UNIVERSITY **POLITEHNICA** of BUCHAREST

Faculty of Applied Chemistry and Materials Science

Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry

DOCTORAL SCHOOL OF APPLIED CHEMISTRY AND MATERIALS SCIENCE
Nr. CSUD Decision No....... from ....

PhD THESIS SUMMARY

DEVELOPMENT OF REAL TIME ANALYTICAL PLATFORMS BASED ON CHEMICAL, ELECTROCHEMICAL AND OPTICAL SENSORS FOR MONITORING, EVALUATION AND CONTROL OF POLLUTION IMPACT ON WATERS QUALITY FROM WATER RESOURCES

DEZVOLTAREA UNOR PLATFORME ANALITICE ÎN TIMP REAL BAZATE PE SENZORI CHIMICI, ELECTROCHIMICI ȘI OPTICI PENTRU MONITORIZAREA, EVALUAREA ȘI CONTROLUL IMPACTULUI ASUPRA CALITĂȚII APELOR DIN RESURSELE DE APĂ

Doctorand: Ing. chim. Cristian OMOCEA

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Bucureşti -2021-

CUPRINS

INTRODUCTION

Due to their tendency to accumulate and the low rate of elimination from the body, heavy metals (eg Hg, Cd, Pb, As, ...) can cause severe health problems. Although these elements can be found naturally in the environment, they become contaminants in high concentrations resulting mainly from anthropogenic activities. Therefore, their detection in the environment is a pressing issue. Among these metal ions, the mercury ion is one of the most dangerous ions for human health. Its toxicity depends mainly on the chemical form, quantity and period of exposure.

In Law no. 311 of 2004 for the amendment and completion of Law no. 458/2002 regarding the quality of drinking water, the values allowed in the drinking water samples are provided for mercury of 1.0 µg / L, and for lead of 10.0 µg / L. Government Decision no. 352 of April 21, 2005 on amending and supplementing Government Decision no. Regulation (EC) No 188/2002 approving rules on the discharge of waste water into the aquatic environment allows for much lower maximum concentrations for these heavy metals: mercury of 0.05 mg / dm3 and lead of 0.5 mg / dm3 [3], from reasons which will be set out below.

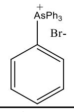
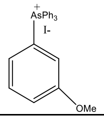
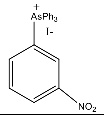
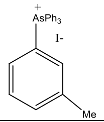
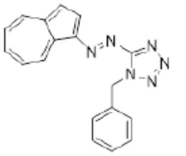
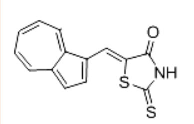
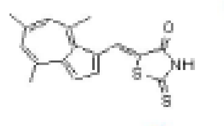
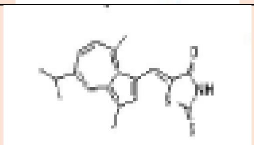
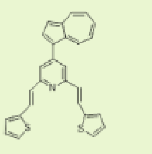
The most used and optimized techniques for determining heavy metals are spectrometric methods (graphite furnace atomic absorption spectrometry, flame atomic absorption spectrometry, inductively coupled plasma mass spectrometry, atomic fluorescence spectrometry, coupled plasma emission spectrometry inductive), 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 staff.

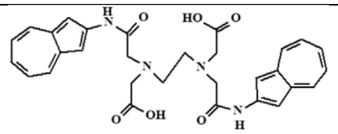
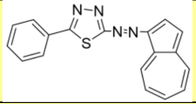
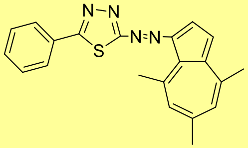
In recent years, new electrochemical methods have been developed using sensors for detecting metals based on modified electrodes. Within the laboratory of Electrochemical Processes in Organic Solvents (PESO) from the POLITEHNICA University of Bucharest (UPB), methods for the detection of heavy metals based on complexing electrodes modified with azulene were initiated and tested. This doctoral thesis is part of these concerns related to testing new ligands for the development of complexing electrodes. The thesis was developed in the laboratories of electrochemical processes in organic solvents in the Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry and General Chemistry of the Faculty of Applied Chemistry and Materials Science at the Polytechnic University of Bucharest.

The thesis brings original contributions related to the electrochemical characterization of new organic compounds: 4 arsonium salts and 8 complexing azulene derivatives (Table I.1) recently synthesized in the CD Nenițescu Center for Organic Chemistry of the Romanian Academy in the Research Team coordinated by Dr. Alexandru C. Răzuș and Dr. Liviu Bîrzan. In the thesis, new ligands were tested and new electrochemical sensors for the detection of heavy metals were proposed (Table I.1) and statistical data for water quality monitoring were processed.

The doctoral thesis presents the spectral and optical electrochemical studies of these new compounds. Derivatives with complexing properties compared to heavy metals were tested for use in the preparation of modified electrodes, with applications for the detection of heavy metals (mercury and lead) in water. Based on the obtained results, new optical, spectrophotometric (based on solution complexation) and electrochemical (based on modified electrodes) sensors were proposed. Electrochemical sensors based on modified electrodes with remarkable detection limits were obtained, an objective difficult to achieve given the low limits allowed by law for these pollutants in water. This goal was achieved by discovering a ligand with favorable properties that was also tested on real samples.

Tabel I.1. Structuri ale sărurilor de arsoniu și structuri azulenice investigate

Nr. crt.	Tip de derivat	Denumire compus azulenic	Structura	Comunicare rezultate*
1	sare de arsoniu	bromură de trifenilarsoniu (L1)		A3, P1, P2
2	sare de arsoniu	iodură de (3-metoxibenzil)trifenilarsoniu (L2)		A3, P1, P2
3	sare de arsoniu	iodură de (3-nitrobenzil)trifenilarsoniu (L3)		A3, P1, P2
4	sare de arsoniu	iodură de (3-metilbenzil)trifenilarsoniu (L4		A3, P1, P2
5	tetrazol	(E)-5-(azulen-1-ildiazenil)-1-benzil-1H-tetrazol (L)		P3
6	2-tioxo-tiazolidin-4-one	(Z)-5-(azulen-1-ilmetilene)-2-tioxo-tiazolidin-4-one (L2349)		P4, P5, P6
7	2-tioxo-tiazolidin-4-one	(Z)-2-tioxo-5-((4,6,8-trimetilazulen-1-il)metilen)-tiazolidin-4-onă (M538)		P5, P6
8	2-tioxo-tiazolidin-4-one	(Z)-5-((5-izopropil-3,8-dimetilazulen-1-il)metilen)-2-tioxotiazolidin-4-onă (M540)		P5, P6
9	tiofen-vinilpiridină	4-(azulen-1-il)-2,6-bis((e)-2-(tiofen-2-il)vinil)piridină		P7

10	acid 2,2′ - (etan-1,2 diilbis ((2- (azulen 2-ilamino) -2-oxoetil) azan-diil)) diacetic (EDTA)	acid 2,2′ - (etan-1,2 diilbis ((2-(azulen 2-ilamino) -2-oxoetil) azan-diil)) diacetic (L5)		A1
11	1,3,4-tiadiazol	2-(azulen-1-ildiazenil)-5-fenil-1,3,4-tiadiazol (L2513)		A4
12	1,3,4-tiadiazol	2-fenil-5-((4,6,8-trimetilazulen-1-il)diazenil)-1,3,4-tiadiazol (L2372)		O1

*P = poster comunicat la conferințe enumerate în Anexe (A2); O = prezentare orală la conferință; A = articol publicat/ în curs de publicare citat în Anexe (A1).

In order to emphasize the relevance of continuous water monitoring and the use of information that can be deduced from the global interpretation of the data, a set of water quality monitoring data was analyzed in the thesis (last chapter). In this sense, the doctoral student followed the quality parameters of some water samples that were taken and analyzed in the period prior to the doctoral internship as an employee of the doctoral student at the Dobrogea-Litoral Water Basin Administration.

During the doctoral internship, the raw data of the evaluations from this institution were requested and the statistical data processing was performed for a period of three years from the Chiciu sampling station, Călărași county. The multivariate analysis method was used in order to identify the main parameters. leading to data variation as well as possible correlations between parameters. The evolution over time of key parameters, such as total nitrogen, is an original contribution that has provided public authorities with useful information on the effectiveness of measures taken to reduce pollution. The statistical methods used in this study were correlation analysis (Pearson and Spearman), principal component analysis (PCA) and linear regression. Because the study examined a large set of experimental data with many parameters, the use of PCA was necessary to identify those who play the most important role in variation. This analysis was also used to validate the results of the correlation analysis, as well as to learn the methods / techniques used to monitor water quality using portable multiparameters.

The doctoral thesis contains 7 chapters and is structured in two parts: BIBLIOGRAPHY RESEARCH and ORIGINAL CONTRIBUTIONS.

BIBLIOGRAPHY RESEARCH includes 3 chapters:

Chapter 1. Electrochemical sensors for environmental monitoring
Chapter 2. Sensors for heavy metals based on modified electrodes with complexing films;
Chapter 3. Reduction and electrochemical oxidation of organic compounds with As, Sb and Bi;

ORIGINAL CONTRIBUTIONS include 4 chapters aimed at the experimental characterization of new organic compounds - arsonium salts and azulene derivatives - and the development of new chemical, electrochemical and optical sensors for water quality analysis of water resources (3 chapters) and statistical analysis studies (1 chapter):

Chapter 4. Experimental details regarding the studies performed
Chapter 5. Electrochemical studies on new triphenyl arsonium derivatives
Chapter 6. Colorimetric, optical and electrochemical sensors for analysis of azulene-based heavy metals in water
Chapter 7. Correlations between water quality parameters

The doctoral thesis ends with the general conclusions, with the original contributions and with the prospects of further development.

The best results obtained in the thesis were published in 3 scientific papers: 2 articles in ISI journals (Journal of Electroanalytical Chemistry and Polytechnic University of Bucharest Scientific Bulletin. Series B), 1 BDI article in REV.CHIM. (Bucharest)) and 1 article being published in the journal ISI Symmetry, or papers at international and national scientific events (7 poster papers and 1 oral paper).

At the end of the thesis are attached the articles published during the doctoral thesis in extenso.

ORIGINAL CONTRIBUTIONS

CHAPTER 4. Experimental details regarding the studies performed

4.1. Experimental details in electrochemical determinations by CV, DPV, RDE

The organic compounds studied in the thesis were synthesized and characterized from a physico-chemical point of view at the Institute of Organic Chemistry „C.D. Nenitzescu ”in the team coordinated by Dr. Eng. Liviu Bîrzan [Liviu Birzan, Mihaela Cristea, Victorita Tecuceanu, Constantin Draghici, A. Hanganu, Alexandru C. Razus, Revista de Chimie 2020, 71 (8), 1-12]. The synthesis plant is shown in Fig. 4.1. Electrochemical studies of compounds, preparation of modified electrodes and recognition studies were performed in the laboratories of Electrochemical Processes in Organic Solvents (PESO) in the Department of Inorganic Chemistry, Physical Chemistry and Electrochemistry and General Chemistry of the Faculty of Applied Chemistry and Science Polytechnic of Bucharest.

Electrochemical studies were performed in organic solvents (acetonitrile, DMF) and aqueous (at different pHs), using as support electrolytes Fluka tetrabutylammonium perchlorate (TBAP) in acetonitrile and acetate or borate buffers, respectively.

The electrochemical experiments were performed using a POLSTAT12 AUTOLAB potentiometer coupled to a three-compartment cell, shown in Fig. 4.2. Glassy carbon discs (3 mm diameter) were used as working electrodes. Before each experiment, the active surface of the working electrode was cleaned with diamond paste (0.25 μm).

Ag / 10 mmol · L-1 AgNO3 in 0.1 mol · L-1 TBAP in acetonitrile (CH3CN) was used for the measurements in the organic solvent as reference. The potentials were related to the potential of the ferrocene / ferricin redox couple (Fc / Fc +) which was in our experimental conditions of +0.07 V.

A platinum wire was used as the auxiliary electrode. Ligand characterization experiments were performed under an argon atmosphere.

All experiments were performed at 25 ° C.

4.2. Experimental details in the development of modified electrodes (CME)

The electrochemical studies of the compounds performed before the preparation of the modified electrodes and the recognition studies were performed in the PESO and

General Chemistry laboratories of the Faculty of Applied Chemistry and Materials Science of the Polytechnic University of Bucharest.

Fig. 4.1. Ligand synthesis plant

Fig. 4.2. Cell for the study of compounds in organic solvents

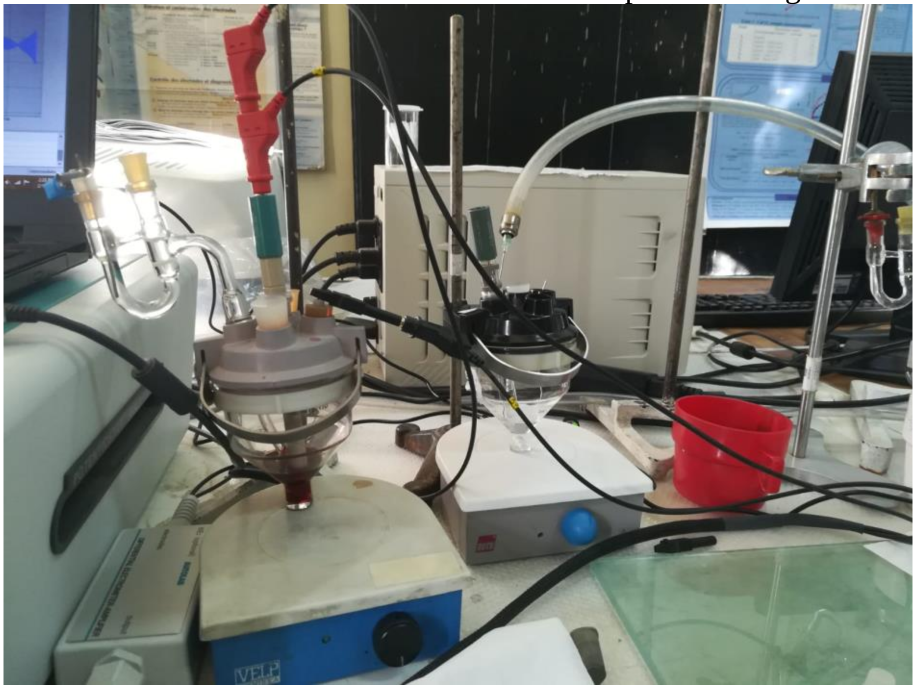


Fig. 4.3. Cells for the preparation of CMEs by electrolysis at controlled potential (left) and for their conditioning (right)

To characterize the electrochemical sensors, electrochemical studies were performed in organic solvents (acetonitrile, DMF) and aqueous solvents (at different pHs), using as support electrolytes Fluka tetrabutylammonium perchlorate (TBAP) in acetonitrile and acetate buffers, respectively. or borate.

Electrochemical experiments were performed using AUTOLAB PGSTAT12 PGSTAT204 potentiostats coupled to a three-compartment cell, shown in Figs. 4.2. Glassy carbon discs (3 mm diameter) were used as working electrodes. Before each experiment, the active surface of the working electrode was cleaned with diamond paste (0.25 µm).

Fig. 4.4. CPE modified glassy carbon electrode

Fig. 4.5. Installation for measuring the pH of the electrolyte used in the conditioning cell for CMEs

For measurements in organic solvent as reference, Ag / 10 mmol · L-1 AgNO3 in 0.1 mol · L-1 tetra-n-butylammonium perchlorate (TBAP) in acetonitrile (CH3CN) was used. The potentials were related to the potential of the ferrocene / ferricin redox couple (Fc / Fc +) which was in our experimental conditions of +0.07 V.

For aqueous solvent measurements, Ag / AgCl, 3 M KCl, H2O were used as reference. Potentials have been reported in this reference. Platinum wires were used as auxiliary electrodes (counterelectrodes).

All experiments were performed at 25 ° C.

Electrozii de carbon vitros au fost modificați prin electroliză la potențial controlat (CPE) în solvent organic (Fig. 4.4) și apoi au fost conditionați în mediu apos la pH controlat, folosind pH-metrul din Fig. 4.5.

4.3. Experimental details in determinations by UV-Vis spectrometry

For chemical and optical sensors, spectroscopic measurements were performed with the Jasco V-670 double-beam spectrophotometer in quartz tanks with an optical path of 1 cm. The ContrAA 700 High Resolution Continuous Source Atomic Absorption Spectrometer (HRCSAAS) was used to determine the amount of mercury in the real samples, with an HS 60M atomization system (CV) cold hydride / vapor generator from Analytik Jena.

All experiments were performed at 25 ° C.

Chapter 5. Electrochemical studies on new triphenyl arsonium derivatives

5.1. Introduction to the interest of the study of arsonium salts

Organic compounds with arsenic have been less studied due to their relatively low abundance of arsenic and their toxicity. However, in recent years, organic arsonium compounds have been shown to be less toxic than their inorganic counterparts [1]. As a result, new research efforts have been made to determine the properties of arsonium ions (especially those containing fluorine) to find new industrial and medical applications [2]. The study of the electrochemical properties of these compounds aims to understand their chemistry and toxicity.

The study performed in the thesis refers to the electrochemical characterization by cyclic voltammetry, pulse-differential voltammetry and rotary disk electrode voltammetry of new triphenylbenzylarsonium derivatives. Their electrochemical study was performed to establish the main characteristics for subsequent applications, such as: treatments for leukemia and other cancers [3], synthesis of gold nanoparticles functionalized with triphenylarsonium as potential nanoparticles for intracellular therapy [4] or extraction of XO4- ions (X = Tc, Re) using arsonium ions [5].

5.2. Experimental part of the study of arsonium salts

Derivatives L1 - L4 were synthesized at the Institute of Organic Chemistry „C.D. Nenitzescu ”in the team coordinated by Dr. Eng. Liviu Bîrzan [6]. Acetonitrile and tetrabutylammonium perchlorate (TBAP) from Fluka were used as solvent and support electrolyte.

Electrochemical experiments were performed by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and rotating disk electrode voltammetry (RDE) using a PGSTAT12 AUTOLAB potentiostat coupled to a three-compartment cell. CV curves were recorded at scan speeds between 0.1 - 1 V / s, and DPV curves were recorded at scan speeds of 0.01 V / s with a pulse amplitude of 0.025V and a pulse period of 0, 2 s. RDE experiments were recorded at 0.01 V / s with rotational speeds between 500 and 2000 revolutions per minute (rpm). A glassy disk (3 mm diameter) was used as the working electrode. Before each experiment, the active surface of the working electrode was cleaned with diamond paste (0.25 μm). Ag / 10 mmol · L-1 AgNO3 in 0.1 mol · L-1 tetra-n-butylammonium perchlorate (TBAP) in acetonitrile (CH3CN) was used as reference electrode. The potentials were related to the potential of the ferrocene / ferricinium redox couple (Fc / Fc +) which was in our experimental conditions of +0.07 V. A platinum wire was used as auxiliary electrode (counterelectrode). The experiments were performed at a temperature of 25 ° C under an argon atmosphere.

5.3. Experimental results for arsonium salts

The electrochemical behavior of these new derivatives on the glassy carbon electrode was studied by electrochemical methods: cyclic voltammetry, differential pulse voltammetry and voltammetry on the rotating disk electrode. The redox processes highlighted by cyclic voltammetry and differential pulse voltammetry were established, analyzed and evaluated according to the functional groups involved, according to the active structures in the molecules.

5.3.1. Study of triphenylarsonium bromide (L1)

Electrochemical experiments were performed for compound L1 by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The anodic and cathodic curves were recorded individually, starting from the stationary potential. The CV and DPV curves (Fig. 5.1.) Were obtained for two concentrations (2.5 and 5 mM) of the compound studied in solutions of 0.1 M tetra-n-butylammonium perchlorate (TBAP) in dimethylformamide (DMF).

From the DPV curves (Fig. 5.1.) We observe two anodic processes (a1 and a2) and two cathodic processes (c1 and c2), noted in the order in which they appear in voltammograms.

Data were obtained from pulse-differential voltammetry (DPV) and rotary disk electrode (RDE) voltammetry in a solution of L1 (2.5 mM) in 0.1 M TBAP, DMF on glassy carbon electrode at different rotational speeds (500 - 1500 rpm) (Fig. 5.2.)

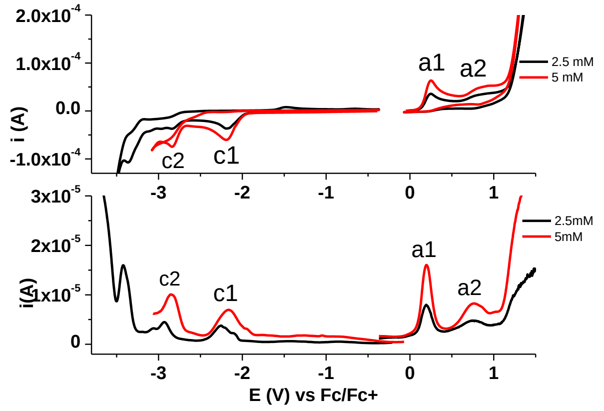


Fig. 5.1. Cyclic voltammograms (0.1 V / s) and differential pulse voltammograms obtained for different concentrations of L1 on glassy carbon in 0.1 M TBAP, DMF

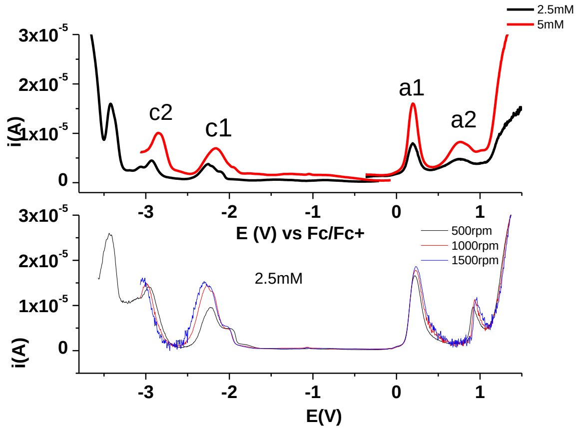


Fig. 5.2. Curves obtained by pulse-differential voltammetry and RDE in a solution of L1 (2.5 mM) in 0.1 M TBAP / DMF on glassy carbon electrode at different rotational speeds (500 - 1500 rpm)

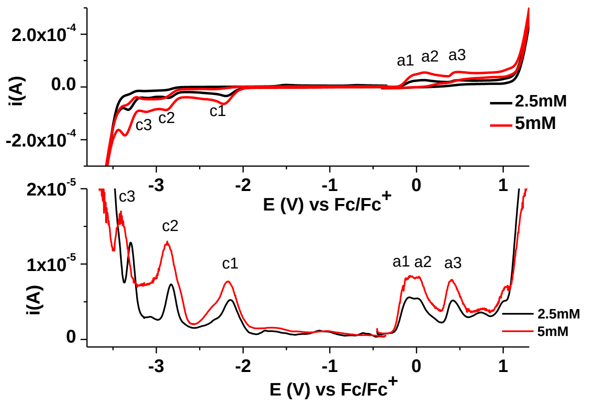
In Table 5.1. the characterization of the pulse-differential voltamogram is presented.

Table 5.1. Potentials (V) vs Fc / Fc + for peaks in DPV curves (2.5 mM)

Pick	c1	c2	a1	a2
E_{DPV} (V)	-2,258	-2,936	0,193	0,760

5.3.2. Study of (3-methoxybenzyl) triphenylarsonium iodide (L2)

Electrochemical experiments were performed for compound L2 by cyclic voltammetry (CV) and pulse-differential voltammetry (DPV). The anodic and cathodic curves were recorded individually, starting from the stationary potential. The CV and DPV curves (Fig. 5.3) were obtained for two concentrations (2.5 and 5 mM) of compound studied in solutions of 0.1 M tetra-n-butylammonium perchlorate (TBAP) in dimethylformamide (DMF).



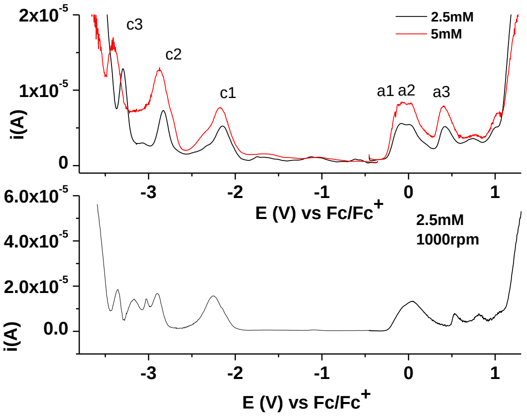


Fig. 5.3. Cyclic voltammograms (0.1 V / s) and pulse-differential voltammograms for different concentrations

Fig. 5.4. Differential pulse voltammetry and RDE curves obtained in L2 (2.5 mM) solutions in 0.1 M TBAP,

of L2 on vitreous carbon electrode in 0.1 M TBAP, DMF

DMF on vitreous carbon electrode at 1000 rpm

From the DPV curves (Fig. 5.3.) Three anodic processes (a1 - a3) and three cathodic processes (c1 - c3) are observed, noted in the order in which they appear in voltammograms.
Data were obtained from differential pulse voltammetry (DPV) and rotary disk electrode (RDE) voltammetry in a solution of L2 (2.5 mM) in 0.1 M TBAP, DMF on vitreous carbon electrode at a rotational speed of 1000 rpm (Fig. 5.3.)

In Table 5.2. the characterization of the pulse-differential voltamogram is presented.

Table 5.2. Peak potentials (V) of DPV curves (2.5 mM) (vs Fc / Fc +)

Pick	**c1**	**c2**	**c3**	**a1**	**a2**	**a3**
E_{DPV} (V)	-2,153	-2,830	-3,297	-0,091	0,014	0,417

5.3.3. Study of (3-nitrobenzyl) triphenylarsonium iodide (L3)

Electrochemical experiments were performed for compound L3 by cyclic voltammetry (CV) and pulse-differential voltammetry (DPV). The anodic and cathodic curves were recorded individually, starting from the stationary potential. The CV and DPV curves (Fig. 5.5) were obtained for two concentrations (2.32 and 4.64 mM) of the compound studied in solutions of 0.1 M tetra-n-butylammonium perchlorate (TBAP) in dimethylformamide (DMF). From the DPV curves (Fig. 5.5) we observe two anodic processes (a1 and a2) and five cathodic processes (c1 - c5), noted in the order in which they appear in voltammograms.

Data were obtained from differential pulse voltammetry (DPV) and rotary disk electrode (RDE) voltammetry in a solution of L3 (2.32 mM) in 0.1 M TBAP, DMF on vitreous carbon electrode at a rotational speed of 1000 rpm (Fig. 5.6.).

In Table 5.3. the characterization of the pulse-differential voltamogram is presented.

Table 5.3. Potentials (V) vs Fc / Fc + for peaks in DPV curves (2.32 mM)

Pic	**c1**	**c2**	**c3**	**c4**	**a1**	**a2**
E_{DPV} (V)	-1,477	-1,624	-2,354	-2,849	-0,113	0,321

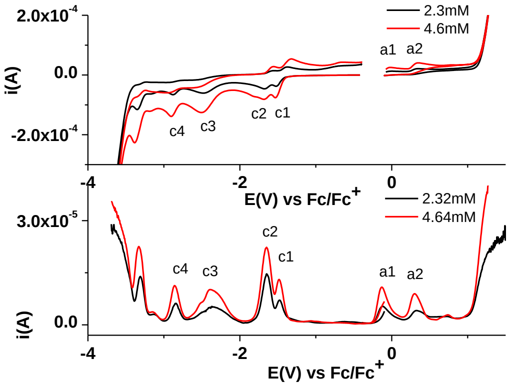


Fig. 5.5. Cyclic voltammograms (0.1 V / s) and differential pulse voltmeters for different concentrations of L3 on vitreous carbon electrode in 0.1 M TBAP, DMF

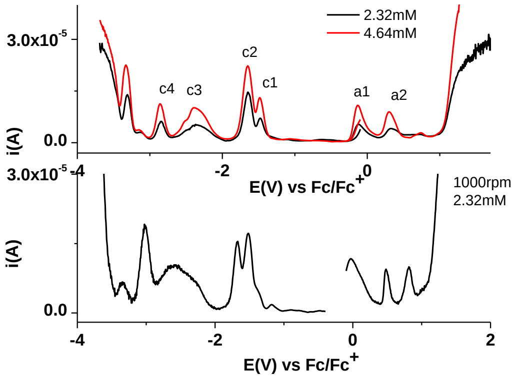


Fig. 5.6. Pulse-differential voltammetry and RDE curves in L3 (2.32 mM) solutions in 0.1 M / DMF TBAP on vitreous carbon at 1000 rpm

5.3.4. Study of (3-methylbenzyl) triphenylarsonium iodide (L4)

Electrochemical experiments were performed for compound L4 by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The anodic and cathodic curves were recorded individually, starting from the stationary potential. The CV and DPV curves (Fig. 5.7) were obtained for two concentrations (2.5 and 5 mM) of compound studied in solutions of 0.1 M tetra-n-butylammonium perchlorate (TBAP) in dimethylformamide (DMF).

From the DPV curves (Fig. 5.7) we observe two anodic processes (a1 and a2) and three cathodic processes (c1 - c3), noted in the order in which they appear in voltammograms.

Data were obtained from differential pulse voltammetry (DPV) and rotary disk electrode (RDE) voltammetry in a solution of L4 (2.5 mM) in 0.1 M TBAP, DMF on vitreous carbon electrode at a rotational speed of 1000 rpm (Fig. 5.8.)

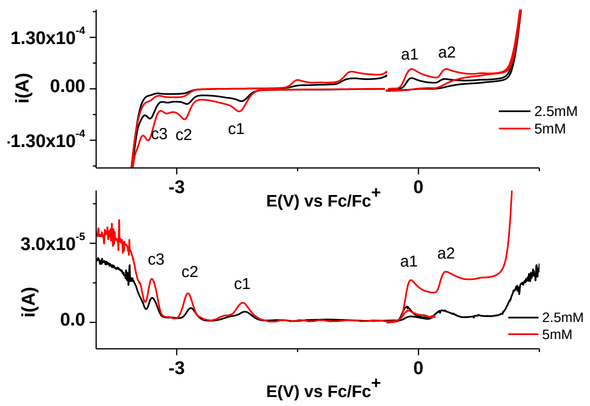


Fig. 5.7. Cyclic voltammograms (0.1 V / s) and pulse-differential voltammograms for concentrations other than L4 on vitreous carbon electrode in TBAP 0.1 M / DMF

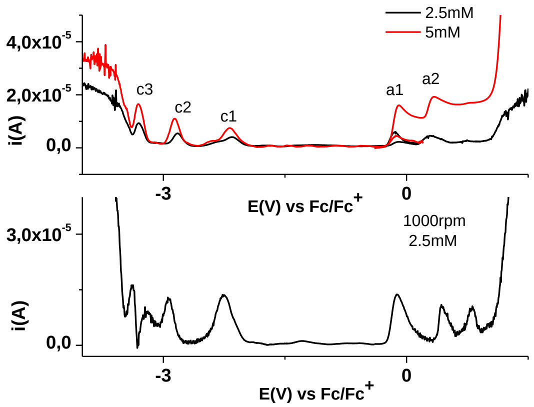


Fig. 5.8. Differential pulse voltage and RDE curves at 1000 rpm in solutions of L4 (2.5 mM) in 0.1 M TBAP, DMF on vitreous carbon electrode

In Table 5.4. the characterization of the pulse-differential voltamogram is presented.

Table 5.4. Potentials (V) vs Fc / Fc + for peaks in DPV curves (2.5 mM)

Pick	c1	c2	c3	a1	a2
E_{DPV} (V)	-2.154	-2.824	-3.31	-0.148	0.296

5.4. Conclusions regarding the electrochemical characterization of L1-L4 arsonium salts

The study performed on arsonium salts L1-L4 focused on the electrochemical characterization by cyclic voltammetry, pulse-differential voltammetry and voltammetry on rotating disk electrode of new triphenylarsonium derivatives, to which is attached a benzyl ring substituted in position 3 with H, OMe , NO2 and Me (Table 5.5). The synthesis resulted in bromides and iodines, so the comparison of data is hampered by different anions of arsonium salts.

Table 5.5. Comparison of arsonium derivatives in terms of reduction and oxidation potentials (V) vs Fc / Fc +

Nr.	Derivat	L1	L2	L3	L4
1	**Anion**	**Br^-**	**I^-**	**I^-**	**I^-**
2	**X**	H	OCH_3	NO_2	CH_3
3	c1	-2,258	-2,153	-1,477	-2,154
4	a1	0,193	-0,091	-0,113	-0,148

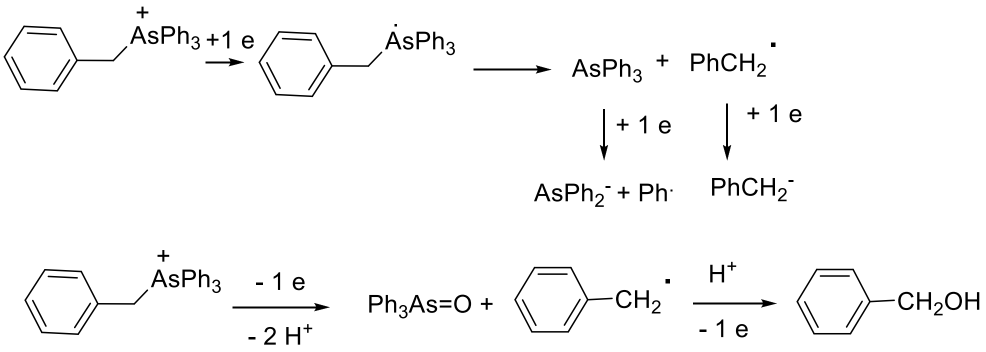
At reduction (potential values for peak c1, Table 5.5) the presence of the anion does not bother, so the data in line 3 of Table 2 may be relevant. It is observed that the NO2-substituted derivative is most easily reduced, consistent with the electron-attracting effect of the group. OCH3 and Me substituents have a repellent effect and are harder to reduce than NO2. The reduction potential of the unsubstituted derivative L1 (H) is not surprising either, because the correlations of the reduction potentials are not conditioned not only by the effects of the substituents, but also by the symmetry of the molecule, which is higher at L1 compared to L2 and L4.

On oxidation (potential values for peak a1, Table 5.5) the following comparisons can be made:

L1 and L4 should have the closest potentials, because the CH3 group has only a weak inductive effect, so the order found is correct, because the Br- anion discharges to a more positive potential (0.193 V) than I- (-0.148 V).

L2, L3 and L4 should have appropriate potentials, because in all cases the same anion (I-) is oxidized; it is observed that, indeed, the values of the oxidation potentials do not differ much for the 3 compounds. The DPV and RDE curves of the 3 compounds are very similar in the anodic domain.

All compounds follow oxidation and reduction schemes with decomposition of the molecule (Scheme 5.1), which involves parallel reactions, which complicates the analysis of peaks, making it difficult to interpret and assign each peak.



Scheme 5.1. Electrochemical reduction and oxidation processes of arsonium derivatives

The electrochemical study of arsonium salts [7] established the oxidation and reduction potentials of these derivatives, which are key features for future applications, such as the use of these compounds in the treatment of leukemia and other cancers, to obtain functionalized gold nanoparticles of triphenylarsonium or for obtaining intracellular nanoparticles.

Chapter 6. Azulene-based colorimetric, optical and electrochemical sensors for the analysis of heavy metals in water

6.1. Introduction to colorimetric, optical and electrochemical sensors for the analysis of heavy metals in water

As a result of their tendency to accumulate and lowclearance rate in the body, heavy metals (e.g. Hg, Cd, Pb, As, …) can cause severe health problems [1]. Although these elements can be naturally found in the environment, they become contaminants in high concentrations resulted mainly through anthropogenic activities. Therefore, their detection from environmental media is a stringent issue. Mercury ion is one of themost threatening for human health among these metal ions. Its toxicity mainly depends on chemical form, amount and exposure period [2]. For instance, each chemical form of mercury (elemental, inorganic, and organic) has his own profile of toxicity [3]. Moreover, a recent study has shown that almost a double amount of mercury is found in the permafrost soils from the Northern Hemisphere compared to the mercury content found in all other natural environments [4]. Also, due to the possibility of permafrost thaws in the following years, an increased amount of mercury is expected to be released into the soil-ocean-atmosphere system, thus underlying the importance of its monitoring and detection.

To detect mercury ions from different environmental media a few techniques such as atomic absorption, emission, fluorescence or mass spectrometry [5] are considered to be the traditional analytical methods to date. These methods involve highly sophisticated apparatus which offers very good sensitivity and elemental coverage. However, their high running costs, expensive instruments, elaborate sample making [6] promoted the development of new alternative techniques for the detection of metal ions to overcome these shortcomings. Such requirements can be fulfilled by colorimetric detection (e.g. by the naked eye or UV–Vis and fluorescence spectroscopy) allowing a qualitative and quantitative detection in a rapid and easy manner [7] of metal ions. This makes possible the fabrication of test kits for on-site detection [8–11]. For instance, colorimetric determination of ionic mercury was achieved using receptors based on organic dyes, metal ions complexes, polymers or nanoparticles [12]. Another approach to detect mercury ions is based on voltammetric stripping techniques which can meet the requirements for low detection limits, higher sensitivity, simplicity and portability. The detection is usually accomplished by usingmetallic based electrodes (e.g. gold, carbon, bismuth, antimony…),metallic electrodes modified with molecules or polymers [13,14] or composites based on conducting polymers and carbon nanomaterials electrodes [15]. The latter received special attention due to the possibility to substitute them with tailored moieties in order to improve detection limits, specificity or selectivity towards a certain target species [16]. One of themost efficient approaches to obtain complexingmodified electrodes is the direct electrochemical oxidation of polymerizable monomers possessing appropriate complexingmoieties [17–20]. This will produce in a single stage functionalised polymers with well-determined dimension and complexation sites concentrations [21].

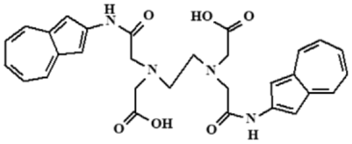
Considering the advantages of both colorimetric and voltammetric techniques, new molecules that can be used in both cases are highly desired. For instance, recently Ayranci and co. reported for the first time a fluorescent conjugated polymer sensor platform based on rhodamine for the detection of mercury by potentiometric, colorimetric and voltammetric means [22]. Also, an hybrid electrochemical/electrochromic sensor based on PANI/ITO-electrode for the generation of multi-signal sensor platforms for Cu(II) metal ion detection is reported by Deshmukh and co. [23].

Teza aduce contribuţii originale legate de caracterizarea electrochimică a unor noi derivaţi de azulenă complexanți sintetizați recent în Centrul de Chimie Organică *C. D. Nenițescu* al Academiei Române în Colectivul de Cercetare coordonat de Dr. Liviu Bîrzan. În cadrul tezei s-au testat 8 noi liganzi azulenici: ((E)-5-(azulen-1-yldiazenyl)-1-benzyl-1H-tetrazole (L), (Z)-5-(azulen-1-ylmethylene)-2-thioxo-thiazolidin-4-one (L2349), (E)-2-(Azulen-1-yldiazenyl)-1,3,4-thiadiazole 4,6,8-Me3 (M538), (E)-2-(Azulen-1-yldiazenyl)-1,3,4-thiadiazoles, 3,8-Me2-5-iPr (M540), 4-(azulen-1-yl)-2,6-bis((e)-2-(thiophen-2-yl)vinyl)pyridine, 2-phenyl-5-((4,6,8-trimethylazulen-1-yl)diazenyl)-1,3,4-thiadiazole, acidul 2,2′ - (Etan-1,2 diilbis ((2- (azulen 2-ilamino) -2-oxoetil) azan-diil)) diacetic (**L5**), 2-(azulen-1-yldiazenyl)-5-phenyl-1,3,4-

thiadiazolede (Tabel 1.1) şi s-au propus noi senzori electrochimici pentru detecţia de metale grele. Rezultatele cele mai bune privitoare la liganzii azulenici testați au fost publicate [20] și comunicate la confetințe internaționale sau sunt în curs de publicare (Anexa 11).

Our group recently reported the synthesis of a new molecule, namely 2,2′-(ethane-1,2-diylbis((2-(azulen-2-ylamino)-2-oxoethyl)azanediyl)) diacetic acid (**L5**) [20]. The structure of this molecule is a combination of chromophore and polymerizable unit (azulene), with a complexing unit (EDTA). Its optical characteristics were exploited to assign the ability to selectively complex Hg(II) ions in methanol, and **L5** capability to electropolymerize was used to obtain complexing modified electrodes for metal ions sensing.

In this chapter of the thesis i was presented the results obtained in the continuation of the study of this ligand in order to obtain an analytical platform based on colorimetric, optical and electrochemical sensors for the analysis of heavy metals in water.Thus, being a continuation of our previous published results [20], thework described in this paper bring newinsights on the analytical properties of azulene-EDTA derivative **L5** towards mercury ions. Hg(II) ions were put in evidence by colorimetric means on paper analytical devices (PAD), by UV–Vis spectroscopy and by electrochemistry. Samples from environment were analysed using the most performant methods proposed in this paper, and the results were validated by the standard Cold vapour-Atomic Absorption Spectrometry method.



L5

6.2. Experimental part for the production of sensors based on 2,2′-(ethane-1,2-diylbis((2-(azulene-2-ylamino) -2-oxoethyl)azandiyl))) diacetic acid (L5)

6.2.1. Reagents and apparatus for the study of sensors based L5-

A Xerox Colour Qube 9300, Japan printer was used for wax printing and Brother MFC-L2720DW Japan scanner was used for acquiring images of paper-based analytical devices (PADs).

Jasco V-670 double-beam spectrophotometer was used for spectroscopic measurements performed in quartz cuvettes with 1 cm path length in the UV–Vis domain from 850 to 200 nm.

Autolab PGSTAT204 potentiostat was used for electrochemical experiments performed in a three-electrode configuration electrochemical cell. A plain or modified glassy carbon disk (Φ = 3 mm, from ALS Co. Ltd) served as working electrode, a platinum mesh was used as counter electrode, and Ag/10 mM Ag+ in CH3CN + 0.1 M LiClO4 and Ag/AgCl, 3 M KCl were used as reference electrodes for non-aqueous and aqueous solutions, respectively.

A High Resolution Continuous Source Atomic Absorption Spectrometer (HRCSAAS) ContrAA 700 with a Hydride/Cold Vapour Generator HS 60M (CV) atomisation system from Analytik Jena was used to determine theamount of mercury ions fromrealwater samples.

6.2.2. Methods and procedures for the study of sensors based L5

For colorimetric detection of Hg(II) ions PADs have been prepared using filter paper Whatman no. 1. Circular areas with a diameter of 0.7 cm were computer designed followed by wax printing. Then, a hydrophobic barrier was created by placing the wax-printed paper for 20 s on a hot plate at 170 °C.

The immobilisation of L on PADs hydrophilic surface was performed by four successive additions of 7.5 μL 2 mM solution of L in methanol with drying for 10min between the additions.

Then, the PADs containing L (further called L-PADs) were immersed for 10 min into 4 mL acetate buffer solution containing mercury ions at pH 4. After drying, the test zone of L-PADs was digitalized using a flatbed scanner in order to keep constant lighting across all images.

The JPEG format image was then imported to ImageJ software (National Institute of Health, USA) for analysis in RGB colour space (the range of each channel is from0 to 255) [24] after exposure to analytes. The colour change of L in presence of mercury ions caused a maximum change in the intensity of the blue channel and less changes in the intensity of the red and green channels, as will be shown below.

Therefore, the differences of the blue channel mean intensity values (ΔI) from the inner 75% of the spot (in terms of the radius) between L-PAD without and with Hg(II) were calculated and used as the sensor response.

Spectroscopic measurements were performed in methanol or methanol:water (2:1 volume ratio). Spectral changes of 2 × 10−5 M L solution were noticed after the addition of different amounts of Hg(II). Before recording the spectra, the solution was stirred for 3 min.

The glassy carbon disks (GC) modified with L polymer films (polyL) were obtained from acetonitrile solutions containing L (1 mM) and 0.1 M LiClO4 + 2-molar equivalents of HNO3 by controlled potential electrolysis (at +0.55 V using 0.5 mC polymerization charge).

Due to the electroactivity of polyazulene at ~0.29 V [20] high background currents resulted. This leads to difficulty to detect relatively small amounts ofmercury ions. To prevent this issue, a complete overoxidation of the polyL matrix preceding tometal ions complexation was performed.

Thus, polyL films were overoxidized by polarizing the electrodes in the potential range −0.2 V to +1.6 V (5 successive cycles) in acetate buffer. The obtained electrodes are called hereinafter GC|polyL.

The complexation of mercury ions by polyL films has been accomplished by accumulation in open circuit - anodic stripping method using the previously described procedure [19,20]. Briefly, the accumulation of mercury ions within the complexing polymeric matrix was obtained by immersing GC|polyL modified electrodes into 30 mL aqueous acetate buffer solutions containing Hg(II) ions and/or another specified metal ion (open-circuit accumulation).

After this stage, the unbounded ions were removed by electrode rinsing with purified water. Then, the electrode was introduced into an electrochemical cell containing only the supporting electrolyte (0.1 M acetate buffer) with the same pH as the accumulation solution.

Further, the retained metal ions on polyL surface were reduced to zero valence by polarization at −1.1 V for 3 min followed by anodic stripping using DPV technique. DPV curves were recorded at 20 mV s−1 with an amplitude of 25 mV and 0.5 s pulse periods under Ar atmosphere.

The electrode surface was cleaned with a 0.25 μm diamond paste (from PRESI) followed by a thorough wash with acetonitrile before the electropolymerization process. Unless otherwise specified, each experiment was realised with a newly prepared GC|polyL electrode [19,20].

Real sample analysis performed by CV-HRCSAAS followed the method described by the standard ISO 12846:2012 [25] for mercury analysis without enrichment (using the “universal” NaBH4 reducing agent instead of the mercury-specific one, SnCl2) and ISO 8466-1:1990 [26] for data processing.

Tapwater (fromour laboratory) and natural riverwater (Dambovita River at about 750 m downstream of Ciurel Lake) were collected and analysed for comparison. Portion of the samples was spiked at two levels with the corresponding volumes of an intermediary solution prepared fromHg(II) acetate in acetate-buffered ultra-pure water.

The low spike level was set to be roughly 3 μg/L (1.5×10^{-8} M) and the high spike level was set at 50 μg/L (2.5×10^{-7} M). After homogenization, the samples were divided and processed according to each analytical technique requirements.

6.3. Experimental results for the study of sensors L5-based

6.3.1. Colorimetric sensing of Hg(II) using L-PAD

One of the easiest ways to detect an analyte is represented by PADs based colorimetric technique due to its relatively simple manufacture, short response time and possibility to beminiaturized leading to portability for on-site measurements [27].

Methanol solution of L instantaneously changes its colour from purple to yellow solely in the presence of Hg(II) ions as it has been previously observed with the naked eye [20]. The results observed by the naked eye could be erroneous because humans have different visual acuity. To that can be added errors due to external factors (e.g., environmental lightning and psychological state). Thus, the ability of PADmodifiedwith L monomer (L-PAD) to sense Hg(II) ions fromsolution was examined, and the results were analysed using dedicated software (see Experimental section).

Fig. 1A shows the colour change of the filter paper from white to purple after adding L. The observed differences of the mean colour RGB channels intensity before and after adding L reveal a decrease of the colour which confirms L immobilisation onto paper. Further, in the presence of Hg(II) ions, the purple colour of L-PAD changes to yellow (Fig. 1B), in agreementwith our previous experiments performed in homogeneous phase [20].

The highest change was noticed for the blue channel when comparing the mean colour intensity variation (ΔI) of RGB channels in absence and in presence of Hg(II) ions. This channel was further considered in metal ions sensing experiments.

In order to enhance the sensitivity of L-PAD sensor towards Hg(II) ions different parameters were optimized: the added volume of L on the paper surface, pH of Hg(II) ions solution and complexation time. The differences in the mean colour intensity values of L-PAD before and after the addition of Hg(II) ions were determined in each case.

Fig. 2 shows the influence of the sampling volumewhen L monomer (from 2 mM stock solution) was added onto blank filter paper on L-Hg (II) complex mean colour intensity. The best response was obtained when a volume of 30 μL of L was added. This volume was used further to prepare L-PADs sensors. The addition of a larger amount of L results in the saturation of the paper surface leading to inhomogeneous areas on the sensor surface.

The pH of acetate buffer containing Hg(II) ions was varied between 3 and 6 (Fig. S1). ΔI of the blue channel increases from pH 3 to 4.5 whereupon a slight decrease is observed, probably due to the partially hydrolyzed mercury ions. Therefore, a pH of 4 was selected in the subsequent colorimetric experiments on L-PADs. The dependence of ΔI on complexation time (up to 30 min) was studied (Fig. S2). An optimum for ΔI after 10 min was obtained and, consequently, this time was used in the following experiments.

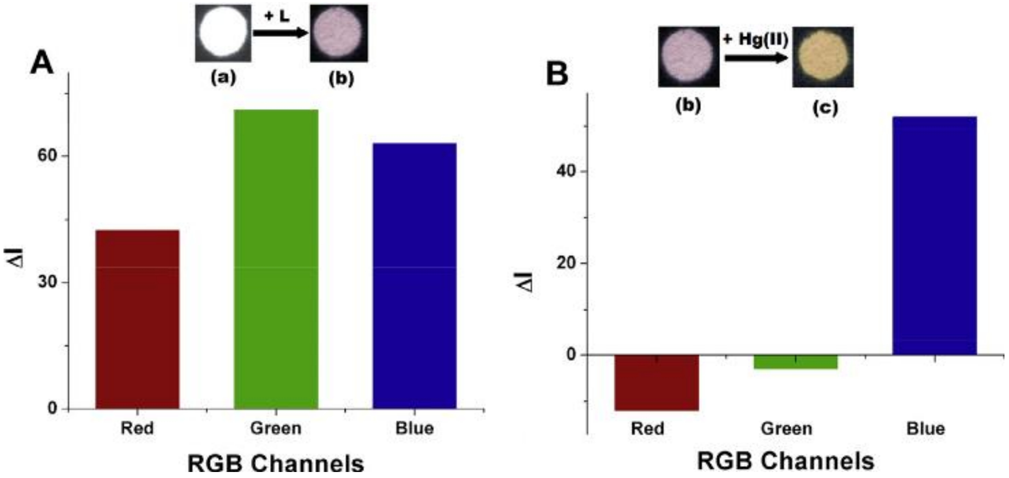


Fig. 6.1. Mean colour intensity variations (ΔI) of RGB channels: (A) before (a) and after the addition of L to filter paper (b) and (B) for bare L-PAD in the absence (b) and in presence of Hg(II) ions (c). Insets: the corresponding images.

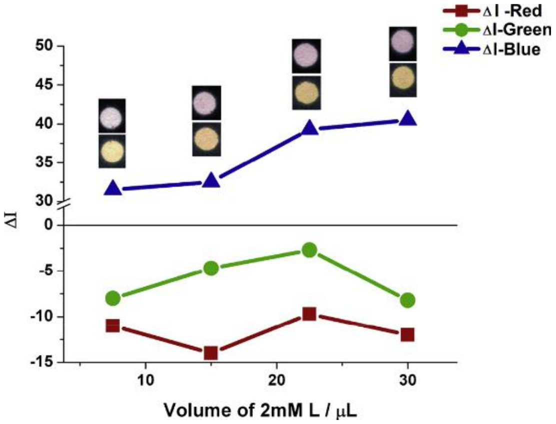


Fig. 6.2 Mean colour intensity differences (ΔI) of RGB channels for different added volumes of L solution (2 mM) after 5 min complexation in 2 × 10−4 M Hg(II) acetate buffer (pH = 4) solution. Inset: the corresponding L-PADs images before (top) and after (bottom) mercury ions addition.

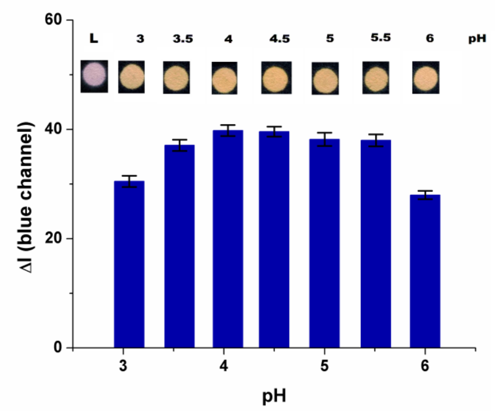


Fig. 6. S1 Influence of pH on the average color intensity difference (ΔI) of the blue channel after 5 minutes of complexation in 2 × 10-4 M Hg (II) acetate buffer. Insertion: corresponding images of PAD without Hg (II) ions (L5) and in the presence of Hg (II) ions at different pHs

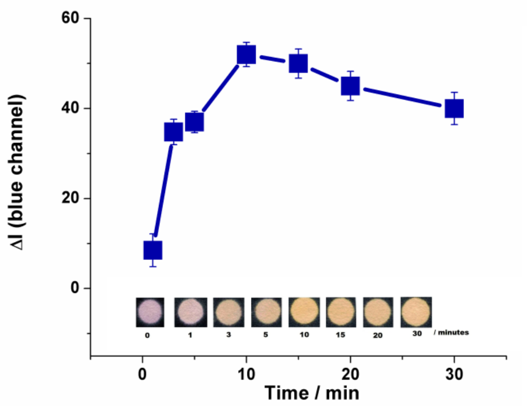


Fig. 6. S2 Influence of complexation time in acetate buffer of 2 × 10-4 M Hg (II) (pH = 4) on the average difference in color intensity (ΔI) for the blue channel. Insertion: appropriate images for L5-PAD without Hg (II) and L5-PAD in the presence of Hg (II) ions at different complexation times (1 - 20 minutes).

Under the previous optimized conditions, L-PAD sensorwas used for the detection of Hg(II) ions fromstandard solutions of different concentrations. A linear dependence on Hg(II) ions concentration in the range 5 × 10−6 M–2 × 10−4 M (Fig. 3) with a correlation coefficient of R2 = 0.968 was established. The limit of detection was estimated at 1.5 × 10−6 M based on 3 times standard deviation/slope.

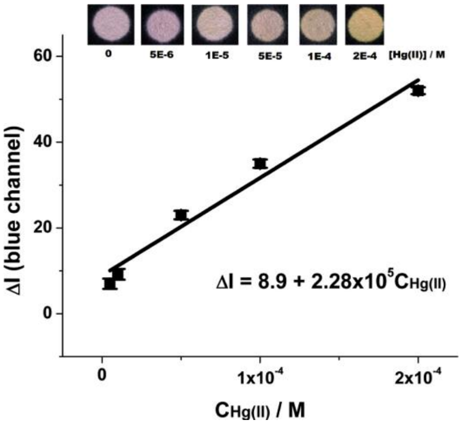


Fig. 6.3 Linear dependence of themean colour intensity difference (ΔI) of the blue channel on Hg(II) concentration for L-PAD sensor. Inset: L-PADs corresponding images.

6.3.1.1. Selectivity and interferences for the determination of Hg(II) using L-PAD

In order to assess the selectivity of L-PAD sensor for Hg(II) ions under optimized conditions, L-PAD response on Hg(II) was examined in the presence of other environmentally relevant metal ions (Cu(II), Pb(II), Cd(II), Ni(II), Co(III) and Cr(VI)). Fig. 4 shows the mean colour intensity variation of the blue channel as determined by ImageJ software for solutions containing 5 × 10−5MHg (II) and 5×10−4Mof all others metal ions. Only the presence ofHg(II) ions changes the colour of L-PAD. Furthermore, these ions were tested as competitive ions in Hg(II) ions detection. Fig. 4 inset shows negligible colorimetric variations of Hg (II) response due to the presence of these ions. Moreover, the high affinity of L for Hg(II) ionswas assessed in a solution containing 5 × 10−4M Hg(II) in the presence of all considered interfering ions each of themat a concentration of 5 × 10−4M. In these conditions, ΔI of L-Hg(II) complex decreases only with ~18%, which indicates a very good selectivity of L towards Hg(II) ions (Fig. S3).

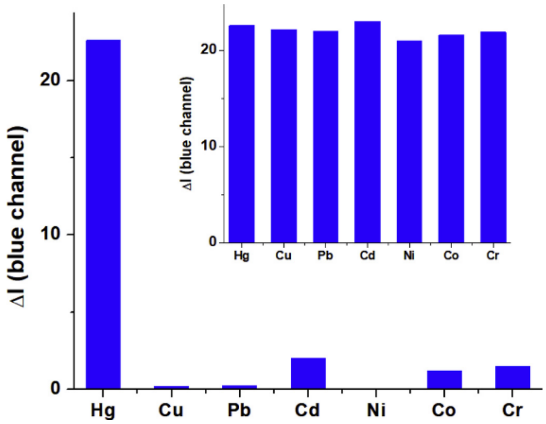


Fig. 6.4. Selectivity of L-PADfor detection of 5 × 10−5MHg(II) and 5 × 10−4MCu, Pb, Cd, Ni, Co and Cr ions. Concentrations of other metal ions 5 × 10−4 M. Inset: Interference effects on the detection of 5 × 10−5 M Hg(II) by Cu, Pb, Cd, Ni, Co and Cr ions (each in a concentration of 5 × 10−4 M).

6.3.2. UV–Vis sensing of Hg(II) using L

The addition of Hg(II) ions to an L solution leads to the occurrence of an additional absorption band in the UV–Vis spectrum at ~440 nm as it has been shown in our previous paper [20]. This new band is due to intramolecular charge transfer interaction that occurs between the two species (L and Hg(II)). Moreover, the presence of an isosbestic point at 384 nm during the titration process reveals that only L and its mercury complex are present in solution.

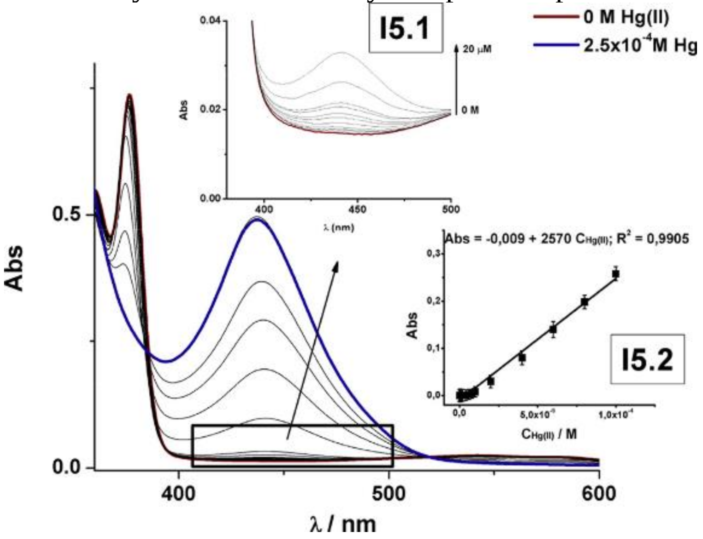


Fig. 6.5. UV–vis titration spectra in the optical active range of L (2 × 10−5 M) interaction with different amounts of Hg(II) ions in methanol. Insets: (I8.1) details of the spectra around 440 nm; (I8.2) the linear domain of the calibration curve absorbance (Abs) read at λ = 440 nm vs. Hg(II) concentrations (CHg(II)).

The sensitivity of Hg(II) detection using L was studied by UV–Vis spectroscopy. Fig. 5 shows the evolution of UV–Vis absorption spectra after the addition of Hg(II) to a 2 × 10−5 M solution of L in methanol. The absorbance at 440nmincreasedwith Hg(II) concentration showing a linear response in the concentration range 10−7–10−4 M (Fig. 5, inset I5.2). A detection limit of 7 × 10−8 M was calculated from the ratio signal/noise = 3. This limit is lower than that obtained with L-PADs (1.5 × 10−6 M).

The detection of mercury ions in methanol implies the addition of a very small volume of sample (usually microlitres) which would lead to very large sample dilutions in case of analyses of real samples of water. Therefore, mercury ions were analysed also in a mixture of methanol: water in a volume ratio of 2:1. The addition of Hg(II) ions to a solution of L (2 × 10−5M) in methanol:water also leads to an increase of the absorption band at ~ 440 nm attributed to the L-Hg(II) complex (Fig. 6).

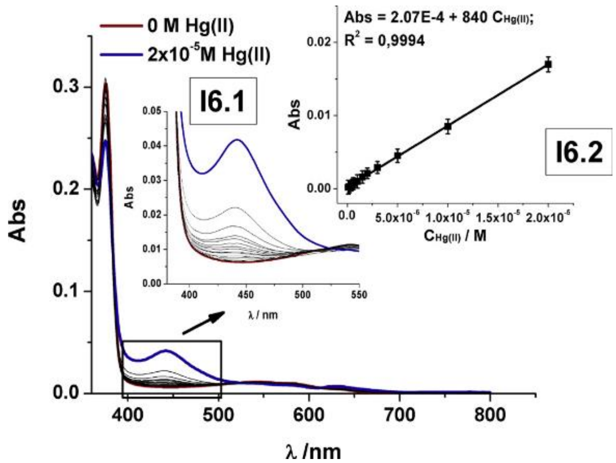


Fig. 6.6 UV–vis titration spectra of L (2 × 10−5 M) on different additions of Hg(II) ions in methanol:water = 2:1 volume ratio. Insets: details of the spectra between 380 and 550 nm (I6.1), and the linear domain of the calibration curve absorbance (Abs) read at λ = 440 nm vs. Hg(II) concentration (I6.2).

The absorbance response vs. Hg(II) concentration varies linearly between 10−7 M and 2 × 10−5 M (Fig. 6, inset I6.2) and a detection limit of 7 × 10−8 M being obtained. Although the same detection limit is attained as in the previous case, the sensitivity and the linearity range are lower. This may be due to a difference in the solubility of the complex formed between the two solvents.

6.3.2.1. Selectivity and interferences in Hg(II) sensing by UV–Vis

The ligand L is selective towards Hg(II) ions among the other studied metal ions (Cu(II), Cd(II), Ni(II), Co(III), Zn(II) and Pb(II) [20]. The corresponding signal for L-Hg complex is only slightly affected (±5.2%) by the presence of 1 M equivalent of all interfering ions (Cu(II), Cd(II), Ni(II), Co(III), Zn(II) and Pb(II)).

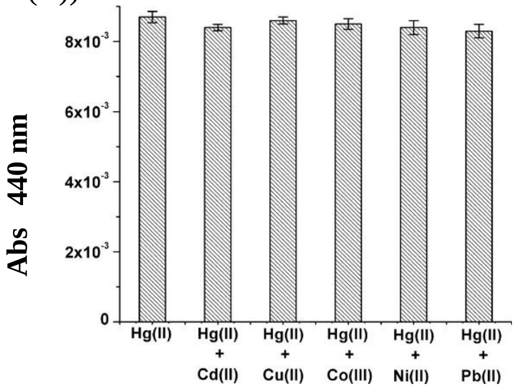


Fig. 6.7 Effect of 5 × 10−5 M (Cd(II), Cu(II), Co(III), Ni(II) and Pb(II)) interfering ions on 5 × 10−6 M Hg(II) ions sensing.

In order to check the influence of each metal ion (Cu(II), Cd(II), Ni(II), Co(III), and Pb(II) on Hg(II) ions detection, the absorbance at the wavelength characteristic for L-Hg(II) complex [20] λ = 440 nm was measured after the addition of 10 fold higher amounts of each interfering ion to a 5 μM Hg(II) solution. The results are shown in Fig. 7. Thus, it can be seen that all considered interfering ions have a negligible effect on UV–Vis absorption peak intensity of Hg(II). This indicates a high specificity of the L-based sensing system towards Hg(II) ions against the considered interfering metal ions.

6.3.2.2. Reversibility of the UV–Vis sensor

The possibility to use the same solution of L for multiple analyses of Hg(II) ions has been investigated. This was accomplished by competitive chemisorption with DDTC. In the presence of Hg(II) ions, L shows a band at 440 nm in the UV–Vis spectrum. This signal was used as an indicator for the complexation/decomplexation process. After the addition of 1 M equivalent of DDTC in L-Hg(II) solution (yellowish colour), the band at 440 nm is strongly diminished to disappearance (Fig. 8). The regeneration of L-free mercury (purple colour) occurs. This confirms that Hg(II) ions were captured by the competitive complexation agent. Using this procedure we could obtain 3 cycles of Hg(II) sensing (Fig. 8, inset) using the same solution of L. This demonstrates the sensor reusability.

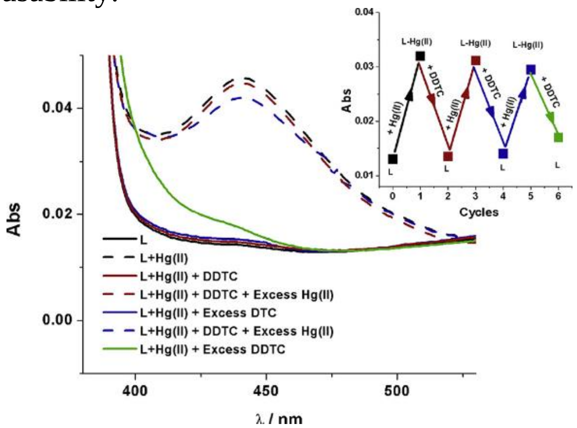


Fig. 6.8. Changes of the UV–Vis spectrum at 440 nm during the sensor complexation/ regeneration processes. Inset: corresponding variation of the absorbance at 440 nm during the sensor complexation/regeneration process..

6.3.3. Voltammetric sensing of Hg(II) ions using GC/polyL modified electrodes

The detection of Hg(II) ions using GC|polyL electrode was achieved by accumulation in open circuit - anodic stripping method [20,21,28]. GC|polyL modified electrodes have been prepared according to the previously published [20] procedure.

In order to optimize GC|polyL electrode sensing properties towards mercury ions, the influence of accumulation solution pH and time, reduction potential and time has been studied.

Because the accumulation of metal ions within polymeric films involves an interfacial process [28,29], the solution pH represent a very important parameter in the metal ions complexation equilibrium. pH influence on Hg(II) stripping peak current was checked in the range 3.0 to 5.5 in 0.1 M acetate buffer solutions (Fig. 9A). A maximum value for the Hg(II) stripping peak current was obtained at pH 4.0. This value is consistent with previous observations that a moderately acidic environment is convenient in EDTA titration for divalent heavy metal cations [30,31]. At higher pH values, Hg(II) peak current intensity decreases due to the hydrolysis of mercury ions [32,33]. Thus, the accumulation solution was kept at pH= 4 in the following electrochemical experiments. The effect of accumulation time of metal ions into complexing polymer films is associated with the type of metallic species presenting various kinetics for complexation and/or through polymeric filmdiffusion [21,28]. The effect of accumulation time in open-circuit on anodic stripping peak currents for Hg(II) ions is shown in Fig. 9B. After20min, the peak currents reach a plateau, hinting that approximately all attainable complexing sites in the polyL film were complexed. This accumulation time for Hg(II) ions is the same with that obtained when using other electrodes modified with complexing polymeric films [34].

To insure a complete reduction of all entrapped mercury ionswithin the polyL film, the reduction potential and time were considered. The effect of the reduction potential was examined between −0.7 V and −1.4 V (Fig. 9C) and the reduction time between 30s and 300 s (Fig. 9D). The highest stripping peak intensity of Hg(II) ions was obtained for a reduction potential of −1.1 V and 180 s. These conditions ensure a complete reduction of complexed Hg(II) ions. The performance of GC|polyL (0.5mC) electrode towards Hg(II) ions at different concentrations (from10−9Mto 2 × 10−6M) was evaluated under the optimized conditions (Fig. 10). A linear domain of Hg(II) ions was obtained in the concentration range from 10−9 to 5 × 10−7M(Fig. 10 inset) with a correlation coefficient of 0.993. A detection limit of 3 × 10−10 M for Hg(II) was estimated based upon the responses for blank solutions (S/N ratio = 3). The obtained detection limit for Hg(II) ions is much lower than that obtained using our colorimetric based sensor (L-PADs) and UV–Vis method. The obtained analytical performances of GC|polyL modified electrodes are comparable, or in some cases better than other EDTA based modified electrodes for Hg(II) ions detection (Table 1).

Tabel 6. 1. Detection limits for voltammetric detection of Hg(II) ions using EDTA based modified electrodes.

Modified electrode	**Detection limit, (M)**	**Ref.**
Poly(ethylenediamine tetra-N-(3-pyrrole-1-yl) propylacetamide)	5×10^{-10}	[21].
Poly(3′,4′-diamino-2,2′;5′,2″-terthiophene)-EDTA	5×10^{-10}	[31]
EDTA-carbon paste electrode	8.6×10^{-9}	[35]
EDTA-polyaniline/SWCNTs[a]	6.8×10^{-7}	[36]
EDTA immobilized graphene-like carbon nitride Nanosheets	5.7×10^{-13}	[37]
PIGE[b]/MWCNT[c]/EDTA/HNO_3	7.68 × 10−7	[38]
Poly(N,N′-ethylenebis[N-[(3-(pyrrole-1-yl)propyl) carbamoyl)methyl]-glycine])	10^{-8}	[39]
Poly(2,2′-(ethane-1,2-diylbis ((2-(azulen-2-ylamino)-2-oxoethyl)azanediyl))diacetic acid)	3×10^{-10}	Present Work

[a] SWCNTs = single walls carbon nanotubes.
[b] PIGE = paraffin impregnated graphite electrodes.
[c] MWCNT = multiwalls carbon nanotubes.

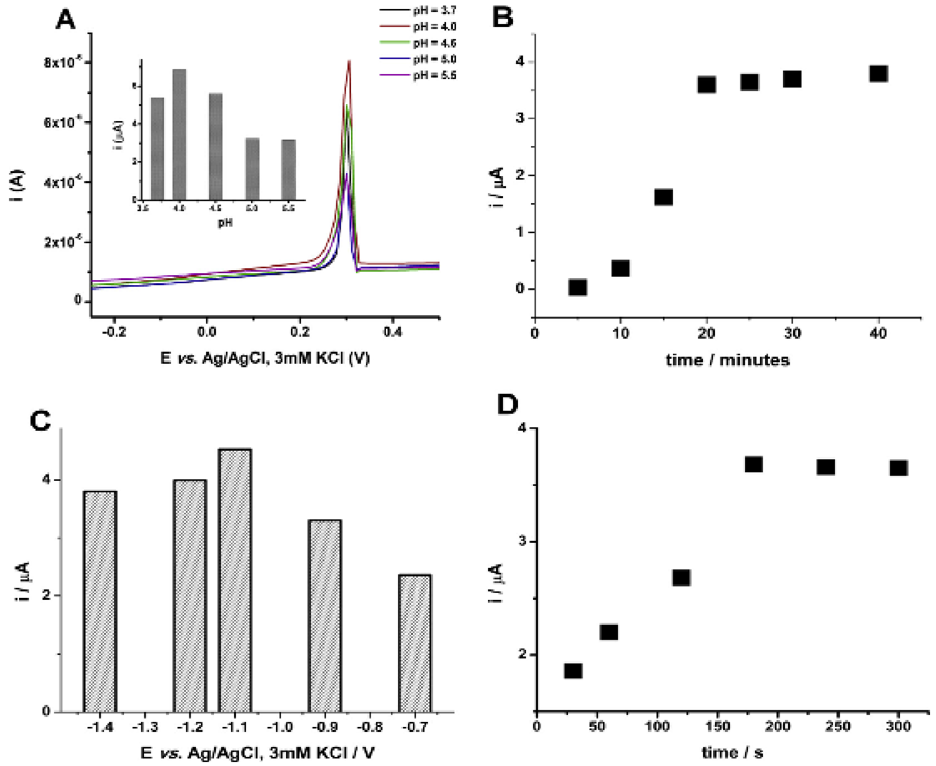


Fig. 6.9. The effect of various factors which influence Hg(II) complexation process by CG|polyL electrode: A) pH of acetate buffer solutions, B) open-circuit accumulation time, C) mercury ions reduction potential and D) mercury ions reduction time; Hg(II) concentration is 5×10^{-7} M.

6.3.3.1. Reproducibility of modified electrodes with L5

The experiments aimed to check the reproducibility of the modified electrodes for Hg(II) ions sensing. Thus, the reproducibility of different electrodes obtained in the same experimental conditions and the reproducibility of the same or freshly prepared modified electrode for repeated Hg(II) ions analyses were evaluated. A solution of 5×10^{-7} M Hg(II) was used to analyse five individual GC|polyL electrodes (0.5 mC). A relative standard deviation (RSD) of 4.32% was attained.

This denotes a very good reproducibility of the preparation and sensing processes, which is very useful in practical applications at a large scale. 10 to 20 successive analyses of the same 5 × 10−7 M Hg(II) solution were performed to evaluate the possibility to use the same GC|polyL electrode. The effective regeneration of mercury-free polyL films was achieved by competitive chemical desorption in 10−3MDDTC aqueous solution for 10min under stirring followed by careful rinsing. Using this procedure, RSD of 4.1% and 12.6%, were obtained after 10 or 20 consecutive measurements, respectively. However, the regeneration of the modified electrode with EDTA or by electrochemical regeneration in 0.1 M HNO_3 at constant/potentiodynamic potential was not possible.

10 successive analyseswere also performed in the same solution of 5 × 10−7 M Hg(II) using each time a freshly prepared electrode. The obtained RDS value of 3.9% denoted a very good electrode preparation process reproducibility.

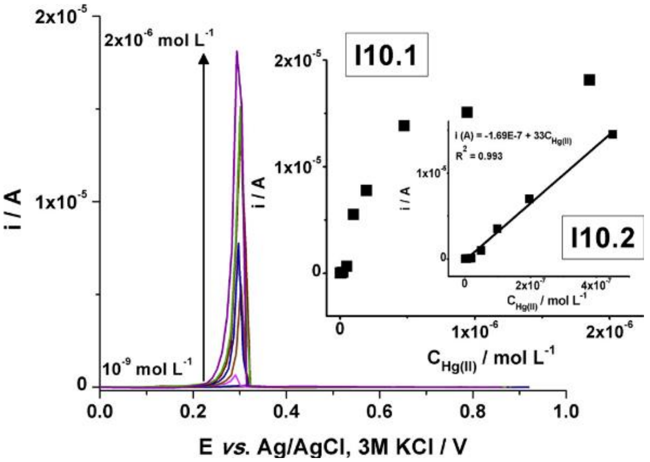


Fig. 6.10. DPV anodic stripping curves at GC|polyL electrodes for different concentrations of Hg(II) ions. Insets: calibration plot (I10.1) and its corresponding linear domain between 10−9 M to 5 × 10−7 M (I10.2).

6.3.3.2. Interferences in Hg(II) sensing using GC/polyL modified electrodes

EDTA can bind a variety of metal cations in solution and its affinity for different metal ions strongly depends on pH [40]. However, in case of chemically modified electrodes, within the accumulation step performed in open circuit, their selectivity can be adjusted by varying the process parameters (e.g. metal ions accumulation time, film thickness and/or imprinted effect of the polymers) [34]. In our previous work [20] it has been showed that polyL films are able to complex Hg(II) Cd (II) and Pb(II) ions from an aqueous acetate buffered solution (0.1 M, pH = 5.0) containing a mixture of metallic ions (Hg(II), Cd(II), Zn(II), Ni(II), Pb(II), Cu(II), and Co(III)).

DPV curves were recorded for increasing amounts of interfering ions (Fig. 11) to determine the selectivity of polyL films towards Hg(II) ions under optimal conditions (pH = 4, accumulation time of 20 min and −1.1 V deposition potential for 180 s). Firstly, GC|polyL modified electrode was introduced for 20 min in acetate buffer solution (pH = 4) containing 5 × 10−7 M Hg(II) and the corresponding DPV curve was recorded. Then, to this solution interfering ions (Cd(II), Pb(II), Zn(II), Ni(II), Co(III), and Cu(II)) were added. These metal ions were further denoted with I.

After adding 1 equivalent of all interfering ions, DPV curve reveals also only two stripping peaks at −0.7 V and −0.54 V attributed to cadmium and lead ions, respectively. When a higher excess of I is added, a new stripping peak appears at −0.1 V assigned to copper ions. The presence of these new peaks indicates that polyL films presents some sensitivity towards these ions being in agreement with others results obtained by using EDTA based electrochemical sensors [21,36,41]. However, the presence of interfering ions in an increased ratio has a negligible influence on the DPV peak current for Hg(II) ions. The peak intensity is maintained in the domain of ±5% from the original value until an excess of 20 eq. I was added (Fig. 11, inset). Moreover, the peak current intensity remains at 90% from the initial height even when 50 equivalents of I are added. This reveals the small influence of considered interferents overHg(II) analysis in the proposed method (Fig. 11 inset). This behaviour suggests that Hg(II) complexation is stronger than that of the other interfering ions and leads to the occupation of themost accessible complexing sites in the polymer film.

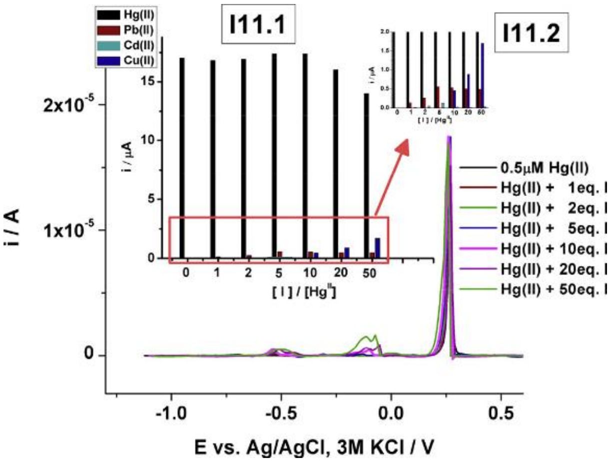


Fig. 6.11. Effect of interfering cations (I: (Cd(II), Pb(II), Zn(II), Ni(II), Co(III), and Cu(II)) on 5 × 10−7 M Hg(II) detection after open-circuit accumulation of 20 min in acetate buffer (pH = 4). Each interfering ions is at the same specified molar equivalent vs. Hg(II) ions concentration. Insets: Variation of Hg(II), Cd(II), Pb(II) and Cu(II) peak currents with [I]/[Hg(II)] ratio in the accumulation solution (I11.1) and detail of small DPV currents (I11.2).

6.3.4. Analysis of real samples using L5-modified electrodes

To demonstrate the feasibility of the obtained sensors in practical applications, real water samples were analysed. Natural river water and tap water mercury spiked samples were analysed for the comparison of the newly developed methods with the consecrated Cold Vapour Atomic Absorption Spectrometric technique.

Both tap water and river water mercury contents were determined by CV-HRCSAAS, UV–Vis spectrometry using L (L/UV–Vis) and GC| polyL-anodic stripping voltammetry (GC|polyL-ASV). Without enrichment, both contents were lower than the stated detection limit of about 0.05 μg/L. The analysed mercury content of the spiked samples together with their ISO 8466-1 confidence level is presented in Table 2. It can be seen that the recovery (between 98% and 101%) is complete for Hg(II) for both sensors. This shows that the proposed sensors can be successfully applied for Hg(II) ion analysis fromenvironmental samples.

Tabel 6.2 Determination of Hg(II) content in tap and river water samples using CV HRCSAAS, L/UV–Vis spectrometry and GC|polyL electrodes.

Sample	Added Hg(II) (M)	Method		
		CV-HRCSAAS	**L5**/UV–Vis	GC|poly**L**-ASV
River water	-	[a]ND	ND	ND
River water spike I	1.5×10^{-8}	$1.31 \pm 0.21 \times 10^{-8}$	ND	1 $1.47 \pm 0.35 \times 10^{-8}$
River water spike II	2.5×10^{-7}	$2.40 \pm 0.19 \times 10^{-7}$	$2.52 \pm 0.52 \times 10^{-7}$	$2.49 \pm 0.15 \times 10^{-7}$
Tap water	-	ND	ND	ND
Tap water spike I	1.5×10^{-8}	$1.32 \pm 0.21 \times 10^{-8}$	ND	$1.51 \pm 0.23 \times 10^{-8}$
Tap water spike II	2.5×10^{-7}	$2.51 \pm 0.20 \times 10^{-7}$	$2.48 \pm 0.32 \times 10^{-7}$	$2.52 \pm 0.19 \times 10^{-7}$

[a] ND - not detected.

6.4. Conclusions on the development of new multi-signal sensor platforms for Hg (II)

In this chapter of the thesis the versatility of the derivative 2,2′-(ethane-1,2-diylbis((2-(azulen-2-ylamino)-2-oxoethyl)azanediyl))diacetic acid (L) in the development of novel multi-signal sensor platforms for mercury ion detection has been demonstrated. By using L, colorimetric (paper based and UV–Vis spectrometry) and voltammetric (based on complexing chemically modified electrodes) sensors were obtained in a very simple manner. These sensors have been successfully used for the detection of mercury ions from aqueous samples and also showing a high selectivity for these ions. Under established optimal conditions, detection limits of 1.5 × 10−6 M, 7 × 10−8 M and 3 × 10−10 Mwere obtained by using PADs, UV–Vis spectrometry, and chemically modified electrodes sensors, respectively. These sensors could be regenerated, so they can be used in repeated mercury ion analyses.

Using these sensors, mercury ions could be detected from natural water samples. The obtained results were consistent with those obtained by atomic spectrometry, indicating that these sensors can be applied in practical situations.

Capitolul 7. Correlations between water quality parameters

7.1. The need to correlate quality parameters

This chapter of the doctoral thesis brings together the results of research conducted on data on Danube water quality, being the first in a series of studies conducted to monitor water quality in Chiciu, Calarasi County, Romania, over a period of 3 years (2010-2012) in order to improve the quality of the Danube water downstream in the years following the period considered. This study serves as a reference basis to evaluate the impact of the second phase of modernization works at the Bucharest Wastewater Treatment Plant (2017-2019).

7.2. Experimental part in correlating the quality parameters in the study

7.2.1. Water sampling and analysis

Chiciu monitoring station is located, in the Lower Danube at 375 km (geographical coordinates: latitude 44°07'60.0"N and longitude 27°16'22.0"E). A number 234 water samples, collected from both banks of the river and from the middle during January 2010 – December 2012, were analysed within the national monitoring programme. Sampling was carried out according to standard EN ISO 5667:2007 (parts 1, 3, 4, 6, 10, 11) and internal specific procedures of National Administration “Romanian Waters” (ANAR). The measurements and analysis methods are presented in Table 1.

7.2.2. Statistical analysis methods

Environmental data were analysed using statistical methods in order to assess the quality of environmental factors against given standards, to identify correlations between different parameters and to see how the parameters have evolved in time, particularly if some changes have occurred in a certain region. The statistical methods used in this study are summary statistics, correlation (Pearson and Spearman) analysis, covariance, principal component analysis and linear regression.

Principal component analysis (PCA) was used for the large experimental dataset with many parameters in order to identify which parameters play the most important role in its variation. This analysis may also be used to validate the results of the correlation analysis. By centring and scaling data, the influence of measuring units and orders of magnitude is removed.

Linear regression was also applied as a modelling method that presumes a linear dependence between two variables and it was used to analyse the evolution in time of studied parameters.

Statistical analyses were carried out using the RStudio software Version 1.1.463, based on R version 3.5.2.

7.3. Results regarding the correlation of quality parameters

7.3.1. Summary statistics

The monitoring data set was checked for consistency, and the parameters with the highest number of observations were selected for further analysis. Data are presented in a statistical Table (Table 2) which includes the maximum and minimum values, mean, median, 1st quartile (the middle value between minimum and median) and 3rd quartile (the middle value between medium and maximum value).

Table 2 allows us to assess the water quality during the study period according to norms. In Romania water quality assessment is regulated by Ministry Order 161/2006 regarding classification of surface waters [13]. The Order defines five quality classes I-V, with class I being best quality and class V the poorest. According to the limits set in this Order, values of measured DO range between class III (minimum value) and class I (maximum value). The median is also in class I (>9 mg/L, which means that more than 50% of measured values are in class I.

BOD5 values up to the 3rd quartile are in class I, which means that more than 75% of measured values are below the 3 mg O2/L limit. There is one extreme outlier, the maximum value, but all the other values are smaller than 5 mg O2/L, which is the class II limit.

Ammonium levels are also in class I, with most values lower than 0.4 mg N/L. Only 4 values are in class II, including the maximum value. Nitrites qualify in classes I to IV. The first quartile in already in class II, being higher than 0.01 mg N/L, and the third quartile is also in class II (<0.03 mg N/L). The maximum value is in class IV. Nitrates values are in classes I-III, with the 1st and 3rd quartiles in class II, while the maximum value is in class III. Total Nitrogen values are in classes I and II. From the 1st quartile to the maximum, all values are in class II (between 1.5 -7 mg N/L).It should also be noted that all values for nitrates are much lower than the EU Water Framework Directive limit of 50 mg/L. Ortho-phosphates are in class I up to the 3rd quartile, while the maximum value is in class II. Total Phosphorus is also in class I up to the 3rd quartile, with the maximum value in class II.

According to Romanian Standards, most values of the analysed parameters from period 2010-2012 in Chiciu are in water quality classes I and II.

7.3.2. Correlation and Covariance Analysis

Correlation and covariance analysis were carried out by calculating Pearson and Spearman coefficients. Figure 1 represents the nine selected variables plotted against each other below the diagonal, with Pearson correlation coefficients above the diagonal and histograms on the diagonal. Histograms show if data are skewed and it can be noticed that BOD, as well as ammonium, nitrates, nitrates and phosphorus data are skewed to the left, which may be an indication of accidental pollution.

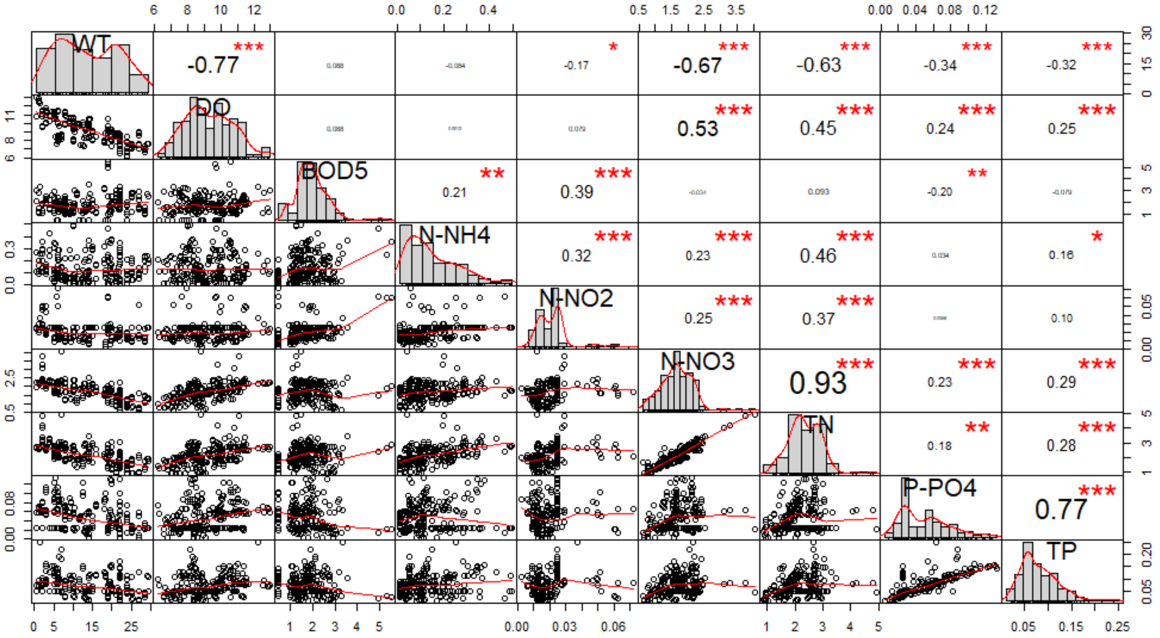


Fig. 7.1 Pairwise scatterplot matrix for the selected parameters with Pearson correlation factors for each pair. Significance codes for p values: $0.01<p<0.05$ (*), $0.001<p<0.01$ (**), $0<p<0.001$ (***). To improve the accuracy of the analysis, one BOD5 outlier was removed

Spearman's rank correlation coefficients are a non-parametric measure of monotonic associations between variables and were calculated in addition to Pearson's correlation because many of the analysed data are skewed and include outliers. Significant positive (a relationship in which both variables move in tandem—that is, in the same direction) linear correlations are noticed between nitrate nitrogen N-NO3 and total nitrogen TN (0.93 Pearson, 0.89 Spearman). Also, significant positive correlations are between ortho-phosphates P-PO4 and total phosphorus TP (0.77 Pearson, 0.81 Spearman) while a significant negative correlation is noticed between water temperature WT and dissolved oxygen DO (-0.77 Pearson, -0.77 Spearman). There are also weaker negative correlations between WT and N-NO3 (-0.67 Pearson, -0.70 Spearman) and between WT and TN (-0.63 Pearson, -0.65 Spearman). These results are supported by the low calculated p values ($p<0.001$).The negative correlations between water temperature, on one side, and DO, N-NO3, TN on the other side may be a reflection of eutrophication during the warm seasons, solar radiation and the availability of nutrients leading to a proliferation of algae, which consume nitrogen and phosphorus for their metabolism, while their decay depletes the waters of dissolved oxygen.

The negative correlation between WT and DO is easily explained by the physical solubility of oxygen in water, which decreases as temperatures rise (Henry's law). However, the seasonal variation of nutrients concentrations (nitrogen and phosphorus) would require further investigation of the factors that influence the values of these parameters (population density, livestock numbers, land use, agriculture activities, physical and metabolic processes).

7.3.3. Principal component analysis

Principal component analysis (PCA) was carried out in order to identify which parameters have a stronger influence on the variation of the data set and relationships between them. Because the selected variables have different units of measure and ranges of variation, data were scaled and centred (normalised) before calculating the principal components. The standard deviation of each of the principal components (PC), and their rotations (or loadings) are presented in Table 3. Loadings are the coefficients of the linear combinations of the continuous variables [14]. The new variables (PC1, PC2, PC3 etc.) are factors or components.

For example, the loadings of PC1 mean that it is mainly influenced by TN (-0.46), N-NO3 (-0.46), WT (0.45) and DO (-0.38), and the proportion of variance means that PC1 explains 39.18% of the variance of the data set. The most important roles in PC2 are played by ortho-phosphates P-PO4 (0.58) and TP (0.49), followed by nitrites N-NO2 (0.35) and ammonium nitrogen N-NH4 (0.35). Together, PC1 and PC2 explain 56.81% of the variation of the data set. PC1 (Dim1) and PC2 (Dim2) are represented in Figure2.

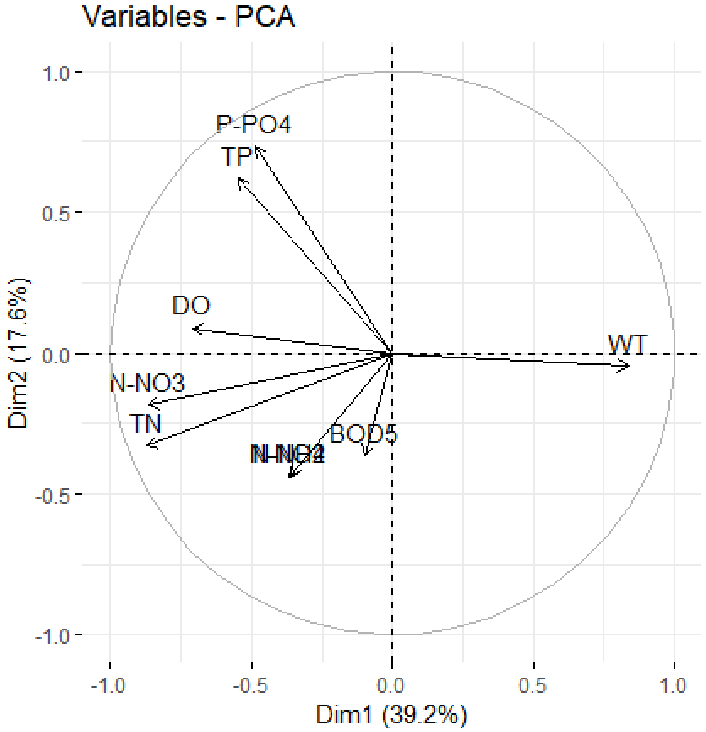


Fig. 7.2. Graphical representation of Principal Component Analysis, PC1 (Dim1) and PC2 (Dim2) of working data set (Chiciu 2010-2012)

The arrows in Figure 2 represent variables, and the longer the arrow, the higher the variable's influence on the data set. The angles between arrows represent correlations between parameters: right angles indicate no correlation, angles less than 90 degrees indicate positive correlation and angles more than 90 degrees indicate increasing negative correlation. The Figure indicates very clearly the strong negative correlation between water temperature WT and dissolved oxygen DO that was identified in the previous section, as well as the positive correlations between N-NO3 and TN on one side and P-PO4 and TP on the other side.

The most important roles play the parameters WT, DO, N-NO3 and TN in PC1, P-PO4 and TP in PC2, N-NH4in PC3, BOD5 in PC4, and N-NO2in PC5, respectively. Together, the first five components explain 89.21% of the variation of the dataset.

7.3.4. Graphical Data Representation and Linear Regression

In this section, the groups of parameters that were identified as correlated are represented in scatterplots according to sampling dates, in order to investigate how they evolved during the study period 2010-2012 (Figures 3-6). To make the charts clearer, only data from the Romanian left bank were represented.

Figure 3 represents the water temperature (WT), dissolved oxygen (DO) and total nitrogen (TN), the variables for which a high correlation was identified. The Figure highlights the seasonal variation, but there is no obvious general trend during the three years when data were collected. It can be noticed that high WT values correspond to low DO and TN, in agreement with the negative correlations highlighted before.

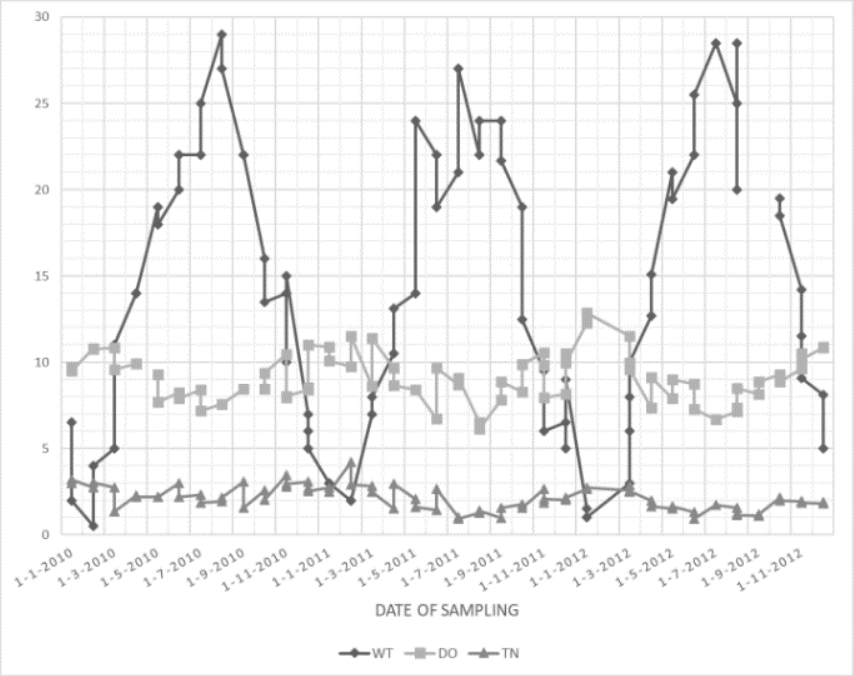


Fig. 7.3 Graphical representation of water temperature WT [°C], dissolved oxygen DO [mg/L] and total nitrogen TN [mg/L] at Chiciu, left bank, 2010-2012.

Fig. 7.4. Graphical representation of NNH4 [mg/L], N-NO2 [mg/L], N-NO3 [mg/L] and total nitrogen TN [mg/L] at Chiciu monitoring station, left bank, 2010-2012.Dotted regression line is for TN

The values of the different nitrogen species (N-NH4, N-NO2, N-NO3) are represented in Figure 4 together with total nitrogen (TN). Values for nitrates and TN are close, showing that nitrates are the most abundant nitrogen species. The chart also shows seasonal variations, with lower values during summer and higher values in the cold season, as well as the positive correlation between N-NO3 and TN identified before. Ammonium nitrogen (N-NH4) could be an indicator for sewerage pollution, as it is rapidly released from urea. However, its variation does not present an obvious pattern.

The regression line indicates a slight decrease in TN values during the study period (p-value < 0.001), which may be a consequence of improved wastewater treatment in Bucharest (first phase of secondary treatment commissioned in the end of 2011).

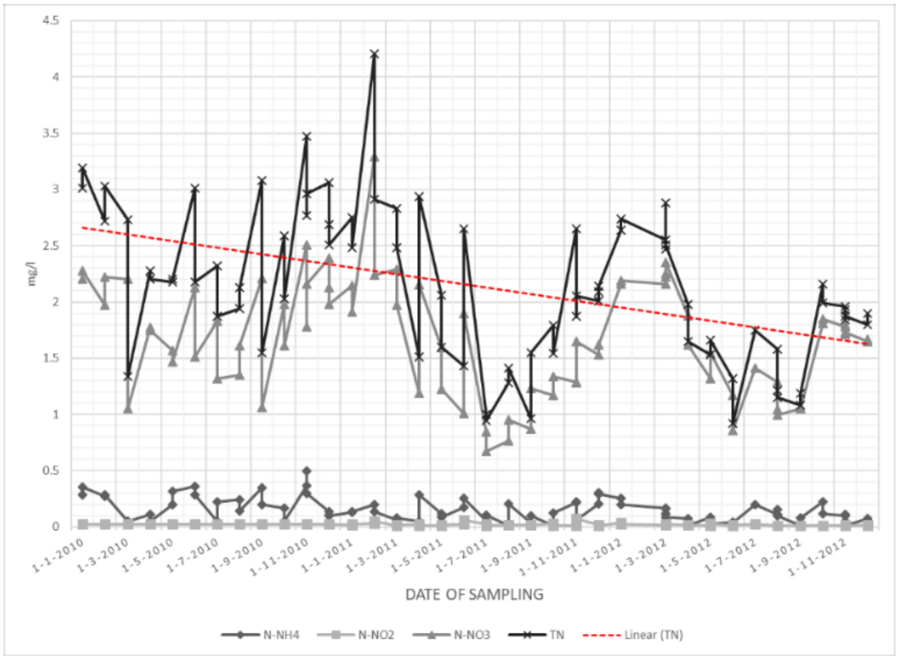


Figure 5 represents the phosphorus parameters P-PO4 and TP. The represented values are close, so it can be seen that ortho-phosphates are the main contributors to total phosphorus. The seasonal variation is more obvious in 2011 and 2012 and the linear regression line for TP also indicates a slight decline of pollution during the analysed period (p-value: 0.006319). Ortho-phosphates are the main phosphorus species, and their levels are close to zero in some of the summer months, so they appear to be the limiting factor for algal growth in this case, like in most surface waters [15].

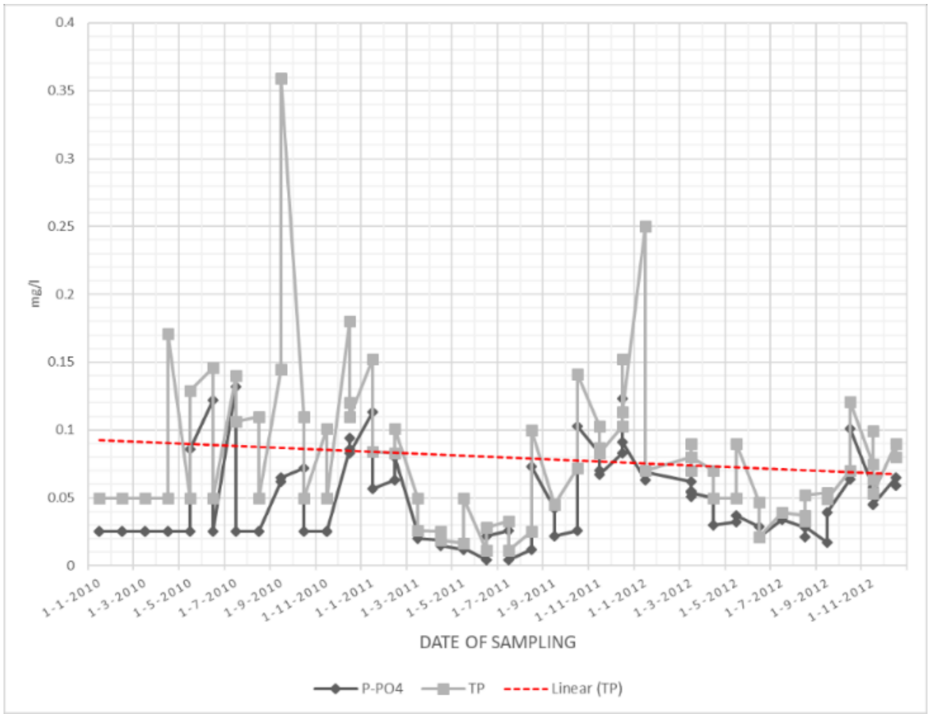


Fig. 7.5. Graphical representation of P-PO_4 [mg/L] and total phosphorus TP [mg/L] at Chiciu monitoring station, left bank, 2010-2012.Dotted regression line is for TP

The minimum level of nutrients reached during hot and dry periods is probably due both to reduced inputs from agricultural land runoff and increased biological activity in the water body. In addition to biological uptake, at low oxygen levels nitrogen is removed from surface waters by denitrification, a process in which different oxidation forms are reduced to molecular N_2 and released to the atmosphere. In this respect, it was estimated that in Northwest Europe about half of the nitrogen released to surface waters is lost by denitrification until they reach the sea [16]. A Joint Research Centre project has successfully modelled nitrates variations in the Danube using the Soil and Water Assessment Tool (SWAT), however for the denitrification process it was not possible to generate an accurate model [6].

Oxygen parameters are used to monitor organic pollution in water and are presented in Figure 6. Chemical Oxygen Demand (COD) was not included in the analysed data set because COD-Cr values are not relevant for 2010, and both COD-Cr and COD-Mn were less frequently determined than BOD5. Biochemical Oxygen Demand (BOD5) is the amount of oxygen needed for decomposition of organic matter by microorganisms (bacteria) in five days and is measured in mg/L. The linear regression for BOD5 (Figure 6) also shows a slight decline during the study period ($p\text{-value} < 0.001$).

Determination of Chemical Oxygen Demand (COD) is also a method to estimate organic loads in water. COD-Mn is carried out using $KMnO_4$ in H_2SO_4 and has similar values to BOD.This method oxidizes biodegradable organic matter and around 30-40% of the non-biodegradable compounds. COD-Cr uses $K_2Cr_2O_7$ as oxidising agent in acid environment and it determines 60 - 70% of organic matter [17].

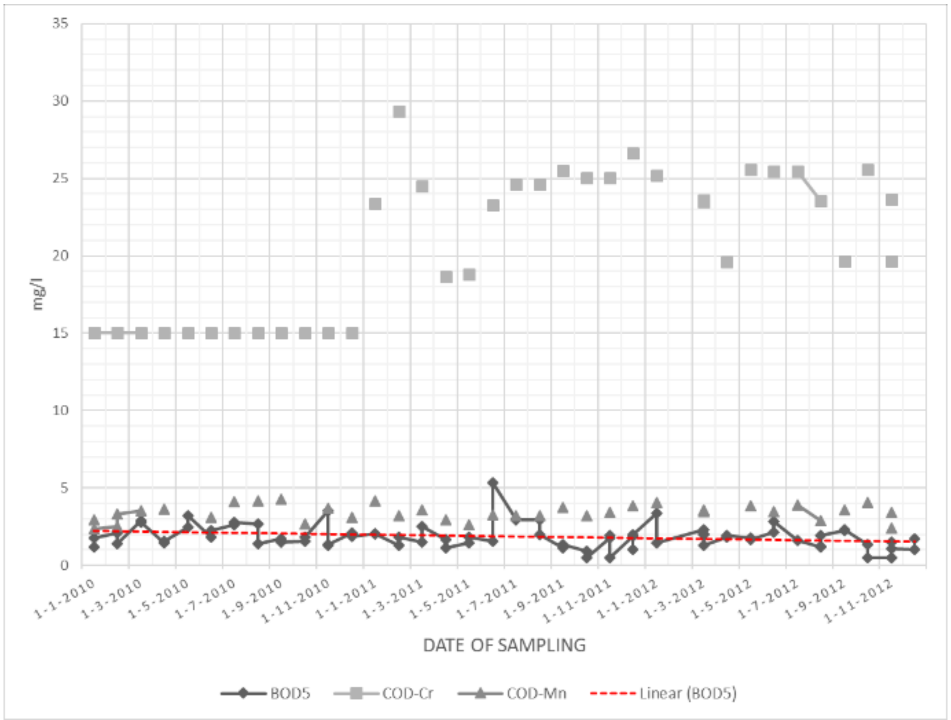


Fig. 7.6. Graphical representation of chemical oxygen demand (COD-Cr, COD-Mn) [mg/L] and biochemical oxygen demand BOD5 [mg/L] at Chiciu monitoring station, left bank, 2010-2012. Dotted regression line is for BOD5

Potassium dichromate is a stronger oxidising agent than potassium permanganate, which is why COD-Cr values are higher than COD-Mn values [18].

The difference between COD-Cr on one side and COD-Mn and BOD5 on the other side, which can be seen in Figure 6, may be an indication that a large part of the organic pollutants present in Danube water are complex compounds, which are not easily biodegradable.

7.4. Conclusions on the correlation of quality parameters

The results of the study on the correlations between the water quality parameters from Chiciu, Călăraşi County, Romania, for a period of 3 years (2010-2012) allowed the monitoring of the Danube waters quality and the monitoring of the evolution of the studied indicators.

According to the socio-economic characteristics of the adjacent regions, the Danube River receives tributaries loaded with various pollutants. The analyzes showed positive correlations between nitrates and total nitrogen, respectively orthophosphates and total phosphorus. Negative correlations were found between water temperatures and dissolved oxygen on the one hand and nitrogen on the other. These parameters have a seasonal evolution, with high temperatures and low concentrations of dissolved oxygen and nitrogen, in the summer months.

A decrease in linear regression of pollution was observed during the monitoring period, which may be due to the improvement of wastewater treatment processes from the treatment plants, which then reach the Danube.

Nutrient pollution appears to be declining, which could be linked to improved wastewater treatment in large cities flowing into the tributaries of the Danube.

The analyzed data indicate that the main source of nitrogen pollution is agricultural land, although low nitrogen levels during the summer may also be the result of increased algae growth, which uses nutrients for their metabolism. Phosphorus seems to be the limiting factor for algae proliferation, as its concentration in the study area is close to zero in some of the summer months.

CONCLUSIONS

C1. GENERAL CONCLUSIONS

In this doctoral thesis, research was conducted in order to develop a real-time analytical platform based on chemical, electrochemical and optical sensors for the analysis of water quality in water resources. Taking into account the experience of the team, the original research was aimed at obtaining modified electrodes with complexing films.

The combination of modern electrochemical techniques with discoveries in microelectronics and miniaturization creates premises for the introduction of new powerful and reliable electrical devices for the efficient control of processes or environmental pollution. This allows to take into account the evolutions of the parameters followed from a distance and in close time to the real one. The development of sensors increases the monitoring capacity and allows sensitive and selective detection capabilities. In recent years, a wide range of electrochemical devices have been developed for in situ monitoring of numerous inorganic and organic impurities. However, there is an awareness that electrochemical sensor technology cannot meet all the needs of environmental monitoring. However, the emergence of new electrochemical devices based on new technology brings the achievements closer to market requirements. Future efforts are needed in the fields of "smart" molecular sensors and devices, multiparametric sensor arrays and remote electrodes. "Smart" devices, capable of "switching" between "screening /

warning" and "detailed analysis" operating modes, are in high demand for various environmental scenarios.

The move to advanced miniaturization brings together areas such as chemistry, engineering and physics. The integration of electrochemical technology in microfluidic platforms, for example, further facilitates on-site and in situ environmental monitoring efforts. Details about the performance, design and perspectives of electrochemical sensors are covered in Chapter 1 of the doctoral thesis.

In the bibliographic study from Chapter 2 of the thesis were analyzed a large number of bibliographic references available on the Internet and deepened the results of several research studies conducted in the laboratory of electrochemical processes in organic solvents (PESO) based on modified electrodes . In order to study new sensors, the obtained results were analyzed and a ligand (4-azulen-1-yl-2,6-bis (2-thienyl) pyridine) with experimentally tested properties was selected as a model for the original researches provided for. be performed in the thesis. This analysis led to the proposal of experiments to characterize the new sensors included in the original part of the thesis. In order to obtain a research method that would quickly lead to a final product, the results of the team for poly films (4-azulen-1-yl-2,6-bis (2-thienyl) pyridine) were analyzed. These showed complexing properties and led to the development of reliable electrochemical sensors, with results consistent with measurements on standard equipment, such as Atomic Absorption Spectrometry Graphite Furnace. The electrochemical method underlying their design is simple and suitable for routine analysis of environmental samples.

In order to extend the range of sensors and their applications in Chapter 3 of the doctoral thesis, the bibliographic works on oxidation and electrochemical reduction of compounds with As, Sb and Bi were studied. The parameters that characterize their oxidation and reduction were highlighted, which take place at close potentials for the 3 investigated metals. The most influenced by the structure are the compounds with As.

Chapter 4 of the thesis presents the main experimental details regarding the studies performed (electrochemical experiments by CV, DPV, RDE, spectral and optical experiments) related to the development of new sensors based on modified electrodes and their characterization by electrochemical and spectral methods.

In Chapter 5 of the thesis are presented the main results related to the study of new organic compounds. would be the use of these compounds in the treatment of leukemia and other cancers, to obtain functionalized gold nanoparticles of triphenylarsonium or to obtain intracellular nanoparticles. The results are being published in the journal Polytechnic University of Bucharest Scientific Bulletin.

Chapter 6 of the thesis presents the results of research related to the development of new chemical, electrochemical and optical sensors based on 8 azulene derivatives. The most relevant results that were the subject of an article published in a prestigious journal (Journal of Electroanalytical Chemistry). These results obtained for the azulene derivative of EDTA, namely for the diacetic acid 2,2 ′ - (ethane-1,2-diylbis ((2- (azulen-2-ylamino) -2-oxoethyl) azanediyl)) (L5) are presented in detail in this chapter of the thesis. The versatility of the L5 derivative in the development of new multi-signal sensor platforms for the detection of mercury ions has been demonstrated. L5-based, colorimetric (paper-based), spectrometric (UV-Vis) and voltammetric sensors were obtained using modified electrodes, based on the chemical complexation of Hg (II) by L5. These sensors have been used successfully to detect mercury ions in water samples and have a high selectivity for these ions. Under the optimal conditions determined for each of the 3 types of sensors obtained, the detection limits of 1.5×10^{-6} M, 7×10^{-8} M and 3×10^{-10} M were established using colorimetric detection (PAD- uri), UV-Vis spectrometry, respectively electrochemistry based on chemically modified electrodes. These sensors could be regenerated so that they can be used in repeated analyzes of mercury ions. Using these sensors, mercury ions could be detected in natural water samples. The results obtained were consistent with those determined by the atomic absorption spectrometry method, indicating that these sensors can be used in practical situations. Through this chapter, the doctoral thesis made significant contributions to obtaining new sensors based on azulene derivatives for the determination of heavy metals in water.

Chapter 7 of the thesis brings together the results of studies on the correlations between water quality parameters in Chiciu, Calarasi County, Romania, over a period of 3 years, which allowed monitoring the quality of Danube waters and monitoring the evolution of the indicators studied. These results are of great importance given the European projects aimed at studying the socio-economic characteristics of the regions bordering the Danube River, which receives tributaries loaded with various pollutants. The analyzes performed at Chiciu point allowed the observation of the decrease in linear regression of the pollution during the monitoring period, which may be due to the improvement of the wastewater treatment processes from the treatment plants, which then reach the Danube. The trend of decreasing nutrient pollution may be related to the improvement of the wastewater treatment process in large cities discharging into the tributaries of the Danube. The highly complex statistical studies carried out in a collaboration with Mrs. Frâncu Mihaela from the National Research - Development Institute for Chemistry and Petrochemistry - ICECHIM Bucharest, allowed monitoring the Danube water quality and following the evolution of the studied indicators.

C2. ORIGINAL CONTRIBUTIONS

The original contributions made by this doctoral thesis are the following:

- electrochemical characterization of new organic derivatives (4 arsonium salts (triphenylarsonium bromide (L1), (3-methoxybenzyl) triphenylarsonium iodide (L2), (3-nitrobenzyl) triphenylarsonium iodide (L3), (3-) methylbenzyl) triphenylarsonium (L4) and 8 derivatives of azulene ((E) -5- (azulen-1-yldiazenyl) -1-benzyl-1H-tetrazole (L), (Z) -5- (azulen-1-ylmethylene) -2-thioxo-thiazolidin-4-one (L2349), (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), 4- (azulen-1-yl) -2,6- bis ((e) -2- (thiophen-2-yl) vinyl) pyridine), 2,2 ′ - (ethan-1,2-diylbis ((2- (azulene 2-ylamino) -2-oxoethyl) azane) diyl)) diacetic (L5), 2- (azulen-1-yldiazenyl) -5-phenyl-1,3,4-thiadiazole (L2513), 2-phenyl-5 - ((4,6,8-trimethylazulen-1) -yl) diazenyl) -1,3,4-thiadiazole (L2372))
- obtaining new modified electrodes based on azulene derivatives L5, L2349, M538, M540 and L2372;
- detection of Hg (II) between heavy metal ions using new chemical, optical and electrochemical sensors based on L5;
- optimization of the parameters of the method for determining mercury in water samples using sensors based on electrodes modified with L5, which allowed the detection of Hg (II) ions in drinking and surface water;

To date, no systematic studies on the reduction of R4M + in aprotic solvents have been performed. The reduction of Ph4As + because in DMF [12] on mercury has been reported to occur in a two-electron process, while the reduction of Ph4Bi + in MeCN has been reported to occur in two single-electron steps [1]. The number of electrons involved in the reduction processes in DME was not mentioned [11].

C3. PERSPECTIVES FOR FURTHER DEVELOPMENT

The elaborated doctoral thesis opens perspectives related to:

- characterization of new organic compounds to obtain modified electrodes;
- characterization of complexing polymeric films by electrochemical, optical and spectral methods;
- obtaining new sensors based on modified electrodes for the detection of heavy metals from different water samples;
- optimization of methods for determining heavy metals based on modified electrodes for other azulene derivatives;
- development of new real-time analytical platforms based on chemical, electrochemical and optical sensors.

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A.3. TUTORING STUDENT SCIENTIFIC CONFERENCES

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