STUDIES

6.1. EXPERIMENTAL DETAILS IN ELECTROCHEMICAL DETERMINATION BY CV, DPV, RDE, CPE

All the other reagents used in voltammetric experiments were of analytical purity. Acetonitrile (Sigma Aldrich, electronic grade 99.999 % trace metals) and tetra-*n*-butylammonium perchlorate (Fluka puriss, electrochemical grade > 99 %) were used as solvent and supporting electrolyte, respectively, without further purification.

The electrochemical experiments were carried out by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and rotating disk electrode voltammetry (RDE), using a PGSTAT 12 AUTOLAB potentiostat to which a three-compartment cell was coupled. The working electrode was a glassy carbon disk (with a diameter of 3 mm). Its active surface was polished before each determination with diamond paste (0.25 μm) and cleaned with acetonitrile. $\text{Ag} / 10 \text{ mmol}\cdot\text{L}^{-1} \text{ AgNO}_3$ in $0.1 \text{ mol}\cdot\text{L}^{-1}$ tetra-*n*-butylammonium perchlorate

(TBAP) in acetonitrile (CH_3CN) was used as the reference electrode while a platinum wire (from ALS Co., Ltd., high purity, length 23 and 0.5 mm in diameter) was used as the auxiliary electrode [244].

Sodium acetate (Roth, 99.99%) and acetic acid (Fluka, >99.0%, trace select) were used for preparing acetate buffer solution. Lead(II) nitrate, $\text{Pb}(\text{NO}_3)_2$ (Sigma-Aldrich, >99.99% trace metal basis), cadmium(II) nitrate, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Sigma-Aldrich, $\geq 99.0\%$), copper(II) acetate, $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ (Fluka, $\geq 99.0\%$) and mercury(II) acetate, $\text{Hg}(\text{CH}_3\text{COO})_2$ (Sigma-Aldrich, $\geq 99.99\%$ trace metal basis) were used as received. Distilled water was obtained using a Millipore Direct – Q 3UV water purification system (18.2 MWcm). Electrochemical experiments involving metal ions analysis were performed in a three electrode cell in aqueous acetate buffer solution (0.1 M, pH=5.5), on glassy carbon disks or C/polyL modified electrodes, with a platinum wire as counter electrode, and Ag/AgCl, 3 M KCl as reference electrode. The experiments were conducted at 25°C under Ar atmosphere [245].



Fig. 6.1. Electrochemical cells used in electrochemical and metal detection

6.2. EXPERIMENTAL DETAILS IN SCANNING ELECTRON MICROSCOPE / ATOMIC FORCE MICROSCOPY DETERMINATION

The morphology and topography of the glassy carbon electrodes modified with polyL was observed by scanning electron microscopy (SEM) using a QUANTA INSPECT F with field emission gun scanning electron microscope with a resolution of 1.2 nm and A.P.E Research A100-SGS AFM, respectively [244, 245].

6.3. EXPERIMENTAL DETAILS IN ELECTROCHEMICAL QUARTZ CRYSTAL MICROBALANCE DETERMINATION

The electrochemical quartz crystal microbalance (EQCM) experiments were performed with a Metrohm Autolab EQCM, using 6 MHz piezoelectric crystal covered with Ti-backed gold layer (5.0 mm diameter), coupled to a PGSTAT 30 AUTOLAB potentiostat [245].

6.4. EXPERIMENTAL DETAILS IN ELECTROSPRAY IONIZATION MASS SPECTROMETRY DETERMINATION

Varian 310 – MS LC/MS/MS triple quadrupole mass spectrometer fitted with an electrospray ionization interface (ESI) was used for ESI-MS experiments. Air was the drying gas at a pressure of 19 psi and 508C. The nebulizer gas was nitrogen to 40 psi, and the needle voltage was established to the potential of 4500 V for negative ionization. The shield voltage was 600 V, the capillary voltage 40 V, and the detector voltage 900 V. The substances were dissolved in acetonitrile:water=1 : 1 and directly infused into the interface by a Harvard 11 PLUS pump, while the flow was set to 10 mL/min. Thus, the obtained deprotonated molecular ion was selected by the first quadrupole. The deprotonated molecular ion was

fragmented into the second quadrupole by collision with an inert gas (argon) to 1.5 mTorr pressure. The fragments were analyzed by the third quadrupole. The tuning of the mass spectrometer using PPG both for positive and negative was performed prior to these experiments [245].

6.5. EXPERIMENTAL DETAILS IN STOCHASTIC DETERMINATION

Graphite powder and nitric acid were supplied by Sigma Aldrich, and paraffin oil by Fluka (Buchs, Sweden). Deionized water used to prepare all the solution has been obtained from the Millipore Direct-Q 3 System (Mosheim, France). Metal cations' salts were purchased from Sigma Aldrich. Working standards metallic cations' solutions, having concentrations between 10^{-3} and 10^{-15} mol/ L were prepared using the successive dilutions method in a solution containing: 50 mL HNO_3 65% + 1000 mL H_2O , pH = 0.61.

All measurements were recorded using a PGSTAT 302 potentiostat/galvanostat (Metrohm) connected to a three-electrode cell, and linked to a computer via an Eco Chemie (Utrecht, The Netherlands) software version 4.9. Ag/AgCl electrode served as reference electrode, platinum electrode served as auxiliary electrode and the new developed stochastic sensors served as working electrode in the electrochemical cell. [246].

CHAPTER 7

ELECTROCHEMICAL CHARACTERIZATION OF SEVERAL AZULENE DERIVATIVES

7.3. ELECTROCHEMICAL CHARACTERIZATION OF (2E)-2-(AZULEN-1-YLMETHYLIDENE) HYDRAZINECARBOTHIOAMIDE (**L2352**)

The electrochemical behaviour of the ligand (2E)-2-(azulen-1-yl-methylidene)hydrazinecarbothioamide (**L2352**) has been investigated by two complementary methods: DPV and CV (Figs. 7.13 și 7. 14).

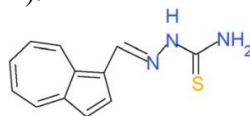


Fig. 7.12. Structura unui monomer azulena-tiosemicarbazona (**L2352**)

Fig. 1 shows the DPV curves obtained for L at different concentrations. Both anodic and cathodic curves are shown. The main anodic peaks are situated at: 0.39 V (a1), 0.70 V (a2) and 0.9 V (a3). Two reduction peaks can be seen in the cathodic region at: -1.79 V (c1) and -2.78 V (c2). The same processes have been identified from the CV curves (Fig. 2) with peaks at: 0.33 V (a1), 0.76 V (a2), 0.99 V (a3), and -1.84 V (c1), -2.88 V (c2), respectively. The inset from Fig. 7.13 and 7.14 shows the linear dependences of the peak currents on **L2352** concentration, as obtained from DPV and CV experiments, respectively. Their equations are given in Table 7.4. The linear dependences of the peaks currents could be used in analytical determination of **L2352** by these electrochemical methods. The slope of a1 peak current (in mA) vs. L concentration (in mM) is smaller (~18) than the absolute slope for c1 (~27). This means that a fast chemical reaction occurs, immediately after the first oxidation step.

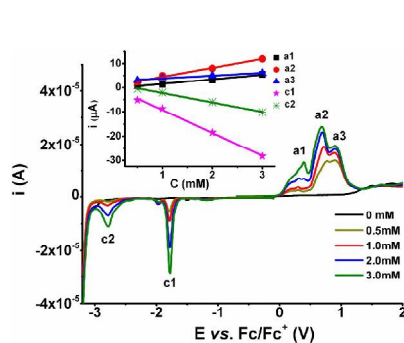


Fig. 7.13 DPV curves for L at different concentrations (mM) on glassy carbon electrode (3 mm in diameter) in 0.1 M TBAP, CH₃CN. Inset: linear dependences of the recorded peak currents (i) on **L2352** concentration (C).

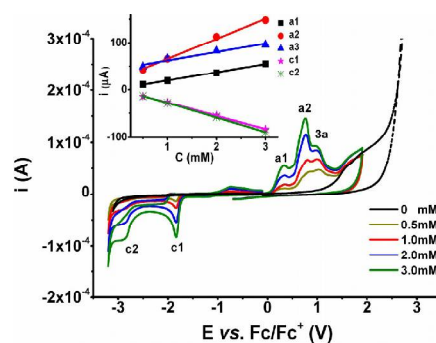
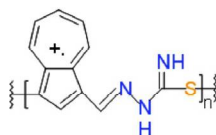


Fig. 7.14 CV curves on glassy carbon electrode (3mm in diameter) at different concentrations (mM) of **L2352** in 0.1 M TBAP, CH₃CN, $v=0.1$ V/s. Inset: Linear dependences of the peak currents (i) on **L2352** concentration (C); total currents are represented.

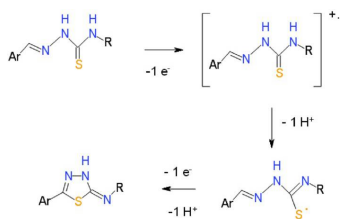
Table 7.4. Equations of linear dependences of the peak current (i in mA) on **L2352** concentration (C in mM) from CV and DPV experiments

Method	CV		DPV	
	Equation of a straight line	Correlation coefficient	Equation of a straight line	Correlation coefficient
a1	$i = 1,86 + 17,58 \cdot C$	$R^2 = 0,998$	$i = -0,4 + 1,89 \cdot C$	$R^2 = 0,990$
a2	$i = 23,13 + 42,56 \cdot C$	$R^2 = 0,992$	$i = 0,67 + 3,77 \cdot C$	$R^2 = 0,990$
a3	$i = 44,08 + 18,10 \cdot C$	$R^2 = 0,946$	$i = 2,62 + 1,15 \cdot C$	$R^2 = 0,970$
c1	$i = -0,1 - 27,48 \cdot C$	$R^2 = 0,999$	$i = 0,16 - 9,4 \cdot C$	$R^2 = 0,996$
c2	$i = 1,48 - 30,06 \cdot C$	$R^2 = 0,999$	$i = 1,56 - 3,85 \cdot C$	$R^2 = 0,996$

Assigning of anodic peaks reported in voltammetric curves (a1, a2, a3) is hard to be done because both azulene and thiosemicarbazide moieties oxidize easily, at close potentials. This fact makes oxidation to become non-selective. By losing 1e⁻ many intermediates could be formed. Radical cation intermediates resulting from oxidations can combine each other at different ends leading to polymer films with undefined structure. One option could involve the azulenic moiety, as in the limiting structure given below:

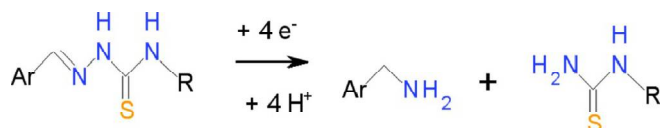


The redox potentials of azulene compounds are influenced by the nature of the substituent. The aryls such as phenyl, halophenyl, etc. stabilize the thiosemicarbazone combination, while the electrodonor groups (EDGs) make these compounds more prone to oxidation. In the last case, the oxidation is less selective (Scheme 7.1)[246].



Scheme 7.1.

The azulene derivatives are similar to phenyl moieties substituted with EDGs, and thiosemicarbazone group oxidation interferes with azulene moiety oxidation, leading to polymeric materials, as in the case of strong electron donor phenyls, like dimethylaminophenyl. Undoubtedly, all the electron transfers are irreversible. Assigning the cathodic peaks reported in voltammetric curves (c1, c2) is easier. The first peak can be attributed to the reduction of the thiosemicarbazide moiety, which is similar to that for other thiosemicarbazone aryl derivatives. It is known that four electrons are accepted at neutral pH, and reduction of C=N and NN bonds occurs in the first stage (peak c1) [250].



It is possible that the first cathodic process (c1) corresponds to the reduction of **L2352** to 1-azulenylmethylamine, a very low stable compound. Taking into account the high number of transferred electrons the reduction is also irreversible. This could be reduced further (c2) with the destruction of azulene structure.

The reversibility of the first anodic and cathodic processes has been carefully evaluated from the CV curves obtained in different domains of scanning. No reversible response can be seen for both anodic and cathodic peaks in the reverse scan (Fig. 7.15).

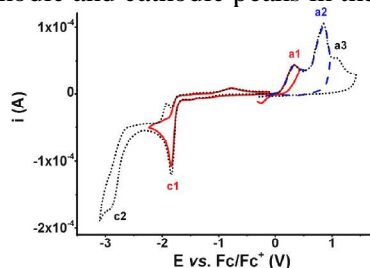


Fig. 7.15. CV curves on different cathodic and anodic scans at 0.1 V/s on glassy carbon electrode (3 mm in diameter) in 2 mM **L2352**, 0.1 M TBAP, CH₃CN

The peak currents from the CV curves recorded at different scan rates (Fig. 7.16) have been plotted vs. the square root of the scan rate. Linear dependences have been obtained (Fig. 16, inset) showing diffusion controlled processes for **L2352**. The peaks a1 and c1 have similar absolute slopes of around 315 mA (V/s)^{-1/2} (mM)⁻¹. In the range of investigated scan rates, both processes are irreversible. Figure 7.16 shows that, in the reverse scan, c1 has a response peak c1' which has a smaller dependence on the scan rate than c1. This is an indication for that c1 is in fact an ECE process [245].

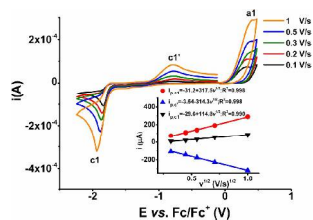


Fig. 7.16. CV curves at different scan rates on glassy carbon (3mm in diameter) in the potential range of the first cathodic and anodic peaks for 2 mM solution of **L2352** in 0.1 M TBAP, CH₃CN. Inset: Dependences of the peak currents on the square root of the scan rate and their equations

CHAPTER 8

MODIFIED ELECTRODES BASED ON AZULENE DERIVATIVES

The main interest of this research was to obtain modified electrodes based on azulene for metal ions complexation. Therefore, the electrochemical polymerization of **L2352** was studied in detail. Glassy carbon electrodes modified with poly**L2352** films (further called C/poly**L2352**) were obtained from millimolar solution of **L2352** in acetonitrile solution containing 0.1 M TBAP as supporting electrolyte, by successive scanning or controlled potential electrolysis (CPE) [245].

8.3. MODIFIED ELECTRODES BASED ON POLY(**L2352**)

Figure 8.9 shows the CV curves of glassy carbon electrode recorded during successive potential scans, in millimolar solutions of **L2352** in 0.1 M TBAP, CH₃CN. When the potential was scanned with an anodic limit situated in the domain of the first peak a1 (Fig. 8.9A) the potential of the first peak is slowly shifting as the number of cycles is increasing. This indicates that the electrode surface is covered stepwise with an insulating film. Indeed, a light brown polymer can be seen on the electrode surface at the end of scanning.

When the upper limit of scanning is situated in the oxidation domain of the processes a2-a3 (Fig. 8.9B), the shift of the first peak potential a1 is more pronounced, and blue films have been obtained at the end of scanning. As the upper potential limit of scanning is increased, the change in the electrochemical response between the cycles is more evident (Fig. 5C). The peak currents are dramatically diminished after only 2 scans (Fig. 8.9D) if the upper limit is increased after the a3 peak potential. That means the electrode is covered faster by a non-conductive polymeric film.

In Figs. 8.9B–D, the currents have important decreases between successive cycles, indicating that the deposited film is rather insulating, unlike the highly effective layer by layer deposition, found in the case of other azulene based conducting polymers.

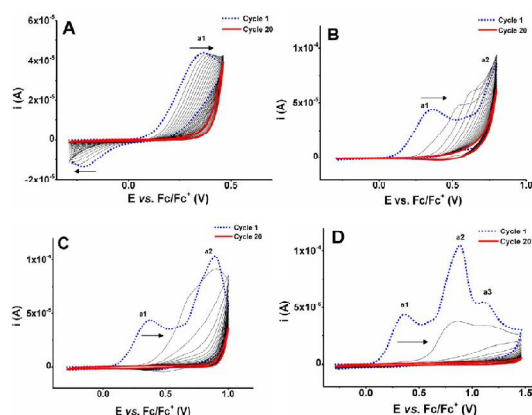


Fig. 8.9. Successive cyclic voltammograms (0.1 V/s) on glassy carbon disc electrode (3 mm diameter) during the oxidative electropolymerization of **L** (2 mM in CH₃CN+0.1 M TBAP) by cycling the potential between 0.25 V and different limits of anodic scanning (A) +0.46 V; (B) +0.8 V; (C) +1.0 V and (D) +1.46 V.

As it is difficult to control the amount of charge used for electropolymerization by scanning and, consequently, the thickness of the polymer layer, the electrochemical polymerization of the **L2352** monomer was achieved by controlled potential electrolysis (CPE). This was performed in 2mM **L2352** acetonitrile solution containing 0.1 M TBAP. The electropolymerization potentials were chosen within the characteristic oxidation potential domains of **L2352**. Because poly**L2352** does not show any electrochemical activity, the

evidence for electrode modification was obtained by redox assay in ferrocene solutions (1mM) in 0.1 M TBAP, CH₃CN. Figure 8.10 shows the ferrocene CV curves on the modified electrodes obtained by CPE (at different potentials). It can be noticed that the ferrocene signal is significantly altered by the presence of polymer film on the electrode surface. The films obtained by CPE at the smallest positive potential gave the smallest change in the ferrocene anodic peak current and potential, while those obtained at higher potentials are very much changed proving that the films are insulating. Thus, the charge transfer process of ferrocene species on polyL2352 modified electrodes depends on the applied potential in CPE, indicating a migration control through the polymer film.

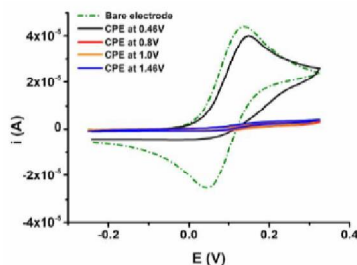


Fig. 8.10. CV curves (0.1 V/s) in 1mM ferrocene solution in 0.1 M TBAP, CH₃CN for the bare electrode (dashed line) and for the modified electrodes obtained by CPE at different potentials using polymerization charges of 1mC (films were obtained on glassy carbon electrodes with 3 mm in diameter in 2 mM solutions of L2352 in 0.1 M TBAP, CH₃CN).

When using higher polymerization charges (for increased thicknesses of the polymer film), there is a slower solute transport across the film, and consequently, a decrease of ferrocene direct oxidation (Fig. 8.11). Thus, upon increasing the thickness of the film, the ferrocene current signals are much altered, in comparison with the bare glassy carbon electrode signal (Fig. 8.11, inset). Thus, in order to reach faster rate constants for substrate and electrons, it is necessary to decrease the polymer film thickness in order to have a more open film structure, as it results from the previously shown studies [245].

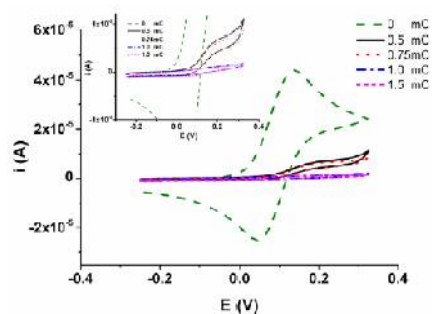


Fig. 8.11. CV curves (0.1 V/s) in 1mM ferrocene solution in 0.1 M TBAP, CH₃CN for the bare electrode (dashed line) and for C/polyL2352 modified electrodes obtained by CPE at different charges (the films were obtained at the polymerization potential of 1 V on glassy carbon electrodes with 3 mm in diameter in 2 mM solutions of L2352 in 0.1 M TBAP, CH₃CN). Inset: detail of the modified electrodes curves.

CHAPTER 9

FILMS CHARACTERIZATION BASED ON POLYAZULENE

9.1. POLYAZULENE FILMS CHARACTERIZATION BY SEM/AFM

SEM experiments were carried out on polyL modified electrodes in order to see if the polymerization conditions (potential or charge) influence the morphology of polyL2352

films. AFM topography studies were also performed in order to check the films roughness and thickness (Table 9.1). [245].

9.1.2. Poly(L2352) films characterization

The films were grown by CPE at different potentials (3 mC polymerization charge), or using different amounts of electric charge at 1.0 V in **L2352** solutions (2 mM) in 0.1 M TBAP, CH₃CN on glassy carbon disks (6 mm in diameter).

The morphological appearance of the polymer films obtained at different polymerization potentials indicates a continuous coverage of the electrodes (see Fig. 9.2). However, in terms of surface roughness (*Rs*), the smallest value (*Rs*=1.0 nm) is obtained at the oxidation potential of 1.46 V. This is probably due to the overoxidation of the polyL film which occurs at this potential. At 1.0 V, the polymer film presents the highest *Rs* of 2.4 nm and for the film obtained at 0.46 V *Rs* is 1.8 nm (see also insets in Fig. S1 A–C from supplementary material).

Table 9.1. Roughness average of the modified electrodes surfaces (*Rs*) and thickness of the film (*d*) for polyL films obtained at different potentials and amounts of electric charge

Potential for CPE (V)	Q (mC)	<i>Rs</i> (nm)	<i>δ</i> (nm)
0,46	3	1,8	20
1	3	2,4	100
1,46	3	1,0	60
1	2	2,5	60
1	1	2,7	35
1	0,7	2,8	25

Table 2 shows the influence of polymerization charge (which determines the polyL film thickness) on the polymer morphology. As the amount of electric charge is increasing as the films is thicker. The electrode coverage with polymeric films is continuous, without pinholes, cracks or evident inhomogeneities at all studied charges (see Fig. 9.3 A–D from supplementary material). The surface becomes rougher (from 2.4 nm to 2.8 nm) when the thickness of the polymer film is decreasing, as it can be seen when examining in detail the AFM images (see also insets in Fig. 9.3 A–D) [245].

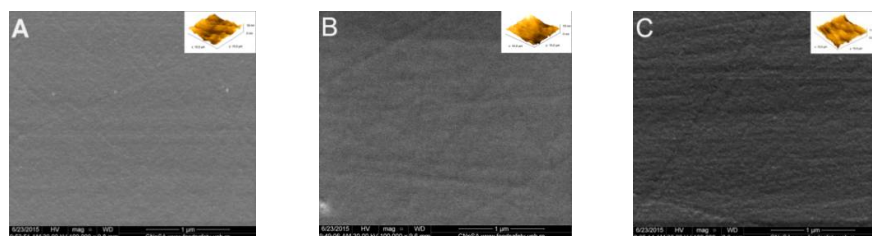


Fig. 9.2. SEM images of the modified electrodes obtained by CPE at: (A) +0.46 V, (B) +1.00 V and (C) 1.46 V. The films were obtained in 2mM solutions of L in 0.1M TBAP, CH₃CN on glassy carbon disks (6 mm in diameter) using polymerization charges of 3mC. Insets: corresponding AFM images

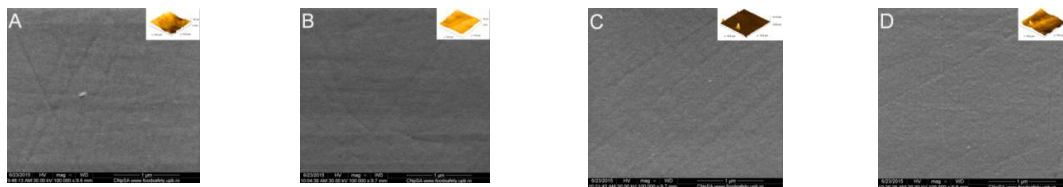


Fig. 9.3. SEM images of the modified electrodes obtained by CPE at 1.00 V. The films were obtained in 2mM solutions of L in 0.1M TBAP, CH₃CN on glassy carbon disks (6 mm in diameter) using different polymerization charges: (A) 3mC; (B) 2mC; (C) 1mC and (D) 0.7mC. Insets: corresponding AFM images

9.2. POLY(L2352) FILMS CHARACTERIZATION BY EQSM

The coverage of glassy carbon electrodes was further evidenced through EQCM experiments at constant potential. The amount of polyL film deposited on the glassy carbon electrode has been measured from the mass change per unit area, by recording the changes in resonant frequency of a quartz crystal oscillator. Figure 9.17 shows the current decay during the electrochemical deposition of polyL film, performed by CPE. The mass change resulted from EQCM experiments is given by Sauerbrey equation (1):

$$\Delta f = - \frac{2f_0^2}{A\sqrt{\rho_q\mu_q}} \Delta m$$

In (1), Δf is the quartz crystal frequency change (Hz), f_0 is the nominal resonant frequency of the quartz crystal (MHz), Δm is the quartz crystal mass change (g/cm^2), A is the crystal area (cm^2), ρ_q is the quartz density (g/cm^3), and μ_q ($\text{g}/\text{cm}^2 \text{ s}^2$) is the quartz shear modulus. For a 6 MHz crystal, the equation (1) can be reduced to (2), where Y_f is $0.0815 \text{ Hz}/\text{ng}/\text{cm}^2$.

$$-\Delta f = \Delta \cdot m \cdot \Psi_f \quad (9.1)$$

Figure 9.17A shows the chronoamperometric curve recorded during the polymerization of **L2352** monomer (2 mM in 0.1 M TBAP, CH_3CN solution) at +1.04 V. The chronoamperogram (Figure 9, inset) presents two characteristic domains: i) the current jump (zone I), which is attributed to the double layer charge and monomer oxidation processes, ii) the nucleation phenomena which occurs simultaneous with continuous and gradual polymer growth (zone II). The corresponding frequency changes during electropolymerization process are shown in Fig. 9.17 B. The frequency decreased in time, while the mass deposited onto the electrode correspondingly increased. After about 90 s, the slope of frequency Δf changes to a lower value and a limit of about 2000 Hz is attained (Fig. 9.17 B). In these conditions, an amount of polyL film $\Delta m = 24.5 \text{ mg}/\text{cm}^2$ results from (2). The overall mass variation in CPE experiments by EQCM is due to the deposition of polymer, but also due to the coordination of surface gold species. Thus, the mass obtained on gold electrode is higher than the one on glassy carbon electrode, so the amount of poly**L2352** deposited at +1 V is less than $24 \text{ mg}/\text{cm}^2$ [245].

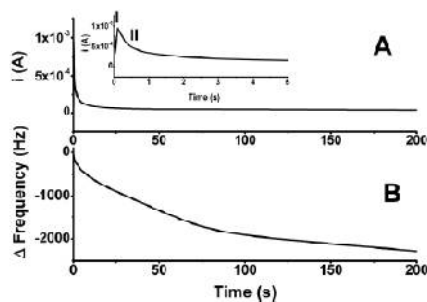


Fig. 9.17. Current decay during the electrochemical polymerization process driven in 2 mM L in 0.1 M TBAP, CH_3CN by CPE at +1 V (A) and the simultaneously obtained EQCM curve (B) on piezoelectric crystal covered with Ti-backed gold layer (5.0 mm diameter).

9.3. POLY(L2352) FILMS CHARACTERIZATION BY ESI-MS

The ability of **L2352** monomer to complex $\text{Pb}(\text{II})$ and $\text{Cd}(\text{II})$ ions was evidenced by mass spectrometry/electrospray (ESI-MS). ESI-MS spectra were recorded for equimolar aqueous solutions of $\text{Pb}(\text{II})$ ions and L (10^{-3} mol/L in 1 : 1 $\text{CH}_3\text{CN} : \text{H}_2\text{O}$). The spectra have

shown signals attributed to $[(\mathbf{L2352})_2\text{Pb}]$ ($m/z=665.1$ (100%)) (Fig.9.18). In case of Cd(II) ions, the ESI-MS signals correspond to the formation of 2 : 1 and 3 : 1 complexes between **L2352** and Cd(II) ions (peaks at $m/z=571.0$ (100%) and $m/z=800.0$ (100%)) have been attributed (Figs. 9.19 and 9.20). These results clearly highlight the efficient metal binding properties of the azulene monomer **L** toward Pb(II) and Cd(II) ions, in agreement with voltammetric experiments in homogenous phase. [245].

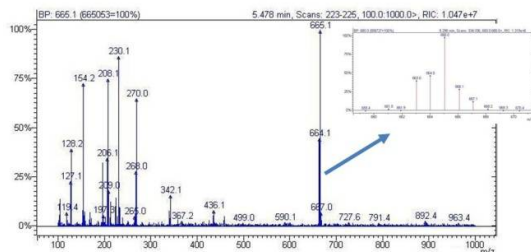


Fig. 9.18. Experimental ES-MS spectra of $[\text{PbL2352}]_2$. Inset: detail of spectra which correspond to $[\text{PbL2352}]_2$ complex.

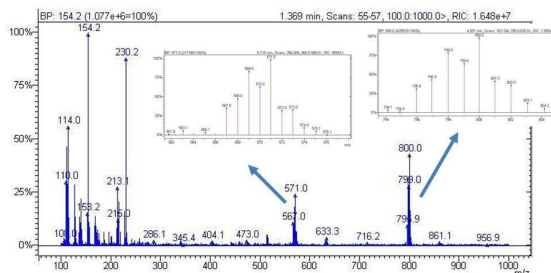


Fig. 9.19. Experimental ES-MS spectra of $[\text{CdL2352}]_2$ and $[\text{CdL2352}]_3$. Inset: detail of spectra which correspond to $[\text{CdL2352}]_2$ and $[\text{CdL2352}]_3$ complexes

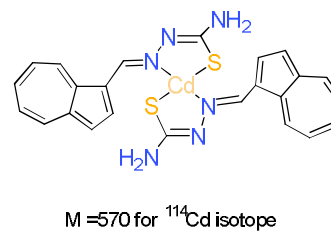
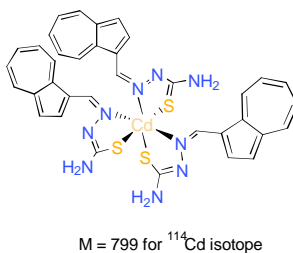
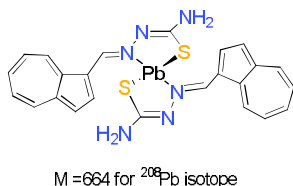


Fig. 9.20. Possible coordination structures for $[\text{PbL2352}]_2$, $[\text{CdL2352}]_2$ and $[\text{CdL2352}]_3$, respectively complexes; H atoms for the amino group are omitted for the reason of clarity

CHAPTER 10

HEAVY METALS RECOGNITION USING MODIFIED ELECTRODES BASED ON POLYAZULENE

Metoda de preconcentrare chimică–stripping anodic a fost utilizată pentru investigarea proprietăților de complexare a electrozilor poli. Ei au fost obținuți din soluții la diferite concentrații în 0,1 M TBAP/ CH_3CN prin electroliză la potențial controlat (CPE). Electrozii au fost imersați în soluție apoasă conținând un amestec de ioni de Pb (II), Cd (II), Cu (II) și Hg (II) (la diferite concentrații), proveniți din azotat de plumb (II), $\text{Pb}(\text{NO}_3)_2$ (Sigma-Aldrich, puritate $\geq 99,99\%$), nitrat de cadmiu (II), $\text{Cd}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$ (Sigma-Aldrich, $\geq 99,0\%$), acetat de cupru (II), $\text{Cu}(\text{CH}_3\text{COO})_2 \times \text{H}_2\text{O}$ (Fluka, $\geq 99,0\%$) și acetat de mercur (II) $\text{Hg}(\text{CH}_3\text{COO})_2$ (Sigma-Aldrich, puritate $\geq 99,99\%$), conducând la complexarea de ioni metalici în polymerul complexant (acumulare chimică în circuit deschis). Apoi, electrodul modificat (clătit) a fost transferat într-o soluție de tampon acetat fără metal. Ioni metalici acumulați au fost reduși la metale zero valente (prin aplicarea unui potențial negativ - suficient pentru a reduce toți ioni metalici complexați), iar curenții de stripping (redizolvare) corespunzători au fost înregistrați folosind DPV (0,01 V/s). După fiecare determinare electrodul de carbon vitros utilizat pentru detecția de metale a fost curățat cu pastă diamantată [242].

10.2. COMPLEXING PROPERTIES OF POLY(L2352)

Thiourea like compounds could present a dual binding character for anions and cations [254]. Taking into account our interest in toxic and heavy metal analysis, the binding ability of L toward the heavy metal ions has been studied in homogeneous and heterogeneous manners. Pb(II), Cd(II), Cu(II) and Hg(II) ions have been successively the analytes in experiments with **L2352** (homogeneous complexation) or poly**L2352** modified electrodes (heterogeneous complexation).

10.2.1. Metal Binding Properties of C/polyL2352

Chemical preconcentration-anodic stripping method [15, 21] has been used to investigate the complexing properties of Cj polyL electrodes. They have been obtained from 2 mM of L in 0.1 M TBAP, CH₃CN solution at different polymerization potentials. Dipping Cj polyL electrodes (for 10 minutes) into aqueous 0.1 M acetate buffer solutions which contain a mixture of Pb(II), Cd(II), Cu(II) and Hg(II) ions (each of them having concentrations of 10⁻⁷ M) led to the complexation of metal ions within the complexing polymer (chemical accumulation at open circuit). Then, the rinsed modified electrode was transferred into metal-free acetate buffer solution. The collected metal ions were reduced to zerovalent metals (by applying a negative potential – enough to reduce all complexed metal ions), and the corresponding stripping currents were recorded using DPV. Typical stripping curves presented peaks only for Cd(II), Pb(II) and Cu(II) situated at around 0.76 V, 0.55 V and 0.35 V, respectively (vs. Ag/AgCl) (Fig. 10.3). The highest response was obtained for Pb(II) ions.

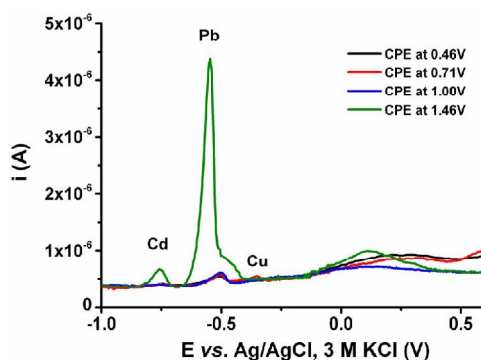


Fig. 10.3. DPV curves recorded at C/poly**L2352** modified electrodes (1 mC polymerization charge) obtained by CPE at various polymerization potentials; accumulation for 10 min in Cd(II), Pb(II), Cu(II) and Hg(II) each at 10⁻⁷ molL⁻¹ in acetate buffer at pH 5.5; reduction of complexed metal ions was performed in acetate buffer (pH 5.5) at -1.2 V for 1 min.

The polymerization potential used in EPC has been found to have a considerable influence on the stripping peak current intensity. As it can be observed in Fig. 10, the films obtained at +1.46 V led to higher responses for Cd and Pb (copper and mercury ions are not seen in these experimental conditions). This behaviour is in agreement with previous studies [66] and can be explained by the overoxidation of the polymeric film at this potential which leads to the increase of the polymer complexing sites (such as keto and carboxyl). The extra binding groups improve the affinity of the polymer for cations. Further experiments regarding the analytical properties of these modified electrodes are under study.

10.2.2. Metal Binding Properties of L2352

The metal ion binding properties of L have been investigated also in homogenous phase by electrochemical experiments with respect to Pb(II) and Cd(II) ions, respectively. The addition of one equivalent of **L2352** monomer into Pb(II) buffered solution has led to 100 mV

shift of the Pb(II) CV reduction peak to more negative potentials, from -0.57 to -0.67 V (Fig. 10.4). For Cd(II) ions, the shift of the reduction peak potential between free and complexed ions is higher (148 mV), from -0.928 V to -1.076 V (Fig. 12). In the reverse scans, the reduced Cd(II) and Pb(II) ions present anodic stripping peaks at about the same potentials of -0.68 V and -0.42 V for Cd(II) and Pb(II), respectively. For both ions, these stripping peaks are not affected by the complexation processes. [245].

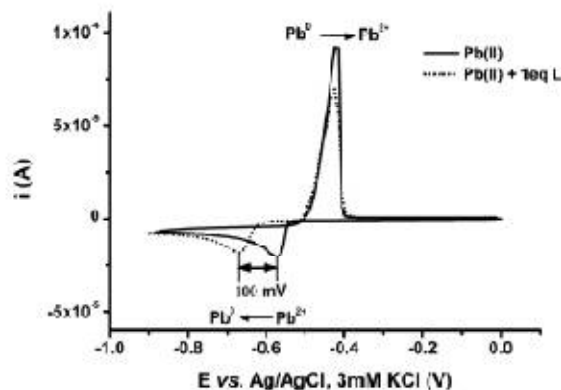


Fig. 10.4. Cyclic voltammograms (25 mV/s) at a bare carbon disk electrode (3 mm in diameter) recorded in acetate buffer (pH 5.5; 0.1 M) containing 10^{-4} M Pb(II) in the absence (solid line) and in the presence (dot line) of 1 eq. **L2352** monomer.

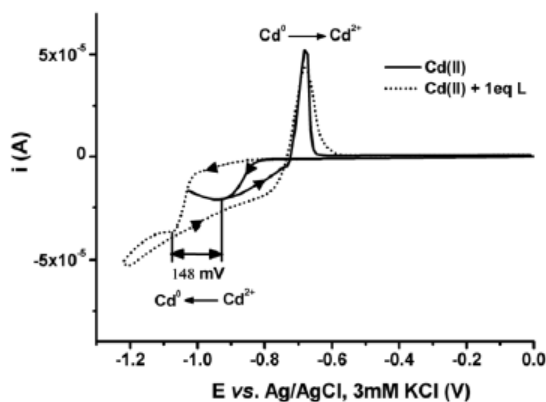


Fig. 10.5. Cyclic voltammograms (25 mV/s) at a bare carbon disk electrode (3 mm in diameter) recorded in acetate buffer (pH 5.5; 0.1 M) containing 10^{-4} M Cd(II) in the absence (solid line) and in the presence (dot line) of 1 eq. **L2352** monomer.

CHAPTER 11

OPTIMIZATION OF ANALYTICAL METHOD FOR THE DETERMINATION OF LEAD FROM TAP WATER USING MODIFIED ELECTRODES BASED ON POLY(L2352)

The electrochemical cell to obtain the film on the surface of the electrode was connected to an AUTOLAB PGSTAT 302N potentiostat.

To obtain the film on the electrode surface a cell with three electrodes was used:

- working electrode: vitreous carbon electrode (Metrohm, d = 3 mm);
- reference electrode: Ag 10⁻² M Ag⁺ in 0.1 M TBAP / CH₃CN;
- counter electrode: platinum wire;
- supporting electrolyte: 0.1 M TBAP / CH₃CN.

A substrate solution (azulene L 2352) of 1 mM (1 mmol / L) was prepared by dissolving the substrate in the support electrolyte (0.1 M TBAP, CH₃ CN). This solution is introduced into the electrochemical cell. The modified electrodes are obtained by potential-controlled electrolysis (CPE) of 1.7 volts with a load of 1 mC.

The previously prepared electrochemical modified electrode (ECM) is introduced into a cell containing an electrolyte buffer acetate buffer pH = 4 (or different pH buffer phosphate buffer, different pH buffer acetate buffer). In the same cell the reference electrode for the aqueous medium (Ag / AgCl) and the counter electrode is introduced. For ECM balancing in the aqueous environment, 15 cycles of -0.9 V ÷ + 0.6 V are performed, the sweep speed is 0.1 V / s. For over-oxidation of ECM in aqueous medium, 10 cycles are performed in the range - 0.2 V ÷ +1.6 V with a scanning speed of 0.1 V / s. For preconcentration, the over-oxidized modified electrode is introduced for 25 minutes (or different complexation times) into the solution containing Pb ions at different concentrations under magnetic stirring (light). After 25 minutes, remove the electrode from the solution, rinse it with distilled water, and insert it into the cell containing the electrolyte support for the aqueous medium. After each determination, the electrode was cleaned with diamond paste and rinsed with acetonitrile. The accumulated metal ions were reduced to zero valent metals (by applying a negative potential - enough to reduce all complex metal ions) and the corresponding stripping currents were recorded using DPV (0.02 V / s).

The determinations were carried out under an atmosphere of argon at 25 ° C.

11.1. OPTIMIZATION OF METHOD

Optimization of the method involves the experimental determination of some parameters, such as:

- reduction time;
- reduction potential;
- complexation time;
- determining the optimum pH of the acetate buffer solutions, phosphate buffer.

11.1.1. Reduction time

Experimental assays were performed at [Pb] = 10⁻⁸M by immersing the electrode for 10 minutes, keeping the complexation time constant and varying the time of reduction as follows: 60 seconds; 120 sec; 180 sec; 300 sec at the -1 V reduction potential, using as an electrolyte acetate buffer pH = 5.5.

Figure 11.1 (A and B) shows the DPV stripping curves for the concentration of 2.07 µg / L Pb (II) and the graphic representation of the peak current as a function of the reduction time.

According to the data processed and shown in Figure 11.1, the highest current was obtained at a reduction time of 120 seconds.

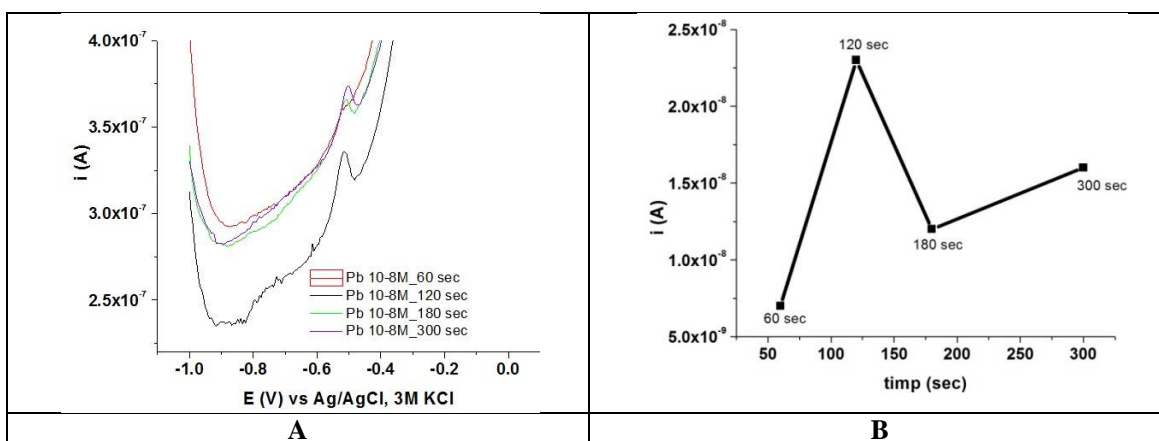


Fig. 11.1. Anodic stripping DPV curves recorded on polyL2352 modified electrodes at different reduction times with a -1 V reduction potential after immersion in 0.1 M acetate buffer pH = 5.5 (accumulation for 10 min) at the concentration of 10^{-8} M Pb (II) (A); graphical representation of stripping currents by reduction time (seconds) (B)

11.1.2. Reduction potential

Experimental tests were performed at the $[Pb] = 10^{-8}$ M concentration by immersing the electrode for 10 minutes while maintaining the complexation time, the reduction time of 120 seconds, the reduction potential varied as follows: -0.8 V; -0.9 V; -1.0 V; -1.1 V; -1.2 V; -1.4 V, using as electrolyte acetate buffer pH = 5.5.

According to the data processed and presented in figure 11.2 (A and B), the best response was obtained at the -1.0 V reduction potential.

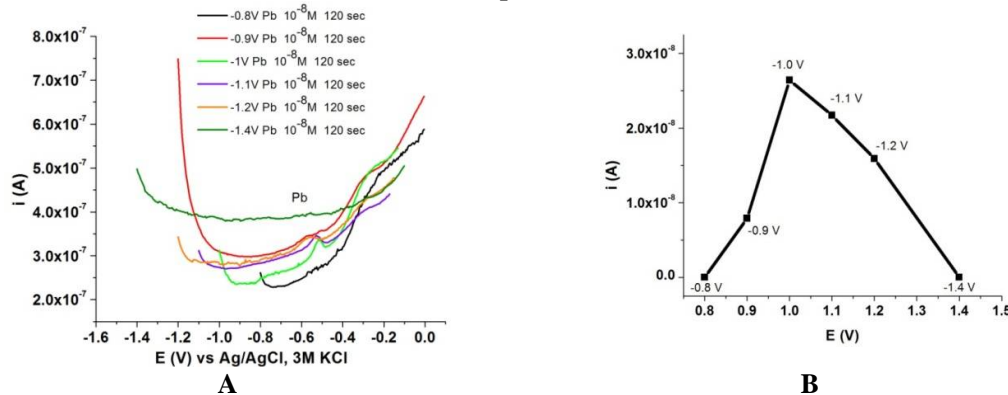


Fig. 11.2. Anodic stripping DPV curves recorded on modified electrodes with polyL2352 with different reduction potentials with a reduction time of 120 s after immersion in 0.1 M acetate buffer pH = 5.5 (accumulation for 10 min) at a concentration of 10^{-8} M Pb (II) (A); graphical representation of stripping currents according to the reduction potential (B)

11.1.3. Complexation time

Experimental tests were performed at the $[Pb] = 10^{-7}$ M concentration by immersing the variable time electrode (1; 3; 5; 7; 10; 12; 15; 20; 25; 30 minutes), keeping the reduction of 120 seconds, reduction potential of -1.0 V (figure 11.3 A and B). Pb solutions were prepared in ultrapure water. For better accuracy, the determinations for 15 minutes and 20 minutes were repeated, and for the graphical representation the mean values were used. The optimal complexing time was 20 minutes.

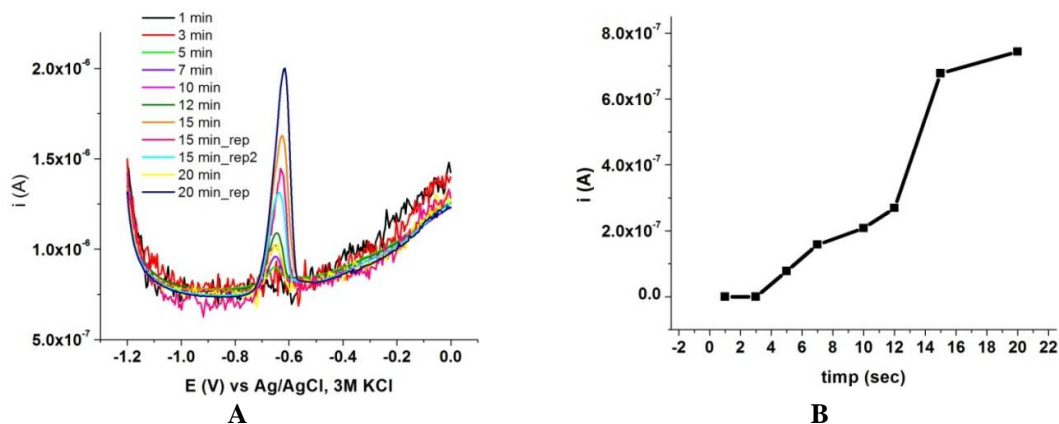


Fig. 11.3. DP curves Recorded on polyL2352 modified electrodes at different complexation times with a reduction time of 120 seconds and a -1 V reduction potential after immersion in 0.1 M acetate buffer pH = 5.5 at a concentration of 10^{-7} M Pb (II) (A); graphical representation of stripping currents by complexation time (seconds) (B)

For better precision, the determinations have been repeated, because better results can be obtained with a longer complexity time and a repeatability of better determinations. Figure 11.4 A and B show the stripping curves for different complexation times and graphical representation of stripping currents according to complexation time.

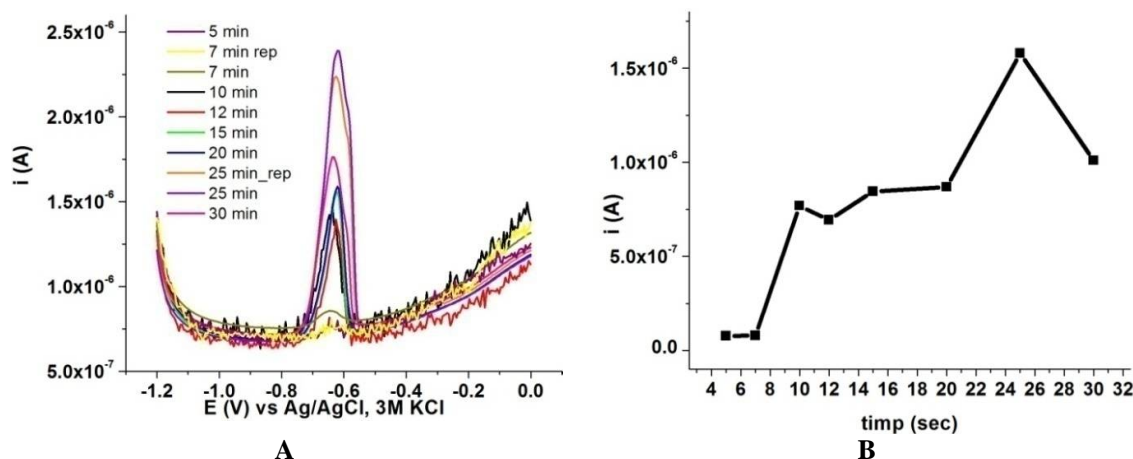


Fig. 11.4. Anodic stripping DPV curves recorded on polyL2352 modified electrodes at various complexation times (120 sec reduction time, -1 V reduction potential) after immersion in 0.1 M acetate buffer pH = 5.5 at the 10^{-7} M Pb (II) (A); graphical representation of stripping currents by complexation time (seconds) (B)

The electrode was immersed for 5 minutes, 7 minutes, 10 minutes, 12 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes in Pb aqueous solution at a concentration of 10^{-7} M. For better accuracy, repeat a few determinations, and for the graphical representation the average of the two values was used.

The optimal complexing time was 25 minutes, with good repeatability (Table 11.2).

Table 11.2. Values obtained for repeating the sample with a 25-minute complexation time

Nr det.	Complexation time	i (A)	E (mV)
1	25 min	$1,67 \cdot 10^{-6}$	-621
2	25 min	$1,49 \cdot 10^{-6}$	-621

11.1.4. pH for stripping

To optimize the support electrolyte, phosphate buffer and acetate buffer pHs were used. The Pb solution [10^{-7} M] was prepared from the 10^{-5} M solution in different pH phosms (5.00, 5.53, 6.01, 6.52, 7.02, 8). Also, a solution of Pb of 20.72 $\mu\text{g} / \text{L}$ in ultrapure water was also carried out.

For these determinations the optimal parameters were used:

- Reduction time: 120 sec;
- reduction potential: -1 V;
- complexing time: 25 min, light magnetic stirring in Pb 10^{-7} M solution in ultrapure water or in different phosphate buffer pHs.

The support electrolyte used for this determination was phosphate buffer with different pHs. Figure 11.5 (A and B) shows the anodic stripping curves for optimizing the support electrolyte and plotting the stripping currents according to the pH of the support electrolyte. It is noted that the best response was obtained at pH 6 using ultra-pure water matrix (a bit of blue).

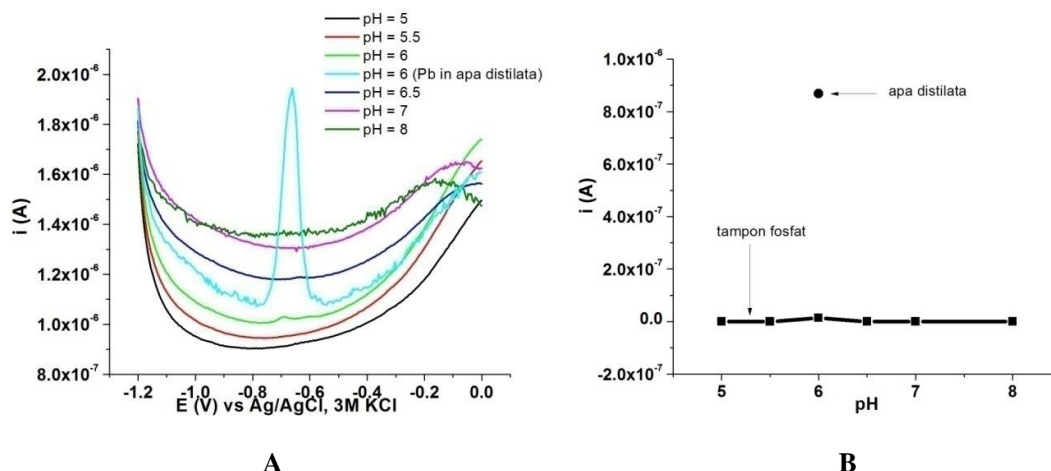


Fig. 11.4. Anodic stripping DPV curves recorded on modified electrodes with polyL2352 using different phosphate buffer phosphate electrolyte pHs (120 sec reduction time, -1 V reduction potential) (A); graphical representation of stripping currents in function of the pH of the support electrolyte (B)

The solution of 20.72 $\mu\text{g} / \text{L}$ Pb [10^{-7} M] was prepared from the 10^{-5} M solution in acetate buffer of different pHs (4.03, 4.5, 5.02, 5.51, 6.00 ; 6,50) as well as in ultrapure water. Figure 11.5 (A and B) shows the DPV curves of stripping currents using different acetate buffer pHs such as support electrolyte and optimal parameters (reduction time: 120 seconds, reduction potential: -1 V, complex time: 25 min, light magnetic stirring in Pb solution 10^{-7} M in ultrapure water or in different acetate buffer pHs).

From Figure 11.5 A and B, it is noted that the best response was obtained at pH 5.5, using ultra-pure water matrix (green bit).

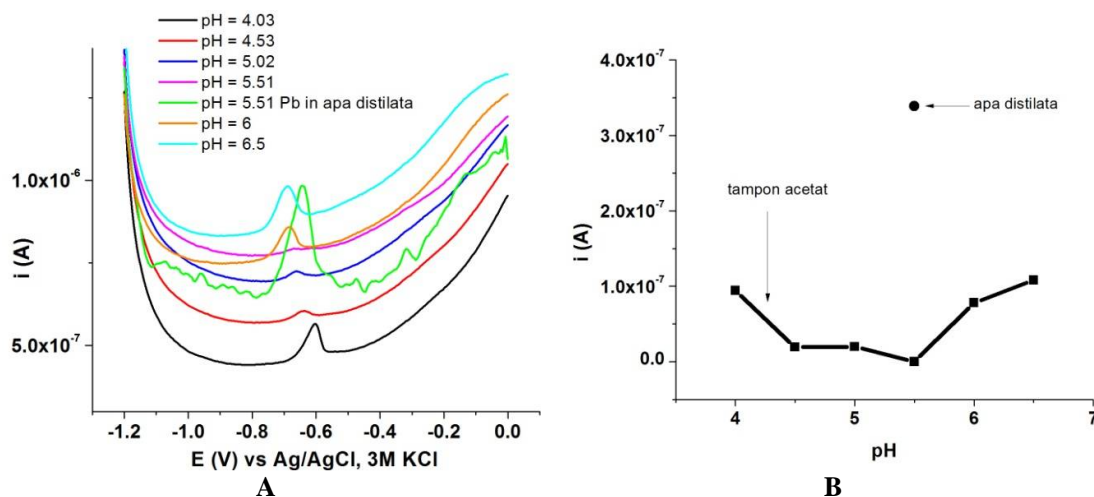


Fig. 11.5. Anodic stripping DPV curves recorded on modified electrodes with polyL2352 using different acetate acetate buffer pH (A) as electrolyte buffer; graphical representation of stripping currents in function of the pH of the support electrolyte (B)

The best response was obtained using ultra-pure water matrices. To optimize the pH for the support electrolyte the acetate buffer was used because a response to different pHs was obtained.

Figure 11.6 (A and B) shows the anodic stripping curves using different pH and ultrapure water matrices (Pb (II) 10⁻⁷ M as electrolyte buffer) and graphical representation of pH stripping currents of the support electrolyte.

For these determinations the optimal parameters were used:

- reduction time: 120 sec;
- reduction potential: -1 V;
- complexing time: 25 min, light magnetic stirring in Pb 10⁻⁷M solution in ultrapure water.

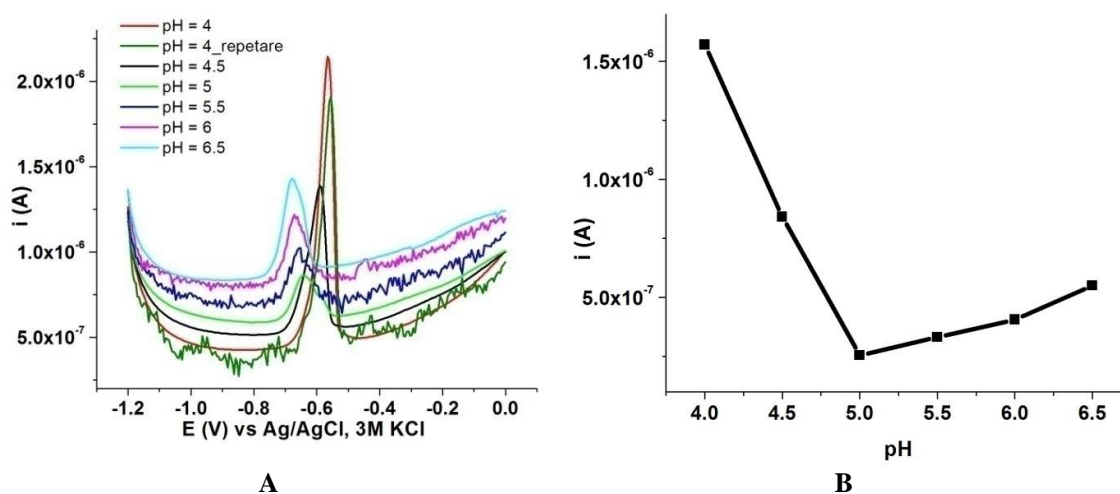


Fig. 11.5. Anodic stripping DPV curves recorded on modified electrodes with polyL2352 using different pH acetate (A) and 10⁻⁷ M Pb (II) matrix in ultrapure water as electrolyte buffer; graphical representation of stripping currents in function of the pH of the support electrolyte (B)

The best response was obtained for pH 4. For better precision, the determination for pH 4 was repeated, giving a similar response (Table 11.5). The mean of the two determinations was used for graphical representation.

Table 11.5. Values obtained for repeating the sample at pH 4 using acetate acetate buffer as electrolyte

Nr det.	pH	i (A)	E (mV)
1	4	$1.67 \cdot 10^{-6}$	-621
2	4	$1.46 \cdot 10^{-6}$	-621

11.2. ASSESSING THE ANALYTICAL PERFORMANCE OF PARAMETERS OF THE METHOD

limit of determination (LOD)	limit of quantification (LOQ)	linearity, domain and range	precision	repeatability	intermediate precision	accuracy/recovery
0,36 µg/L	1,20 µg/L	0.621 – 20.7 µg/L	s= 1,26 µg/L	s= 1,26 µg/L Repetabilitatea (r)= 3,54 µg/L RSDr = 12,55%	Xmediu(det.) = 11,02 µg/L s _R =1,83 µg/L Precizia intermediară (Ri)= 5,13 µg/L RSDR=16,61%	Xmediu (cu adaos) = 4,10 µg/L Recuperare = 82,00 % Acuratete = Xmediu – X adaugat = 5,0 – 4,1 = 0,9 µg/L

Final result:	3,33 µg/L	Final result:	12,17 µg/L
Compound uncertainty:	0,97 µg/L	Compound uncertainty:	3,09 µg/L
Extensive uncertainty (2*0.97):	1,94 µg/L	Extensive uncertainty:	6,18 µg/L

RsDr	12,55	%
RsDR	16,61	%
Uex	50,77	%

11.3. COMPARATIVE RESULTS FOR DETERMINATION OF Pb IN TAP WATER, SURFACE WATER

In the tests on real samples were used together with the optimized method three other methods of determination of Pb: inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption with graphite furnace (ETAAS), optical emission spectrometry with inductively coupled plasma using ultrasonic nebulizer (ICP-EOS-USN).

The calibration curves were in the 10 ÷ 50 µg / L range, the abscissa (y) representing in each case another size. Table 11.8 shows the abundance values (ICP-MS), absorbance (ETAAS), peak height (ICP-EOS-USN), and the intensities (electrochemical) currents.

To represent the four calibration curves on the same graph, the abscissa values were normalized, considering the value from 10 µg / L as 1 (y₁ / y₁), and the other values were obtained by dividing the y values to y₁. The values obtained for all four curves are shown in Table 11.9.

Table 11.8. Calibration curves (ICP OES, ICP-MS, AAS graphite, Electrochemical)

Concentration (µg/L)	ICP-MS Abundance	AAS graphite furnace absorbance	ICP-EOS-USN Peak hight	Electrochemical Current	Abscissay _i i=1÷5
10	91303	0,120	3480	7,36E-07	y ₁
20	171790	0,223	6761	1,5E-06	y ₂
30	244003	0,334	10306	2,18E-06	y ₃
40	338313	0,442	13982	2,92E-06	y ₄
50	436188	0,550	17451	3,65E-06	y ₅

Table 11.9. The slopes of the calibration curves

Concentration (µg/L)	ICP-MS	AAS graphite furnace	ICP-EOS-USN	Electrochemical
10	1,00	1,00	1,00	1,00
20	1,88	1,86	1,92	2,04
30	2,67	2,79	2,96	2,96
40	3,71	3,69	4,02	3,97
50	4,78	4,59	5,01	4,96

The graphical representation of the data in Table 11.9 is shown in Figure 11.7. It can be seen that the slope of the curves is not significantly different.

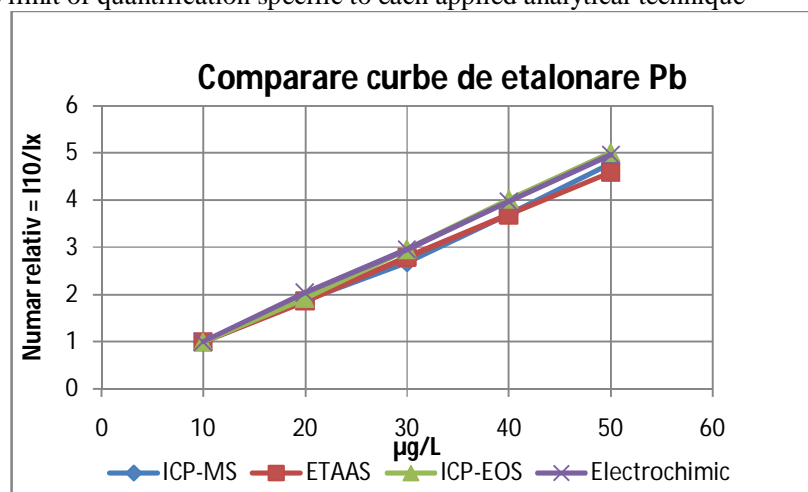
For the comparison of the results, a potable water sample was collected, the sample which was enriched with 1 µg / L (sample 1); with 3 µg / L (sample 2); with 4 µg / L (sample 3); with 5 µg / L (sample 4).

The samples were analyzed as such, the results obtained being reported in Table 11.10.

Table 11.10. Comparative Pb concentration in Pb enriched drinking water

Concentration µg/L	Blank Ap water	Sample 1	Sample 2	Sample 3	Sample 4
ICP-MS	<0,6*	0,94	2,75	3,76	4,40
AAS- graphite furnace	<1,0*	0,81	3,17	3,87	4,95
ICP-EOS USN	<0,5*	0,92	2,81	3,72	4,36
Electrochemical	< 1,2*	< 1,2	2,78	3,90	4,35

* The limit of quantification specific to each applied analytical technique

**Fig. 11.7.** Comparison of calibration curves used to determine Pb in drinking water

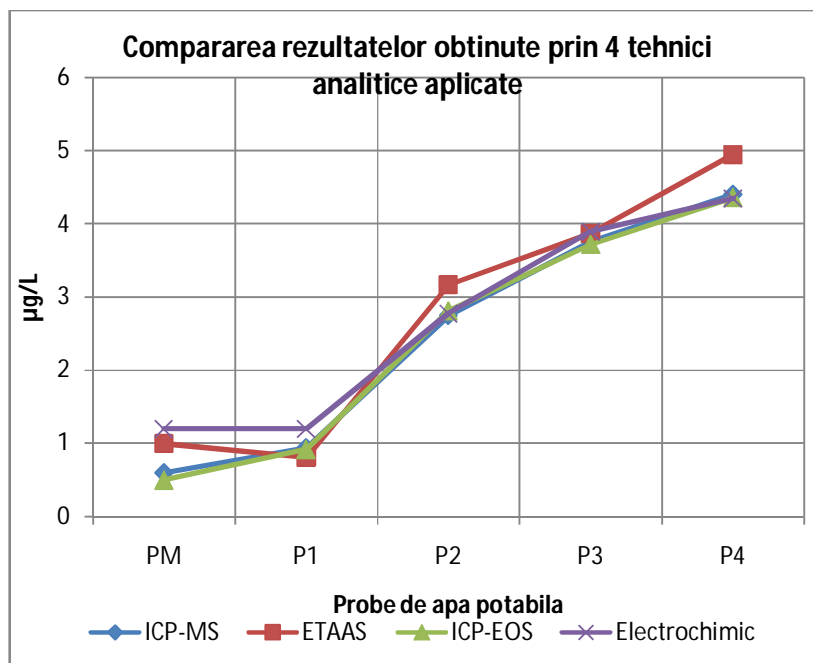


Fig. 11.8. Compare results obtained on Pb enriched drinking water samples

From Figure 11.8, it can be seen that the results obtained by the four applied analytical techniques are comparable.

Several real water samples were analyzed: surface water, drinking water, monitoring water drilling. The results obtained are presented in Table 11.11 and Table 11.12.

Table 11.11. Comparative Pb concentration in feed water

Concentratie µg/L	F1	F2	F3	F4	F5
ICP-MS	<0,6	<0,6	<0,6	<0,6	<0,6
AAS- graphite furnace	<1,0	<1,0	<1,0	<1,0	<1,0
ICP-EOS USN	<0,5	<0,5	<0,5	<0,5	<0,5
Electrochemical	<1,2	<1,2	<1,2	<1,2	<1,2

Table 11.12. Comparative Pb concentration results in surface water and drinking water

Concentratie µg/L	P1	P2	P3	P4	S1	S2	S3
ICP-MS	<0,6	<0,6	<0,6	<0,6	<0,6	<0,6	<0,6
AAS- graphite furnace	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0	<1,0
ICP-EOS USN	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Electrochemical	<1,2	<1,2	<1,2	<1,2	<1,2	<1,2	<1,2

P1 ÷ P4 - Drinking water from various sources;

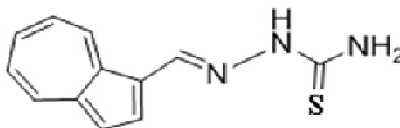
S1, S2, S3 - surface water Ilfov county and Bucharest.

The lead concentration of the 12 samples analyzed was in all cases below the quantification limit of the methods.

CHAPTER 12

STOCHASTIC MICROSENSORS BASED ON L2352 AZULENE

This chapter proposed a new reliable stochastic microsensor based on an azulene (able to provide the required pores/channels for stochastic sensing) derivative immobilized on graphite paste. Four metallic cations: Cu(II), Pb(II), Hg(II) and Cd(II) served as model analytes to prove the sensitivity and selective of the proposed microsensor. The metal cations were determined directly from nine waste water.



Structure of **L2352**

12.1. DESIGN OF THE MICROSENSOR

A solution of **L2352** (100 μ L solution 10^{-3} mol/L) was used as modifier for 100 mg graphite paste (obtained by mixing graphite powder with paraffin oil). The modified paste was pressed into a plastic tube with an internal diameter of 200 μ m. The length of the sensor was 1 cm. Electrical contact between the modified paste and external circuit was obtained using an Ag wire inserted into the modified paste. The surface of the microsensors was renewed by polishing with alumina paper. The microsensors were stored in a dry place, at room temperature.

12.2. STOCHASTIC MODE FOR HEAVY METALS DETERMINATION

All stochastic measurements were performed using chronoamperometry at a potential of 125 mV. Solutions of each metallic cation (Cu(II), Pb(II), Hg(II) and Cd(II)) with concentrations varying between 10^{-3} and 10^{-15} mol/L were measured, and equations of calibration were obtained for each cation using linear regression method. t_{off} and t_{on} values were measured for the diagrams obtained for qualitative and quantitative analysis, respectively of each metallic cation. The qualitative analysis of the cations was done based on their signature (t_{off} values, see Table 12.1 and Figure 12.1) identified in the diagram obtained using chronoamperometry. For quantitative analysis, the $1/t_{\text{on}}$ values (Figures 12.1, 12.2) were plotted against the concentration of each cation ($1/t_{\text{on}} = A + B \times \text{Conc}_{\text{cation}}$). The unknown concentrations of the metallic cations were determined by inserting the $1/t_{\text{on}}$ values obtained in the diagrams obtained for the sample measurements in the calibration graph obtained for each of the cations. All measurements were performed at 25°C.

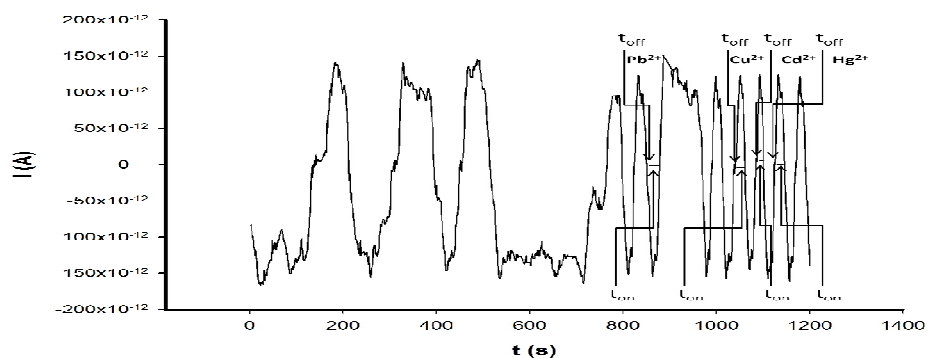


Fig. 12.1. Diagram obtained for the simultaneous assay of Cu(II), Cd(II), Pb(II), and Hg(II) in waste water

Table 12. 1. Response characteristics of the stochastic microsensor used for analysis of Cu^{2+} , Hg^{2+} , Cd^{2+} , and Pb^{2+}

Cation	t_{off} (s)	Equation of calibration and correlation coefficient *	Linear concentration range (mol/L)	Sensitivity ($\text{s}^{-1} \text{mol L}^{-1}$)	Limit of determination (mol/L)
Cu^{2+}	1.9	$1/t_{\text{on}} = 0.017 + 2.74 \times 10^6 \times C$ $r = 0.9995$	$10^{-10} - 10^{-8}$	2.74×10^6	1.00×10^{-10}
Hg^{2+}	2.7	$1/t_{\text{on}} = 0.045 + 2.47 \times 10^5 \times C$ $r = 0.9999$	$10^{-10} - 10^{-7}$	2.47×10^5	1.00×10^{-10}
Cd^{2+}	3.3	$1/t_{\text{on}} = 0.031 + 7.90 \times 10^6 \times C$ $r = 0.9936$	$10^{-11} - 10^{-9}$	7.90×10^6	1.00×10^{-11}
Pb^{2+}	2.2	$1/t_{\text{on}} = 0.033 + 1.82 \times 10^7 \times C$ $r = 0.9977$	$10^{-11} - 10^{-9}$	1.82×10^7	1.00×10^{-11}

* $1/t_{\text{on}} < \text{s}^{-1} >$ and $C < \text{mol/L} >$

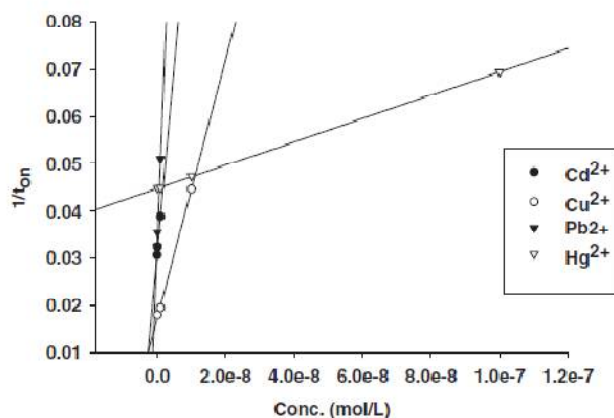


Fig. 12.2. Calibration graphs obtained when the stochastic sensor based on azulene was used for the assay of Cd(II), Cu(II), Pb(II), and Hg(II)

12.3. RESPONSE CHARACTERISTICS OF THE STOCHASTIC MICROSENSOR USED FOR THE SIMULTANEOUS ASSAY OF Cu(II), Cd(II), Hg(II), AND Pb(II)

Stochastic sensing is based on channel conductivity: the analyte in the first stage is going through the channel and blocking it (the time when the channel is blocked is called t_{off} and its value is giving the signature of the analyte); in the second stage the analyte is binding on the channel wall favorizing electrochemical processes, the time spend on this stage is called t_{on} and it is used for the quantitative analysis of the analyte, using the equation: $1/t_{on} =$

$$A + B \times \text{Conc}_{\text{cation}}$$

The process taking place inside the channel was: $\text{Ch}(i) + \text{C}(i) \rightleftharpoons \text{Ch} \cdot \text{C}(i)$ where Ch is the channel, C is the metallic cation, and i is the interface. The signatures (values of t_{off}) of the metallic cations determined using the proposed stochastic microsensor, are shown in Table 12.1. t_{on} values served to obtained the other response characteristics: linear concentration ranges, limits of determination, equation of calibration, and sensitivities. The calibration graphs are shown in Figure 12.2.

Limits of determination of 100 pmol/L were recorded for the assay of Cu(II), and Hg(II), while limits of determination of 10 pmol/L were recorded for the assay of Cd(II), and Pb(II). High sensitivities were recorded for all four cations analysis. The differences in the signatures recorded for the four cations made possible their simultaneous assay, showing the selectivity of the sensor. Other metals tested gave different signatures, proving the selectivity of the sensor over other metallic ions. The proposed microsensor is reproducible, and reliable. Five different pastes were prepared accordingly with the proposed design, and tested for a period of 30 days. The RSD(%) values recorded for the sensitivity in between the sensors based on graphite paste, and for each of them when used every day for a period of 30 days were lower than 1.00%.

12.4. PATTERN RECOGNITION OF Cu(II), Cd(II), Hg(II), AND Pb(II) IN WASTE WATER SAMPLES

The main analytical application of the new stochastic microsensor is for the pattern recognition of Cu(II), Cd(II), Hg(II), and Pb(II) in waste water samples. No special processing of the water samples is needed when the stochastic microsensor, because its response is independent on the complexity of the matrix. Therefore, the wastewater samples were used as taken by point samples from the National Institute of Research and Development for Industrial Ecology (INCD ECOIND), without any sampling steps performed – for the proposed method of analysis. Their analysis with ICP for Cu(II), Cd(II), and Pb(II) and with atomic fluorescence spectroscopy for Hg(II). Recovery tests of each cation in the presence of the others were performed, when the following results were recorded for: Cu(II) $98.72 \pm 0.07\%$; Cd(II) $98.99 \pm 0.05\%$, Pb(II) $99.00 \pm 0.05\%$, and Hg(II) $99.02 \pm 0.04\%$. These results proved that the divalent cations can be determined one in the presence of the others using the proposed stochastic sensor. The results obtained for the pattern recognition in nine water samples are shown in Table 12.2. There is a good correlation between the results obtained using the proposed microsensor and the ICP method for the assay of Cu(II), Cd(II), and Pb(II). The Hg(II) concentration was too low in the water samples, and therefore it was not possible to determine it using the conventional method – fluorescence spectroscopy. The RSD(%) values proved the reliability of the measurements [246].

A new stochastic microsensor based on an azulene derivative was designed. Metallic cations: Cu(II), Cd(II), Pb(II), and Hg(II) were used as model analytes. The microsensor exhibited good response characteristics for the assay of these cations: low limits of determination (10, and 100 pmol/L), high sensitivity, and high selectivity (given by the differences in the signatures of the cations). The pattern recognition test performed for nine samples proved that there is a good correlation between data obtained using standard methods

and the new method of analysis. The sensing mechanism of the stochastic sensor proposed in this paper, increased the selectivity, sensitivity, and the overall reliability of this type of analysis. Compare with other types of analysis such as stripping analysis, when the redox potentials (dependent on the composition of the matrix) may be used for the qualitative analysis, utilization of the pores, and the signatures of the metals increased the reliability of the qualitative analysis, the matrix not influencing in any way the qualitative and also the quantitative analysis [246].

Table 12.2. Determination of Cu^{2+} , Hg^{2+} , Cd^{2+} , and Pb^{2+} in waste water samples

Sample Nr.	Method used	Metal cations (MC)			
		Cu^{2+} (mg/L)	$\text{Hg}^{2+,\diamond}$ (mg/L)	Cd^{2+} (mg/L)	Pb^{2+} (mg/L)
1	ICP*	0.195	–**	0.033	0.013
	Stocsens	0.193±0.002	0.0027±0.0003	0.036±0.004	0.013±0.002
2	ICP*	0.319	–**	0.045	0.036
	Stocsens	0.318±0.003	0.0012±0.0002	0.040±0.003	0.031±0.002
3	ICP*	0.235	–**	0.037	0.002
	Stocsens	0.227±0.003	0.0026±0.0004	0.034±0.002	0.0020±0.0004
4	ICP*	0.141	–**	0.048	0.044
	Stocsens	0.132±0.004	0.0019±0.0004	0.047±0.003	0.043±0.001
5	ICP*	0.173	–**	0.008	0.025
	Stocsens	0.158±0.003	0.0028±0.0003	0.0075±0.0004	0.023±0.002
6	ICP*	0.275	–**	0.0092	0.011
	Stocsens	0.308±0.005	0.0026±0.0003	0.010±0.003	0.011±0.003
7	ICP*	0.193	–**	0.0064	0.045
	Stocsens	0.189±0.003	0.0012±0.0003	0.0060±0.0003	0.046±0.003
8	ICP*	1.11	–**	0.212	0.029
	Stocsens	1.060±0.003	0.0083±0.0004	0.200±0.004	0.024±0.004
9	ICP*	1.12	–**	0.292	0.024
	Stocsens	1.320±0.003	0.0020±0.0004	0.240±0.003	0.025±0.004

*ICP – standard method; Stocsens – the results obtained using the stochastic sensor.

\diamond atomic fluorescence spectroscopy method was used for the assay of $\text{Hg}(\text{II})$.

**– the quantity of $\text{Hg}(\text{II})$ could not be determined using this method.

CONCLUSIONS

C1. GENERAL CONCLUSIONS

PhD thesis lead to to new azulene derivatives based sensors for the determination of heavy metals in the water.

New azulene derivatives weere electrochemical characterized (E)-ethyl 3-(azulen-1-yl)-2-nitroacrylate (**L2184–2**), (5-(azulen-1-yl)methylene)-2-thioxothiazolidin-4-one (**L2349**), (2E)-2-(azulen-1-ylmethylidene) hydrazinecarbothioamide (**L2352**), (e)–2–(azulen–1–ildiazenil)–5–fenil–1,3,4–tiadiazol (**L2363**), ((E)-2-(azulen-1-ylmethylene)hydrazine carboxamide (**L2366–2**), 2-(azulen-1-ilmetilen)propanbis(tioat) by s,s-diethyl (**L2370–2**), ((E)- 5-phenyl-2-(4,6,8-trimethylazulene-1-ylidiazenil)-1,3,4-thiadiazole (**L2372**), (Z)-2-thioxo-5-((4,6,8-trimethylazulen-1-yl)methylene)thiazolidin-4-one (**M538**), (Z)-5-((5-isopropyl-3,8-dimethylazulen-1-yl)methylene)-2-thioxothiazolidin-4-one (**M540**) analyzing the redox behavior in both anodic and cathodic potential domains. For compounds **L2184–2**, **L2352**, **M538**, **M540** were calculated the diffusion coefficient using Randless-Sevcik equation.

Modified electrodes based poly (**L2184–2**, **L2349**, **L2352**, **L2366–2**, **L2370–2**, **L2372**, **M538**, **M540**) and for poly(((Z)-5-(azulen-1-ylmethylene)-2-thioxoimidazolidin-4-

one)) (poly**L2353**), poly((Z)-5-((5-isopropyl-3,8-dimethylazulen-1-yl)methylene)-2-thioxoimidazolidin-4-one) (poly(**M541**)) were obtained by successive scanning or controlled potential electrolysis at different charges and potentials. The film were transfer in ferrocene solutions, it can be noticed that the ferrocene signal is significantly altered by the presence of polymer film on the electrode surface.

Modified electrodes based poly(**L2349**), poly(**L2352**), poly(**M541**) were characterized by SEM/AFM.

The ability of **L2352** monomer to complex Pb(II) and Cd(II) ions was evidenced by mass spectrometry/electrospray (ESI-MS).

The optimization of analytical method for the determination of lead from tap water using modified electrodes based on poly(**L2352**) was performance.

Modified electrodes based poly azulene were used for heavy metals detection in acetate buffer at pH 5.5. The best response was for Pb(II) ions.

In the thesis, a study was carried out to optimize the method for the determination of lead in drinking water and other types of surface (drilling, surface) using poly**L2352** modified electrodes. The electrochemical method was compared to three other methods: inductively coupled plasma mass spectrometry (ICP-MS), graphite furnace atomic absorption (ETAAS), inductively coupled plasma emission spectrometry using ultrasonic nebulizer (ICP-EOS-USN). The method for determining lead ions using **L2352** modified electroplating stripping voltammetry allows the determination of lead content in low-lead water. The method can be used for the determination of Pb (II) in surface water, wastewater, but for the determination in drinking water it does not have the necessary performance characteristics, namely: precision of less than 1 µg /L, the accuracy for our method is 1.83 mg/L.

Stochastic sensors (using as **L2352** modifier) were used for the detection of Cu (II), Cd (II), Hg (II), Pb (II) in waste water. The reconnaissance test performed for nine samples showed that there is a good correlation between the data obtained using the standard methods and the new analysis method.

C2. ORIGINAL CONTRIBUTIONS

The original contributions are:

- electrochemical characterization of several azulene derivatives and preparation of the modified electrodes;
- the modified electrodes were characterized by SEM, AFM, EQCM;
- the heavy metals detection using modified electrodes;
- optimization of the lead lead determination method, which allowed detection of ion in drinking water and other types of surface (drilling, surface) with poly**L2352** modified electrodes;
- pattern recognition of Cu(II), Cd(II), Hg(II), and Pb(II) in waste water samples

C3. OUTLOOK

The elaborated doctrinal thesis opens perspectives related to:

- characterization of new azulene derivatives for the production of modified electrodes;
- characterization of complex polymeric films by SEM / AFM, etc .;
- obtaining new sensors for detecting metals from different samples;
- optimizing the method for the determination of metals by electrochemical and stochastic methods for other azulenics derivatives.

APPENDICES

A.1. LIST OF PAPERS ELABORATED DURING PhD THESIS

1. G.-O. Buica, L. Birzan, V. Tecuceanu, A.C. Razus, **G.-L. Arnold**, E.-M. Ungureanu, *Modified Electrodes Based on Poly [(2E)-2-(Azulen-1-ylmethylidene) hydrazinecarbothioamide] for Heavy Metal Ions Complexation*, ELECTROANALYSIS, 2017, 29, 93 – 102. **FI= 2.471, SRI = 0,153 (0.919)**,
2. E. Diacu, G.-O. Buica, I. Chilibon, L. Birzan, **G.-L. Arnold**, E.-M. Ungureanu, *Chemically Modified Electrodes Based on 5-(Azulen-1-yl)methylene)-2-thioxothiazolidin-4-one*, Journal of Solution Chemistry, November 2016, Volume 45, Issue 11, pp 1588–1597, **FI= 1.256, SRI = 0,083 (0,500)**
3. **G.-L. Arnold**, R.-I. Stefan-van Staden, I. Moldoveanu-Ionita, E.-M. Ungureanu, L.-R. Popescu-Mandoc, *Azulene Based Stochastic Microsensor*, Journal of the Electrochemical Society, 163 (10) B563-B566 (2016). **FI= 3.014, SRI = 1.797 (1.797)**
4. L.R. Popescu, **G.L. Arnold**, E.M. Ungureanu, M. Iordache, L.F. Pascu, C.B. Lehr, *Electrochemical Studies for New Azulene Compounds*, REV.CHIM.(Bucharest) 67, No. 8, 2016, **FI = 0.956, SRI = 0,0305 (0.183)**.
5. L. Birzan, M. Cristea, C. C. Draghici, V. Tecuceanu, M. Maganu, A. Hanganu, **G.-L. Arnold**, E.-M. Ungureanu, A. C. Razus, *1-vinylazulenes e potential host molecules in ligands for metal ion detectors*, Tetrahedron 72 (2016) 2316-2326, **FI = 2.645, SRI = 0.128 (1,154)**.
6. C. Cioates (Negut), E.-M. Ungureanu, **G.-L. Arnold**, C. Tanase, L. Birzan, *Electrochemical characterization of two oleamide analogues anti-obesity agents*, Studia Universitatis Babes-Bolyai, Chemia (STUDIA UBB CHEMIA), LX (3), 2015 (p. 77-85), **FI = 0.191, SRI = 0,021 (0.105)**.
7. **G.-L. Arnold**, I.G. Lazar, E.-M. Ungureanu, G.-O. Buica, L. Birzan, *New azulene modified electrodes for heavy metal ions recognition*, Bulgarian Chemical Communications, Bulgarian Chemical Communications, Volume 49 Special Issue C (pp. 205 – 210) 2017 **FI = 0.229, SRI = 0.192 (0.192)**,
8. I.G. Lazar, E. Diacu, **G.-L. Arnold**, E.-M. Ungureanu, G.-O. Buica, L. Birzan, *Synthesis and characterization of poly(azulene-thiophene vinyl pyrylium) salt*, Bulgarian Chemical Communications, Bulgarian Chemical Communications, Volume 49 Special Issue C (pp. 227 – 232) 2017, **FI = 0.229, SRI = 0,032 (0.192)**
9. **G.-L. Arnold**, I.-G. Lazar, G.-O. Buica, E.-M. Ungureanu, L. Birzan, *New Azulene Based Modified Electrodes For Heavy Metals Sensing*, U.P.B. Sci. Bull., Series B, Vol. 79, Iss. 2, 2017, pag. 89 – 100.

FI (poz. 1+2+3+4+5+7) = 10.57, SRI (poz. 1+2+3+4+5+7) = 2.38

A.2. LIST OF SCIENTIFIC COMMUNICATIONS ELABORATED DURING PhD THESIS

1. **G.-L. Arnold**, E.-M. Ungureanu, C. Cioates (Negut), C. Tanase, L. Birzan, *Characterization of several oleamide analogues anti-obesity agents by electrochemical methods*, The International Workshop "FOOD CHEMISTRY & ENGINEERING", Univ. Ovidius Constanța, May 15 2015, KN-4.
2. **G.-L. Arnold**, C. Negut (Cioates), E.-M. Ungureanu, *On the electrochemical behaviour of oleamides*, 5th Satellite Student Regional Symposium on Electrochemistry South - East Europe Institute of Electrochemistry and Energy Systems at the Bulgarian Academy of Sciences (IEES-BAS) Sofia, Bulgaria, June 7, 2015, SS-O-06.
3. M. Pelmus, M. Dina Stănescu, E.-M. Ungureanu, **G.-L. Arnold**, L. Tarko, *Electrochemical and QSPR investigations of some phenols and aromatic amines*, 5th Satellite Student Regional Symposium on Electrochemistry South - East Europe Institute of Electrochemistry and Energy Systems at the Bulgarian Academy of Sciences (IEES-BAS) Sofia, Bulgaria, June 7,

- 2015, SS-O-01. -EAST EUROPE RIU PRAVETS | PRAVETS, BULGARIA, 7-11 JUNE, 2015, PEA-O-01.
4. C. Negut, E.-M. Ungureanu, C. Tanase, **G.-L. Arnold**, F. Harja, *Electrochemical characterization of some obesity regulation n oleoylamides*, 5TH International Symposium On Electrochemistry South-East Europe Riu Pravets | Pravets, Bulgaria, 7-11 June, 2015, MOE-P-01.
5. **G.-L. Arnold**, M.R. Bujduveanu, M. Cristea, E. Diacu, E.-M. Ungureanu, L. Birzan, *Chemically modified electrodes based on polyazulene for heavy metals detection*, 19th Romanian International Conference on Chemistry and Chemical Engineering (RICCCE19), Sibiu, Romania 2-5 September 2015, S3-149-OP.
6. **G.-L. Arnold**, E.-M. Ungureanu, E. Diacu, G.-O. Buica, *Modified electrodes based on (Z)-5-((5-Isopropyl-3,8-Dimethylazulen-1-Yl)Methylene)-2-Thioxoimidazolidin-4-one for heavy metal ions*, International Conference Chimia 2016 "New Trends In Applied Chemistry", Univ. Ovidius Constanța, May 26-28 2016, PB-2.
7. G.-O. Buică, E.-M. Ungureanu, L. Bîrzan, **G.-L. Arnold**, *Films based on azulene-thiouree-like for heavy metals ions complexation*, 16th International Conference on Electroanalysis (ESEAC), Bath, UK, 12-16 June 2016, P16.
8. E.-M. Ungureanu, G.-O. Buica, E. Diacu, L. Birzan, **G.-L. Arnold**, *Azulene-Thiourea-Like Chemically Modified Electrodes for Heavy Metal Ions Detection*, The 67th Annual Meeting of the International Society of Electrochemistry, 21-26 August, 2016, The Hague, The Netherlands, Ps01-050.
9. **G.-L. Arnold**, E.-M. Ungureanu, G.-O. Buica, I.-G. Lazar, L. Birzan, *Modified Electrodes based on 2-thioxo-5-((4,6,8-trimethylazulen-1-yl)methylen)imidazolidin-4-one for Heavy Metal Ions Detection*, The 67th Annual Meeting of the International Society of Electrochemistry, 21-26 August, 2016, The Hague, The Netherlands, Ps15-020.
10. **G.-L. Arnold**, M.-R. Bujduveanu, E.-M. Ungureanu, *Chemically modified electrodes based on (z)-2-thioxo-5-((4,6,8-trimethylazulen-1-yl)methylene)thiazolidin-4-one for heavy metals ions detection*, 3rd International Conference on Analytical Chemistry (ROICAC), Iasi, Romania, August 28 – 31, 2016, P12YN – **best poster premiu**.
11. E. Diacu, I.G. Lazar, E.-M. Ungureanu, **G.-L. Arnold**, L. Birzan, *Détection voltammetrique des métaux lourds des eaux de surface en utilisant des electrodes modifiées avec perchlorate de poly (2,6-bis ((e)-2-(thiophen-2-yl)vinyl)-4-(4,6,8-trimethylazulene-1-yl)pyrylium*, 3rd International Conference on Analytical Chemistry (ROICAC), Workshoop Atelier scientifique Nouveaux Matériaux pour la Reconnaissance Electrochimique des Minéraux et des Espèces Biologiques (NOMARES), Iasi, Romania, August 28 – 31, 2016, PIN.
12. A. Oprișanu, **G.-L. Arnold**, E.-M. Ungureanu, L. Bîrzan, *Electrodes chimiquement modifiées basées sur le perchlorate de 4- (azulen-1-yl) -2,6-bis ((e) -2- (2-yl furan) de vinyl) pyrylium pour la détection des ions de métaux lourds*, 3rd International Conference on Analytical Chemistry (ROICAC), Workshoop Atelier scientifique Nouveaux Matériaux pour la Reconnaissance Electrochimique des Minéraux et des Espèces Biologiques (NOMARES), Iasi, Romania, August 28 – 31, 2016, P4N.
13. **G.-L. Arnold**, I.G. Lazar, E.-M. Ungureanu, E. Diacu, *Electrochemical Characterization of 4-(azulen-1-yl)-2,6-bis((E)-2- (thiophen-3-yl)vinyl)pyrylium in View of Heavy Metals Detection in Water Samples Using Modified Electrodes*, 19th International Symposium "Environment and Industry, 13-14 October 2016, PLT9.
14. L.R. Popescu, E.-M. Ungureanu, M. Iordache, L.F. Pascu, **G.-L. Arnold**, *Studii electrochimice privind testarea de noi materiale nanocompozite utilizate pentru elaborarea de senzori electrochimici*, A XXXIV-a Conferință Națională de Chimie Călimănești – Căciulata, 4-7 October 2016 Călimănești-Căciulata, Vâlcea, Romania, P.S.IV. – 14.
15. E.-M. Ungureanu, G.-G. Vasile, **G.-L. Arnold**, L. Birzan, G.-O. Buica, *Electrochemical determination of lead in water samples using modified electrode based on polyazulene*

derivates, 17 International Multidisciplinary Scientific GeoConference SGEM 2017, 27 June – 6 July 2017, Albena, Bulgaria.

A.3. LIST OF STUDENTS SCIENTIFIC COMMUNICATIONS

1. Petruța Mocanu, Cornelia Dumitru, Valentina Ștefan, *Studii electrochimice de derivați vinilici heterociclici de azulen*, Sesiunea de Comunicari Stiintifice a Studentilor de la Cercul de Chimie Fizica Aplicata si Electrochimie, FCASM, Universitatea Politehnica din Bucuresti, coordonator: E.-M. Ungureanu, **G.-L. Arnold**, 15 Mai 2015.
2. Alina Mihaela Constantin, an 3 CATB Emilia-Julia Deliu-Guteanu, an 3 CISOPC, *Investigații electrochimice ale oleamidelor*, Sesiunea de Comunicari Stiintifice a Studentilor de la Cercul de Chimie Fizica Aplicata si Electrochimie, FCASM, Universitatea Politehnica din Bucuresti, coordonator: E.-M. Ungureanu, C. Neguț, **G.-L. Arnold**, 15 Mai 2015.
3. Alexandru Sebastian Iorgu, Radu Iulian Lăudat, *Studii electrochimice de derivați azulenici*, Sesiunea de Comunicari Stiintifice a Studentilor de la Cercul de Chimie Fizica Aplicata si Electrochimie, FCASM, Universitatea Politehnica din Bucuresti, coordonator: E.-M. Ungureanu, **G.-L. Arnold**, 13 Mai 2016.
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5. Cojocar Denisa-Mihaela, Tureac Denisa-Georgiana, *Caracterizarea electrochimică a unui derivat azulenice de interes pentru obținerea de electrozi modificați*, Sesiunea de Comunicari Stiintifice a Studentilor de la Cercul de Chimie Fizica Aplicata si Electrochimie, FCASM, Universitatea Politehnica din Bucuresti, coordonator: E.-M. Ungureanu, M.-R. Bujduveanu, **G.-L. Arnold**, 12 Mai 2017.

The present summary contains in a concise form the content of chapters 6-12 of original contributions. The numbering of chapters, subchapters and tables corresponds to the sentence. The bibliographic references used in the paper are presented.

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