“BABEŞ–BOLYAI” UNIVERSITY
CLUJ-NAPOCA
Faculty of Chemistry and Chemical Engineering
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Contributions regarding the redox and adsorbtion properties of some phenolic derivatives applied in the environmental analysis

PhD. Thesis Abstract

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Scientific advisor: Prof. Dr. Luminiţa SILAGHI-DUMITRESCU

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Cluj-Napoca
2013

CONTENTS

Key words: nitrophenols, aminophenols, cyclic voltammetry, square wave voltammetry, electrochemical reduction, adsorption, biosensorr, chronoamperometry, electrochemical impedance spectroscopy.

INTRODUCTION

The main goal of this thesis was to reduce the concentration of nitrophenols and nitrophenols derivatives concentration from wastewater or synthetic water containing nitrophenols exceeding the maximum permissible concentration of European and international standards. It also aims to enable a biosensor to determine and quantify other phenolic compounds from synthetic samples and pharmaceutical products.

Nitrophenol derivatives are important organic compounds commonly used in industrial and agricultural applications [1-6]. These derivatives resulting from natural processes occurring in the biosphere and ecosystems are found as pollutants in many developed countries [7], and are produced by microbial hydrolysis [8,9] or the photodegradation of organophosphorus pesticides or containing nitrophenol structure [10,11]. Some pharmaceutical products, like 4-(acetylamino)phenol (acetaminophen) contain the 4-nitrophenol schelet in their molecule.

Nitrophenols are one of the most toxic classes of pollutants for the environment. Nitrophenols are toxic and biorefractory compounds and are classified between the first 129 most toxic pollutants by USEPA [12,13], while the U.E Directive 67/544/EEC on 2006 [14] classifies nitrophenol derivatives as toxic and dangerous for the environment. Dinitrophenols act as inhibitors for the oxidative phosphorylation process, which is why the majority of this class of compounds has been banned in the U.S. since the '80s.Their use has been restricted in some countries[19,20], but they are still used in underdeveloped countries. Derivații nitrofenolici în general şi cei studiați în particular nu sunt biodegradabili Nitrophenol derivatives in general and in particular the the ones analyzed in the present study are not biodegradable [21], therefore they can still be detected in the waste water from industrial sites, soil and even in surface waters in areas where these products have been extensively used.

Considering all these aspects, it is very important to follow the transformation of these compounds in the environment and to develop new and efficient method for identifying, measuring and remove them from wastewaters.

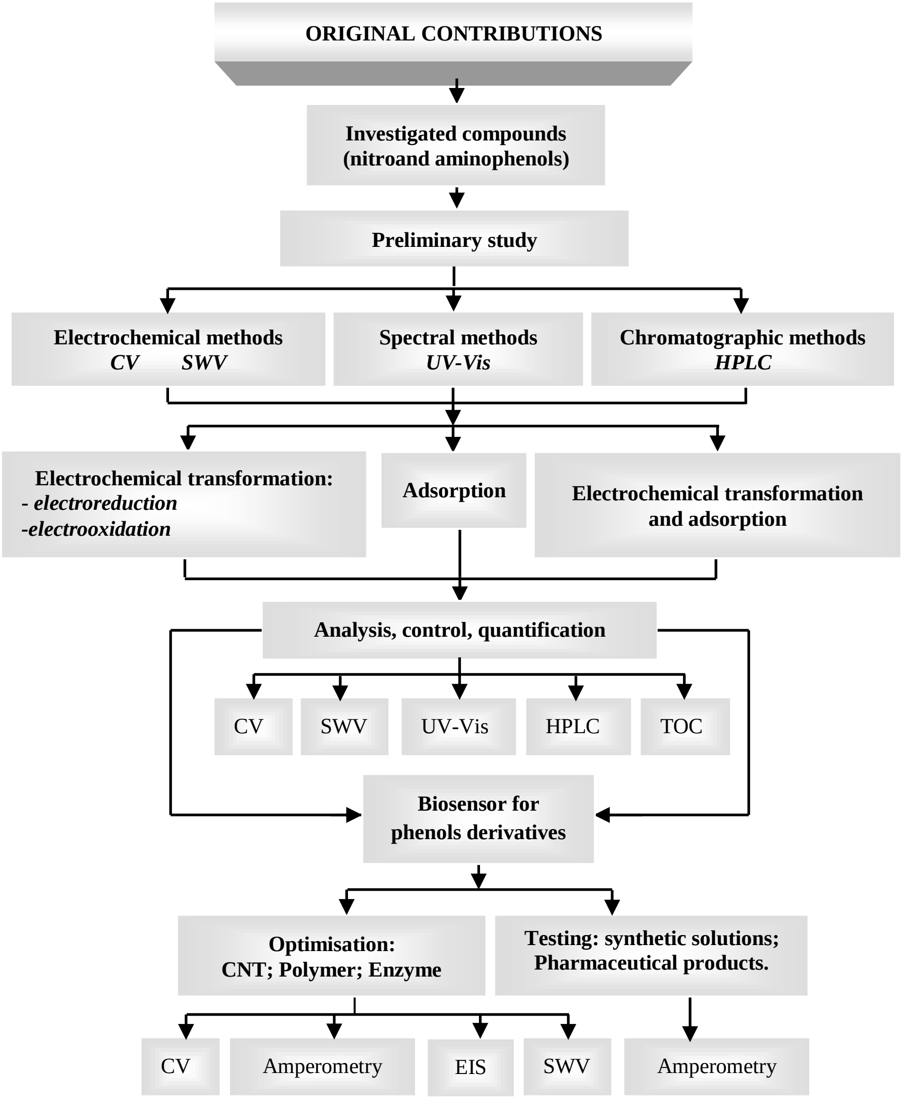
4-(Acetylamino)phenol is an aromatic acylated amide used as antipyretic and active ingredient in numerous medicines used against cold and flu and in some analgesic which can be obtained without prescription. Recent studies have shown that

4-(acetylamino)phenol is associated with liver toxicity and kidney failure.In normal therapeutic doses it is rapidly and completely metabolized by glucuronidation and sulfation to yield inactive metabolites that are excreted in [22]. Overdosing with 4-(acetylamino)phenol yields of toxic metabolites and is a frequent cause of liver blockage in Europe and the U.S..Pharmaceuticals and their metabolites contribute to the environmental pollution as most are able to pass through treatment plants due to polarity, persistence and high solubility in water medium.These compounds are less adsorbed in soil and sludge and thus are polluting groundwater and even surface water and its consequent degradation of water quality and causing the need for monitoring effluents from sewage, surface and tap water, classifying these substances as pollutants relevant to the environment and their analysis should be done usually in trace[22].

The control of the 4-(acetylamino)phenol concentration require effective analytical methods for their quantification in pharmaceutical, biological (serum or urine), and in environmental samples.

This thesis is structured in two parts: *the first part* includes literature data that highlights the current state of knowledge in the field.The state of the art part of the thesis is divided into three chapters which gather information on nitro and aminophenol derivatives, electrochemical transformation mechanisms of these compounds, the technologies used to remove these pollutants from the environment and methods used to analyze the electrochemical processes. A separate chapter covers the study of the current state of knowledge regarding methods of determining aminophenols (4-(acetylamino)phenol) and on the biosensors used to determine 4-(acetylamino)phenol.

The content and structure of the original contributions part of the thesis are illustrated in the following diagram, which shows the important chapters and their succesion.This part of the thesis is structured in 7 chapters describing methodology, equipment and materials used for experimental measurements, electrochemical, spectral and chromatographic behavior ofnitrophenol derivatives in the electrochemical processes. The results obtained in the case of electrochemical conversion (reduction and oxidation) process, in adsorption processes and in the case of the combination of these two processes applied to the mono and dinitrophenols and to some dinitrophenolic pesticides are also presented.



There are also presented the research results on the development, optimization and testing of a biosensor based on glassy carbon electrode modified with carbon nanotubes and horse radish peroxidase immobilized in a polymeric film of polyethilenimine for both synthetic and real samples (pharmaceutical products) containing 4-(acetylamino)phenol (acetaminophen).The thesis contains some chapters namely: *Introduction,Working hypothesis and Objectives*, *Conclusions, References* and *Annexes*, has 201 pages and 296 references indexes (the references list is presented at the end of the paper for all the work).

The results were obtained in two well known research centres: the Research Centre LAF-INT-ECOL, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj-Napoca (Assoc. Prof. CSI. Dr. Maria Jitaru) and the Research Centre from the Analytical Chemistry and Instrumental Analysis Department, Faculty of Pharmacy, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca (Prof. Univ. Dr. Robert Săndulescu). The results presented in this work were published, disclosed or are pending. List of works produced and included in the thesis theme is attached to the end of the paper. A part of the studies were conducted under a research grant for young researchers financed by Balkan Environmental Association (BEnA) and TITAN SA and some research grants, namely*: Contribution to the preparation, characterization and application of some nanostructure materials, for direct/photoassistated electrochemical degradation of pollutants-NANELPOL (Project CEEX-RO MATNANTECH No. 68/2006)*; *Regional and european consolidation and enlargement of cooperation on the electrochemical methods applied for wastewaters control and deppolution–EurElDep (Project CEEX Corint Modul III No. 47/2006)*and *Amperometric immunosensors for ovarian and uterine cancer biomarkers (Project CNCS-UEFISCDI PN-II-ID-PCE-2011-3-0355).*

The motivation for choosing this theme for doctoral thesis

The thesis contributes to the improvement of knowledge in a field of international interest: environmental protection and economic development of rapid methods for detection and removal of these pollutants in the environment.Regarding this, the electrochemical processes are promising paths for treating wastewaters by converting or destroying toxic organic substances and have gained great importance in the last few years. The electrochemical methods used for removing nitrophenol from wastewater are: simple electrochemical reduction, electrochemical reduction coupled with electro-Fenton oxidation, assisted electrochemical coagulation, direct or mediated electrochemical oxidation, etc. Recently, the sensors/biosensors fields have gained a fast development with many applications in the detection of phenolic compounds.

To achieve the objectives various methods have been performed in order to achieve the proposed objectives: the electrochemical characterization of phenolic compounds in direct and mediated charge transfer processes; the study of the spectrophotometric and chromatographic behavior for some phenolic derivatives, in direct and mediated electrolysis experiments and adsorption on different materials in the case of model molecules (mono and dinitrophenols). A comparative study was made between the simple adsorption and the electrosorption processes formodel molecules on different adsorbent materials, and the results were used for the implementation of a combined procedurecoupling the adsorption on granular activated carbon with the electrochemical transformation, procedure that was then applied for the removal of more complex molecule of nitrophenol derivativesin aqueous solution.Elaborarea si optimizarea unui biosenzor amperometric pentru determinarea compuşilor fenolici din soluții apoase şi testarea acestuia pentru determinarea 4-(acetilamino)fenolului (paracetamolul), din soluții apoase sintetice şi din probe reale este o completare a celorlalte cercetări efectuate pe parcursul elaborării acestei teze de doctorat. The elaboration and optimization of an amperometric biosensor for the determination of phenolic compounds from aqueous solutions and its application for the determination of the 4-(acetylamino)phenol (acetaminophen), from aqueous solutions of synthetic and real samples comes to completion of the research done for the elaboration of this thesis.

II. ORIGINAL CONTRIBUTIONS

Chapter II.2. Spectral and electrochemical behavior of nitrophenol derivatives

2.1. Electrochemical behavior of nitrophenols

The voltammetric methods of analysis used for nitroaromatic compounds are: cyclic voltammetry (CV) and square wave voltammetry (SWV). Voltamperometric determinations were conducted in order to establish the optimal conditions for the processes of electrochemical transformation of the nitrophenol derivatives, as well as their dependence on a number of factors, such as: pH, electrode materials, the concentration of the reactant in the electrolyte solution, the type of electrolyte, the position of the NO_2 group on the benzene ring, etc. From the analysis of the cyclic voltammograms of the nitroderivatives obtained with different electrode materials (Hg, graphite, Pb, etc), it can be observed:

- at the first negative scanning of potential, a reduction peak is observed in the range of -0.6 to -1.1 V vs Ag/AgCl, depending on the working conditions, due to the process reduction of the $–NO_2$ group to the corresponding hydroxylamine, stage in which takes place a transfer of 4 e^-;
- afterwards, the formation of hydroxylamine takes place, which is oxidized according to the conditions at the appropriate/corresponding nitrozoderivate, process which opens up the prospect of the electrochemical synthesis for these types of compounds;

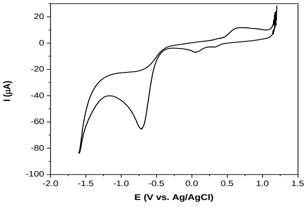
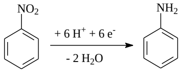


Figure 6. Cyclic voltammograms obtained for 10^{-3} M 4-NP electrochemical reduction.
(Britton-Robinson buffer solution pH 2.2; working electrode of glassy carbon).

According to the cyclic voltammograms form (Figure 6), it is observed that at the electrode potential beyond the reduction potential, in the presence of protons, the $–NO_2$ group is reduced to $–NH_2$ (eq. (1)):



(1)

The curve for the reduction of a nitroderivate to hydroxylamine can be used to calculate the concentration of nitrocompound, because the intensity of the peak current is proportional to the concentration.

2.1.1. Cyclic voltammetry

The overlapped cyclic voltammograms obtained for 10^{-3}M solutions of the nitrophenol compounds, in Britton-Robinson buffer solution (pH 2.2) on a glassy carbon electrode (GCE) are shown in Figure 7. Measurements were performed in a three electrode electrochemical cell using an Autolab PGSTAT12 potentiostat. Before each determination the solutions were de-aerated with nitrogen for 15 minutes to remove oxygen.

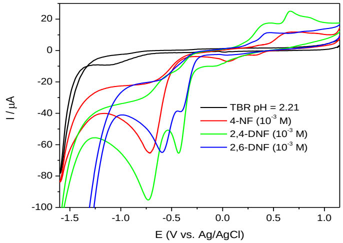


Figure 7. Cyclic voltammograms for 10^{-3} M 4-NP solutions (red), 2,4-DNF (green) and 2,6-DNF (blue) in Britton-Robinson buffer solution pH 2,2, compared with the electrolyte (black) on GCE.
(Reference electrode: Ag/AgCl; counter electrode: Pt wire; scan rate: 0.1 Vs^{-1}.)

It is observed that the voltammograms corresponding to the dinitrophenol derivatives exhibit two reduction peaks. Each reduction peak corresponds to a $-NO_2$ group, and these groups are not reduced to the same value of the potential due to limitations for the different positions on the benzene ring. For 2,4-DNP the reduction peak potential value of the reduction peak, which occurs at the same potential as in the case of 4NF (-0.75 V), may be due to the -NO_2 group in the position 4. The reduction of -NO_2 group from the position 2 occurs at a potential of -0.4V, in the case of 2,4-DNP and also 2,6-DNP in case of both 2,4-DNP and 2,6-DNP.

The influence of various factors was investigated, in order to determine the optimum conditions for their electrochemical transformation. Thus there was important to appraise the influence of the concentration, the scan rate, the pH and the nature of the electrolyte, the position of the functional groups on the benzene ring and the electrode material to the redox processes for conversion of nitrophenol derivatives.

Regarding the concentration of the compounds, the range in which the concentration varies linearly with the intensity of the peak current was determined, as well as the detection limits for each electrochemical method and for each component individually. The determination of the detection limit was calculated by the ratio between three times the standard deviation of the intercept and analytical slope for each compound [243-245].

Using CV, the voltammetric signals and calibration data corresponding to the treated nitrophenols were determined in phosphate buffer medium. The results obtained in the reduction potential are shown in Table 2.

Table 2. The voltammetric and calibration data for the nitrophenol derivative.

Nitrophenols	**-E_p (V)**	**C_{NF} (µM)=f(I_P) (µA)**	**Dinamic range (mg/mL)**	**R^2**
4-NF	0.65	$C_{4-NF} = \frac{I_P(\mu A)}{0.062}$	0.001÷90	0.993
2,6-DNF	0.37	$C_{2,6-DNF} = \frac{I_{P,1}(\mu A)}{0.0598}$	0.002÷90	0.997
	0.55	$C_{2,6-DNF} = \frac{I_{P,2}(\mu A)}{0.0512}$		0.991
2,4-DNF	0.37	$C_{2,4-DNF} = \frac{I_{P,1}(\mu A)}{0.1176}$	0.002÷90	0.995
	0.73	$C_{2,4-DNF} = \frac{I_{P,2}(\mu A)}{0.1468}$		0.991

From statistical measurements we obtained a relative standard deviation of 3.20% and a relative error of 3.05% for 4NP. In the case of 2,4-DNP the relative standard deviation is 4.06% and the relative error 3.28%, and in the case of 2,6-DNP, the relative standard deviation is 2.8% with a relative error of 1.85% obtained from the five determinations. There is a shift in the reduction potential value in cathodic direction with the increasing concentration of the working solutions, probably due to the formation of reduction intermediates which are desorbed more diffcult, hindering the access to the electrode surface. The main cause of the stronger adsorption for the reduction intermediates on the electrode surface can be attributed to the presence of hydroxyl group in the nitrophenol structure and *p-π* conjugations which can occur.

In the case of the determinations carried out on solutions of 4NP with various concentrations in TBR solution of pH 8.32 in the oxidation potential range a single peak is observed whose intensity varies linearly with concentration in the range of 14÷140 mgL^{-1} ($R^2=0.988$) without a significant shift of the potential. The calibration curve does not pass through the origin, probably due to interactions between nitrophenol derivative and some of the electrolyte components (in basic environment the condensation reactions of oxidation intermediates are more likely to occur). The limit of detection is 33.5 μM, and the calibration equation is shown in equation (2):

$$C_{4-NF} = \frac{I_P(\mu A) - 10{,}385}{0{,}0182} \qquad (2)$$

The scan rate provides information about the mechanism of the process, if the diffusion is the rate determining step and if the mechanism of redox transformation changes depending on the pH of the electrolyte. Randles-Sevcik equation expresses the relationship between the peak current intensity and the scan rate value.

$$I_p = (2{,}99 \cdot 10^5) \cdot n \cdot \sqrt{\alpha \cdot n_a} \cdot C \cdot \sqrt{D} \cdot \sqrt{v} \cdot S \qquad (3)$$

The voltamperometric behavior of nitrophenol was determined with scan rate values ranging from 10 to 200 mVs^{-1}. It is noted that the oxidation peak potential moves in the anodic direction, when the scan rate is increased, while in the reduction potential, the shift of the potential value takes place in the cathodic direction. For 4-NP a linear dependence of the intensity of the peak current to the root of scan rate was observed, for both electrochemical reduction and oxidation measurements, and the equations describing this change have good correlation coefficients.

According to Randles-Sevcik equation (3) due to this linear dependence, it can be mentioned that charges transport to the electrode surface is controlled by the diffusion of electroactive species at the electrode surface. Anodic potential shift for oxidation and cathodic shift for reduction proves irreversible electrochemical transformations corresponding to these compounds.

In order to determine the influence of the environmental pH on the electrochemical behavior of nitrophenol, the experiments were performed in Britton Robinson buffer medium. The pH was adjusted with 0.1 M NaOH solution. The cyclic voltammograms of 10^{-3} M solution of 4-NP in 0.1 M TBR+NaOH medium, both in acidic and alkaline pH, in the range of the reduction potential are shown in Figure 12 (A)) and (Figure 12 (B)) in the range of oxidation potential respectively. It is noted that the reduction of 4-NP occurs at a potential about 0.8 V vs. Ag/AgCl in an acid medium and at a potential about -1.2 V vs. Ag/AgCl in a basic medium, so it is preferable for the electrochemical reduction of nitrophenol procedures to be carried out in acidic medium. In the oxidation potential the peak is more well-defined and reaches higher values of intensity in alkaline solutions, and the potential is shifted in the cathodic direction which recommends the use of solutions with a pH of 8 to 10 for the electrochemical oxidation of 4–NP.

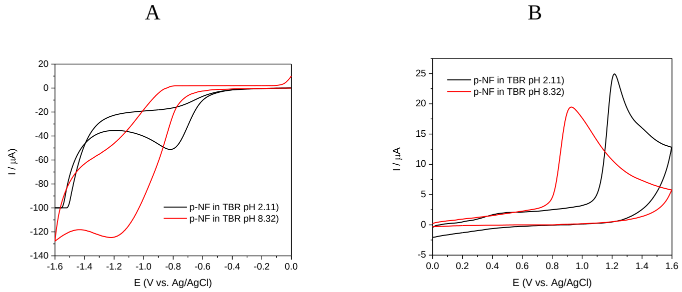


Figure 12. Cyclic voltammograms for a 10^{-3} M 4-NP solution in TBR with acid pH (black) and basic pH (red) in the reduction **(A)** and oxidation **(B)** domain of potential. (Working electrode: GCE; reference: Ag/AgCl,KCl; counter electrode Pt (plate); scan rate 50 mVs^{-1}; sensitivity 100 μAV^{-1}).

The peak potential varies linearly with the pH value for all the analyzed compounds, and the peak is shifted in cathodic direction in the reduction potential, while in the oxidation potential the anodic shift takes place with increasing the increase in the pH. These shifts confirm the irreversibility of the reduction processes, respectively electrochemical oxidation involving nitrophenol derivatives. Side reactions are promoted in an alkaline pH represented by additional peaks visible in the voltammograms of 2,6-DNP and of the binary mixtures [63].

For mononitrophenol compounds, in an acid medium (pH 2.21), electrochemical reduction of the $-NO_2$ group is manifested by the appearance of a well-defined peak (Figure 17 (A)). The potential of the peak is influenced by the mutual position of the two groups -OH and $-NO_2$. $-NO_2$ group located in the vicinity of -OH group is more easily reduced (the values of the potential shifted in the anodic direction).

According to literature data [5], in the case of dinitrophenol derivatives (2,4-DNP and 2,6-DNP) the voltammograms show two well separated, defined peaks corresponding to the electrochemical reduction processes (ΔE=0.35 V for 2 and ΔE=0.4, DNP 0.2 V to 2.6, DNP) (Figure 17 (B)).

For 2,4-DNP and 2,6-DNP, the presence of the second $-NO_2$ group increases the electrochemical reactivity of the first $-NO_2$ group due to its electron attractive effect. The basic medium (Figure 17 (C) and 17 (D)), all nitrophenol derivatives have three reduction peaks, two irreversible and a reversible one.

For the cyclic voltammetry measurements glassy carbon electrode, Pb and Ni were used and experimental measurements have been carried out in several electrolytic medium (TBR solution of pH 2.11 and 8.32, solution of H_2SO_4 0.2 N and pH 2.10). In the determinations carried out in TBR at pH 2.11, the best results are obtained on GCE, while at the same electrolytic medium at pH 8.32, the best results were obtained on the working electrode of Pb. In H_2SO_4 (0.2 N, pH 2.10) the best results are obtained on Pb and Ni electrodes, but on GCE only one reduction peak appears which makes this material more accessible to experimental measurements. For these reasons the glassy carbon electrode was chosen as electrode material in order to determine the concentration of nitrophenols during their removal processes in wastewater.

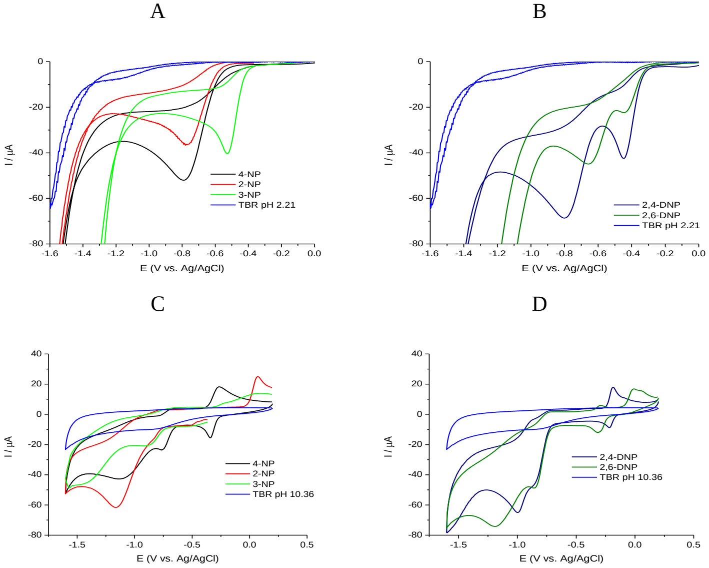
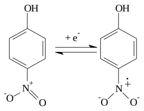


Figure 17. Cyclic voltammograms for nitrophenols in the reduction domain of potential at different pH values: 2.21 **(A)** and **(B);** and 10.36 **(C)** and **(D)** on GCE. (Reference electrode Ag/AgCl, KCl; counter electrode Pt; scan rate 50 mVs^{-1} [63,239].).

Due to the different reactivity of the two nitro groups, in the case of 2,6-DNP two very well separated reduction peaks are observed (ΔE=0.3V) [62]. In the case of 2,6-DNP the electrochemical reduction of both nitro groups occurs more easily than with 4NP (values of the reduction potential of -0.37 V vs. Ag/AgCl and -0.55 V vs. Ag/AgCl, compared with -0.65 V vs. Ag/AgCl). The anion radicals of nitrophenols are unstable and they suffer autoprotonation reactions [49]. As expected, in the protic medium, it is more difficult to determine the anionic radical formation through the first electron transfer step, for all 4-NP, 2,4-DNP and 2,6-DNP. The mechanism for the reduction of 4NP in protic medium is shown in eq.7-10[49]:



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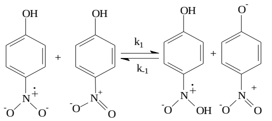

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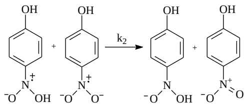

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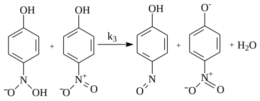


(10)

The reduction of the nitro group to the corresponding hydroxylamine is the most important intermediate step in the process of electrochemical reduction to formamines, reaction (11):

$$Ar-NO_2 \xrightarrow{4\,e^-,\,4\,H^+} Ar-NHOH \xrightarrow{2\,e^-,\,2\,H^+} Ar-NH_2 \qquad (11)$$

Conclusions

The cyclic voltammetry determinations were carried out in order to determine the electrochemical behavior of the studied mono- and dinitrophenol compounds and the influence of various parameters (concentration, scan rate, the pH, the nature of the electrolyte, and the electrode material) on the electrochemical activity of these compounds, thus establishing the optimum conditions for the electrochemical measurements. In the reduction potential domain, the voltammograms of the mononitrophenol derivatives present a single irreversible reduction peak, while for the dinitrophenol derivatives two reduction peaks are observed, each due to the process of electrochemical reduction of a -NO_2 group which does not have the same reduction potential values due to the different position on the benzene ring. This peak was attributed to the formation of the corresponding hidroxilamine derivates, process that occurs with the transfer of 4e^- in acidic medium. The data obtained by using cyclic voltammetry measurements were used to expand the studies using square wave voltammetry.

2.1.2. Square wave voltammetry

Square wave voltammetry (SWV) was used to confirm the results obtained by CV for the electrochemical behavior of 4-NP, in both reduction and oxidation potential ranges. The square wave voltammograms were recorded on GCE and suspended mercury drop electrode (HMDE) for 4-NP solutions of different concentrations in Britton Robinson buffer solution (pH 2.11) using a CHInstruments 630 potentiostat type and a conventional three-electrode electrochemical cell. SWV curves obtained on GCE for 4-NPsolutions of various concentrations in the Britton Robinson buffer solution (pH 2.11) show a single peak at about 0.4 V. The results are shown in Figure 21(A).

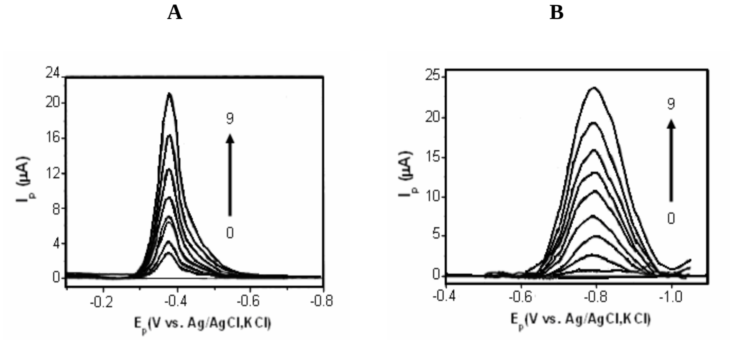


Figure 21. SWVs recorded on: GCE **(A)** and HMDE **(B)** for the electrochemical reduction of 4-NP different concentration: 1 (1); 5 (2); 7.5 (3); 10 (4); 12.5 (5); 15 (6); 20 (7); 30 (8) and 40 μM (9) in TBR medium (0.1 M; pH 2.11). (f 120 s^{-1}, a 40 mV, ΔEs 2 mV, reference electrode Ag/AgCl, contraelectrode Pt wire) (**data included in the final Report for the research grant for young researcher type II, financed by BEnA–Titan SA, 2009).**

In order to compare the response obtained on GCE with the one obtained on HMDE, we used the same methodology described above. SWV curves for the reduction of 4-NP at different concentrations on HMDE are shown in Figure 21 (B). They have a single reduction peak at about -0.8 V reductions vs. Ag/AgCl in Britton Robinson buffer medium (pH 2.11). The difference between the reduction peaks values obtained on the two types of electrodes may be due to the strong dependence of the reduction process of -NO_2 group on the pH value [246]. The values of the current peak intensity are very similar, suggesting that the number of changed electrons in the reduction process is the same on both types of electrodes ($4e^-$). Changes in the intensity of the current peak is linear with the concentration in both cases (graph not shown), and the corresponding equations are presented in Table 6. In the case of GCE the detection limit is 0.43 μM, whereas the limit of detection on HMDE was 0.26 μM.

Table 6. The variation of the reduction/oxidation current intensity with 4-NP concentration.

Electrode	$I_p(\mu A) = f(C_{4-NF})(\mu M)$	**Dinamic range (μM)**	**R^2**	**LOD (μM)**
GCE	$I_p = 0.686 \cdot C_{4-NF} - 0{,}669$	1÷40	0.998	0.43
HMDE	$I_p = 0.601 \cdot C_{4-NF} + 0.817$		0.990	0.26

The reduction process of 4-NP on HMDE and on GCEworking electrode is controlled by the diffusion of this compound to the electrode surface. According to literature data, 4-NP is electrochemically oxidized to the corresponding o-benzoquinone [247,248] by a mechanism similar to that of pentachloro phenol oxidation [249] by the electrochemical transfer of $4e^-$. Square wave voltammograms were carried out between 0.8 and 1.15 V vs. Ag/AgCl in Britton Robinson buffer solution (pH 8.32) and the results are shown in Figure 22. In this case the process is controlled by the diffusion of 4-NP and/or of the oxidation products at the electrode surface. The dependence of the current peak intensity on the concentration is linear in the concentration range of 1÷50 μM and the calibration equation is described by the relation: $I_p = 0.664 \cdot C_{4-NF} - 2.314; (R^2 = 0.992)$

Conclusions

SWV determinations confirmed the results obtained by CV for the electrochemical behavior of the 4-NP, for both reduction and oxidation potential. We determined the dependence of the peak current intensity on the concentration of 4-NF solution and detection limits obtained for the two types of electrodes are of the order of 10^{-7}M.

2.2. The spectrophotometric behavior in UV-Vis domain

In the molecular absorbance spectra recorded in the UV-Vis domain for studied mono-and dinitrophenols in aqueous medium, (Figure 23), one can observe that the absorption peaks are clearly separated allowing the identification of these compounds in the mixture.

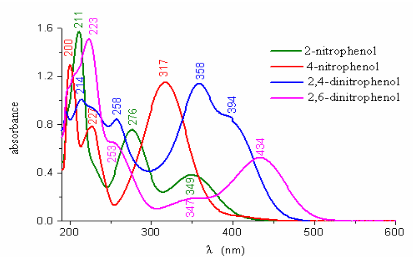


Figure 23. UV-Vis spectra for mono and dinitrophenols registred in aqueous medium [239].

Two different adsorption bands with different values of the wavelength at which the absorbance is maximal appear for 4-NP, 2,4-DNP and 2,6-DNP, in the UV-Vis spectra (λ_{max}: 223 and 317 nm for 4-NP, 222 and 430 nm for 2,6- DNP, 258 and 358 nm for 2,4-DNF respectively). Although the calibration data are derived for both values of λ_{max}, for each compound, only one of them has been used for study, due to the high intensity of the adsorption bands ($\varepsilon=9330$ at 317 nm for 4-NP, $\varepsilon=5970$ at 358 nm for 2,4-DNP, and $\varepsilon=6280$ at 430 nm for 2,4-DNF). All the calibration curves recorded for the studied nitrophenol derivatives in an aqueous medium pass through the origin and show a good correlation coefficient ($R^2>0.99$), which allows the estimation of the concentration of the solution for these compounds by means of spectrophotometric measurements and of the equations describing the dependence of absorbance - concentration. The optimal concentrations that can be applied in UV-Vis spectrophotometry to determine the analyzed nitrophenol in an aqueous medium are: 0.15 to 15 mg $L^{-1}$for 4 NP (accepted concentration limit is 0.33 mg L^{-1}) 0.2÷20 mg L^{-1} for 2,6-DNP and 2,4-DNP (accepted concentration limit of 0.5 mg L^{-1}).

Conclusions

Based on these considerations, it can be concluded that the UV-Vis spectrophotometry is a suitable method for fast determination of the nitrophenols concentration from the solution by the electrochemical process and adsorption, and the combined processes of nitrophenol derivatives removal from wastewater. This method can be successfully used as a second method of determination along with the voltammetric methods previously proposed.

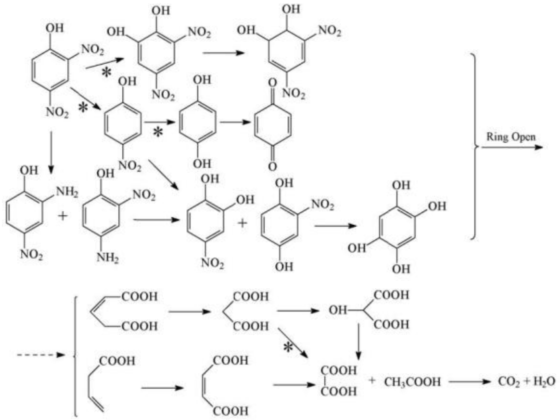
Chapter II.3. Electrochemical transformation of nitrophenols derivatives

3.1. The electrochemical oxidation of nitrophenols

In order to propose a possible degradation pathway of nitrophenols it is essential to detect the intermediates and their evolution as the electrolysis takes place. LC/MS and HPLC were used, in general, for the qualitative and quantitative determination of the intermediates and the products generated during the oxidation processes. Three kinds of intermediates were detected, i.e., polyhydroxylated intermediates, reduction products of NPs and carboxylic acids. Due to the electrophilic attack of hydroxyl radicals, the reaction takes place preferentially at those positions with higher electron density. If the position would have been ocuppied by a nitro group, the denitration from aromatic rings would occur. If the position would have been vacant, the addition of hydroxyl group to the benzene ring would result in the formation of polyhydroxylated intermediates. The substitution of nitro group and hydrogen atom on the aromatic rings by hydroxyl radical also takes place.

The denitration or addition of hydroxyl group that occured in the first part of the degradation process was dependent on the structure of the organic compounds. The polyhydroxylation would subsequently lead to the opening of aromatic rings to form carboxylic acids. There might be another pathway in which the opening of aromatic rings can take place before the substitution of the nitrogroup and the subsequent formation of nitrated aliphatic compounds. In addition, some reduction products of NPs were also detected. Aminophenols were detected in terms of the results of LC–MS through the electrolysis of 2,4-DNP [250]. This phenomenon indicated that the reduction of NPs to aminophenols took place at the cathode. Then, these aminophenols disappeared gradually during the process of electrolysis.

Organic acids were formed as a result of the opening of aromatic rings. Oxalic, acetic, maleic, malonic and formic acid were detected. It could be considered that almost the whole quantity of dinitrophenols was eventually converted to carbon dioxide via formic acid. Either polyhydroxylated intermediates or carboxylic acids were oxidized into CO_2 and H_2O. A possible mechanism for electrochemical oxidation of 2,4-dinitrophenol is presented in Scheme 1.



Scheme 1. Possible degradation pathway of 2,4-DNP during the electrochemical oxidation process [250].

The decrease in the 4-NP concentration over time (calculated from absorbance data using the calibration equation determined for 4-NP in aqueous medium) is presented in Figure 28.The electrochemical oxidation experiments were carried out by batch processes and the apparatus consisted mainly of a potentiostat, a magnetic stirrer and a glass reactor (experimental setup D, page 74). The anode and cathode were positioned vertically and parallel to each other. 4-NPs with an initial concentration of 50 mg L^{-1} were selected as model organic pollutants and the volume of the solution was 300 mL. A 0.1 M phosphate buffer solution at pH 4.00 was used as a supporting electrolyte. The electrochemical oxidation was performed under galvanostatic control at a current density of 30 mA cm^{-2}. During the experiments, samples were drawn from the reactor at certain intervals and then analyzed.The complete degradation of the 4-NPs was achieved within 2 h. The electrochemical oxidation for the degradation of 4-NP is satisfactory. During the first 1 h, the 4-NP degradation was over 96%.

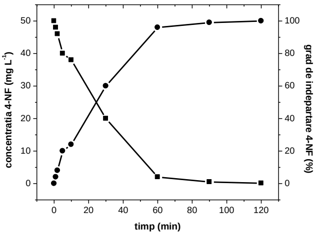


Figure 28. 4-NP removal and concentration decrease in time durring the electrochemical oxidation process on Ti/Pt-Ir anode.
(Experimental conditions: initial 4-NP solution concentration 50 mgL^{-1} in phosphate buffer solution (0.1 M; pH 4); i=30 $mAcm^{-2}$.) (**data included in the final Report for the research grant for young researcher type II, financed by BEnA–Titan SA, 2009)**.

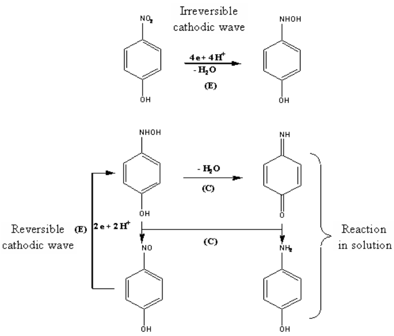
Conclusions

By using electrochemical oxidation at the anode of Ti/Pt-Ir, about 96% of the 4-NP was removed after the first hour of electrolysis, results confirmed by UV-Vis measurements, total organic carbon determination and cyclic voltammetry.

3.2. The electrochemical reduction of nitrophenols

In Scheme 2 a schematic mechanism is presented for the electrochemical transformation of 4-NP in aqueous medium.

Electrochemical transformations (E) are alternated or followed by chemical reactions (C) that could take place either in solution or between the proucts and the intermediates. In agreement with the literature and our data [5,62], nitrophenols cyclic voltammograms, present two peaks in the reduction potentials domain, the first reversible ($E_p \approx -0.2$ V) and the second irreversible (E_p from 0.6 to 0.8 V). From the quantitative point of view the $4e^-$ second irreversible peak can be used for the determination of NP concentration.



Scheme 2. Electrochemical transformations of 4-NP in aqueous media (**data included in the final Report for the research grant for young researcher type II, financed by BEnA–Titan SA, 2009).**

The cyclic voltammograms of 4-NP and 2,4-DNP present only one cathodic peak in the investigated potential range. This behavior is typical for totally irreversible systems [252,253].

Assuming an irreversible reduction of $–NO_2$ group, the following equation was used (equation (13)), in order to calculate the number of electrons involved in the process:

$$j_p = \left(2.99\times10^5\right)\cdot n\cdot\left(\alpha\cdot n_a\right)^{1/2}\cdot C_0^*\cdot D_0^{1/2}\cdot v^{1/2} \tag{13}$$

where j_p is the peak current density ($1.413\cdot10^{-4}$ A cm^{-2}), n is the total number of electrons involved in the reaction, α is the electron transfer coefficient, $n_a$is the number of electrons involved in the rate-determining step, D_o represent the diffusion coefficient of the electro active species, C_o^*represent the concentration in mol cm^{-3} (10^{-4} mol L^{-1}), and v is the scan rate of the potential (0.1 V s^{-1}). The values of $\alpha\ n_a$ were calculated according to equation (14) [242], for the irreversible reduction of $–NO_2$ group, and a value of 0.95 was obtained in the case of 4-NP.

$$\alpha\cdot n_a = 47.7/\left(E_p - \frac{E_p}{2}\right) \tag{14}$$

Using a 100 μmol L^{-1} 4-NP solution and a diffusion coefficient of $9.06\cdot10^{-6}\ cm^2s^{-1}$, the number of total electrons calculated was about 3.96, suggesting an electron transfer mechanism of four electrons for the electrochemical reduction of 4-NP in aqueous solution. This value is in agreement with other authors [253,254], and it demonstrated that in moderately acidic media, the nitro-aromatic compounds are firstly reduced to the hydroxylamine derivative with the implication of 4 e^- and 4 protons, equation (15):

$$R{-}NO_2 + 4e^- + 4H^+ \rightarrow R{-}NHOH + H_2O \tag{15}$$

The electrochemical reduction experiments for mono and dinitrophenols were carried out in three types of electrochemical reactors, with diferent hydrodynamic conditions and using different electrolyts (the experimental conditions are described in detail in Chapter II.1.).

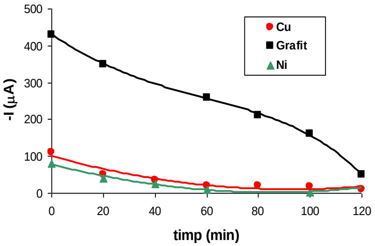


Figure 31. The reduction peak intensity variation during the 4-NP electrolyse in H_2SO_4 solution, (pH 2.10) on different cathodic materials.
(Experimental conditions: 4-NP initial concentration: 10^{-3} M; i=20 $mAcm^{-2}$; WE: glassy carbon; RE: Ag/AgCl; CE: Pt.).

Figure 31 presents the variation of the current peak for the electrochemical reduction of a 10^{-3} M solution of 4-NP monocompartmented electrochemical reactor (experimental setup B, page 73), at different cathode materials in a solution of H_2SO_4 (0.2N; pH 2.10).

The intensity of the reduction peak that appears at a potential of about -0.9 V vs. Ag/AgCl, is decreasing during the electrochemical reduction withby about 90% on graphite and 98% on Ni and Cucathodes after 120 minutes of galvanostatic electrolysis (i=20 mA cm^{-2}, while the theoretical calculated time is 100 minutes).

The decrease of the concentration of nitrophenols was confirmed by the UV-Vis spectroscopy measurements, used as a second method of determination.

The electrochemical reduction of $5\cdot10^{-4}$ M solutions of 2,6-DNP in H_2SO_4 (0.2 N, pH 2.10) was carried out in a two-compartment reactor type cell H (experimental setup C, page 74), using 400 mL of 2,6-DNP solution and 300 mL of H_2SO_4, (0.2 N; pH 2.10) solution. The absorbance value of the 2,6-DNP solution decreases during the electrolysis by about 80% of the initial amount of graphite cathode and by 65% on Ni cathode in the first 15 minutes of electrolysis (theoretical calculated time is 20 min.), Figure 33. The removal of nitrophenols from the solution continues up to 99% after 100 minutes of electrolysis.

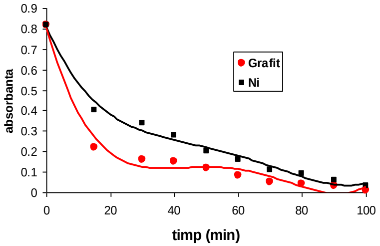


Figure 33. Spectrophotometric control of electrochemical reduction process for 2,6-DNP on graphite and Ni cathode.
(Experimental conditions: 2,6-DNP initial concentration: $5\cdot10^{-4}$M in H_2SO_4, pH 2; i: 20 mA cm^{-2}. λ_{max}=440nm.).

Conclusions

The parameters corresponding to the reduction peak attributed to the formation of hydroxylamine derivatives have been calculated from the data obtained using cyclic voltammetry. A number of 4 e^- was obtained by calculation, confirming our initial assumption. During the electrolysis, the color of the solution changes from colorless to brown probably due to the formation of colored condensation compounds. This phenomenon has been noted in other papers, [5,255] and is consistent with the characteristic properties of 4-aminophenol, which proved to be the most likely product of the 4-NP electrochemical reduction.

Regarding the 4-NP electrochemical reduction, the best results were obtained using a two compartment filter-press electrochemical reactor (experimental set-up A) on Ni cathode (about 98% of 4-NF removal). The results obtained in the case of 4-NP are presented in Figure 35 [266].

In the case of 2,6-DNP satisfactory results were obtained only when diluted initial solution were used (i.e. $5 \cdot 10^{-4}$ M). The electrochemical reactor used was a H type glass cell (experimental setup C, page 73); the 2,6-DNP recovery of about 99%).

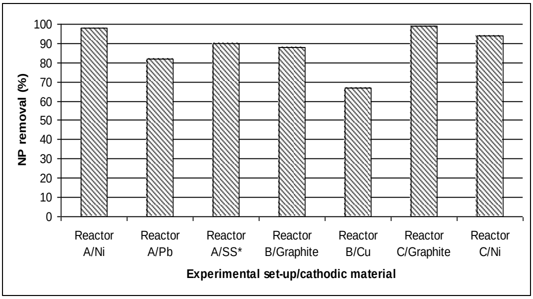


Figure 35. Schematic presentation of the efficiencies for the nitrophenols removal from aqueous solution using various types of electrochemical reactors(* SS–stainless steel [266]).

Chapter II.4. Nitrophenols derivatives adsorption on various adsorbent materials [238,251]

The aim of this research was to reduce the concentration of nitrophenols, such as: 4-NP and 2,6-DNP from synthetic samples (wastewater containing controlled nitrophenols concentration) by: adsorption on granular activated carbon type NORIT GAC 1240, adsorption on powder type activated carbon NORIT SAE SUPER, graphite (MERK) and adsorption on modified clays[238,251].

4.1.1. The characteristics for the considered nitrophenols derivatives adsorption processes [238,251]

In aqueous solutions, phenolic compounds can exist as phenolate ions, depending on the pH of the solution. Conversion of phenol and nitrophenol derivatives to phenolate anions is negligible in water solutions, taking in consideration its small acidic dissociation constants [250,256]. In water and acid solution (H_2SO_4, 1M), nitrophenols derivatives are in neutral form, with inessential amount of ionization products, while in aqueous solutions of0.1 M NaOH, this compounds are almost completely dissociated in their anionic forms, with negligible amounts of neutral species.

For the adsorption experiments volumes of 0.2 L of 4-NF solution with various concentrations were used, in which 1 g NORIT granular activated carbon type GAC1240W was added. The experiments ran for one hour, with stirring provided by a stirrer AGITUVAR 10W type (350 rpm) for maintaining the adsorbent material in suspension. After each experiment, the solid was filtered off, washed with distilled water, and then dried in an oven at 105°C. The samples collected for spectrophotometric measurements (UV-Vis UNICAM HELIOS, and HACH DR2800-LANGE) were diluted with distilled water at a 1:50 ratio prior to determination.

4.2. Determination of the thermodynamic parameters for the adsorption isotherms for nitrophenols adsorption on granular activated carbon [238]

The adsorption isotherms for the adsorption processes of nitrophenol derivatives 4-NP and 2,6-DNP on granular activated carbon type GAC 1240W NORIT were determined based on the experiments performed in the simple batch type glass adsorber. The constant temperature was provided with a thermostatic bath type TERMOMIX GRANT LTD6G, where the samples were maintained at a constant temperature of 25^{o}C for 48 hours, until the adsorption equilibrium was reached. The concentration of the solution was determined from the equations of the calibration curves obtained by the UV-Vis spectrophotometric measurements.

The isotherm data were analyzed according to the three well known isotherm equations: Langmuir, Freundlich and Tempkin, whose liniarized forms are given in equations (17)-(19) respectively [257-259].

- The liniarized form for Langmuir isotherm equation:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{\max}} + \frac{1}{bq_{\max}} \tag{17}$$

- The liniarized form for Freundlich isotherm equation:

$$\ln q_e = \ln K_F + \left(\frac{1}{n}\right)\ln C_e \tag{18}$$

- The liniarized form for Tempkin isotherm equation:

$$q_e = k_1 \ln k_2 + k_1 \ln C_e \tag{19}$$

where q_e is the amount of adsorbate adsorbed per unit mass of adsorbent at equilibrium (mg nitrophenol g^{-1} NORIT GAC 1240W); $C_e$is the final concentration at equilibrium (mg L^{-1}); q_{max} is the maximum adsorption at monolayer coverage of surface (mg nitrophenol g^{-1} NORIT GAC 1240W); b is the adsorption equilibrium constant related to the energy of adsorption (L mg^{-1}); K_F is a Freundlich constant representing the adsorption capacity (mg g^{-1})(L mg^{-1})$^{1/n}$; n is a constant depicting the adsorption intensity; $k_1$the Tempkin isotherm energy constant (L mg^{-1}) and k_2 the Tempkin isotherm constant.

The main difference between these three isotherm models is in the variation of the heat of adsorption with the surface coverage. Langmuir model assumes uniformity, Freundlich model assumes logarithmic decrease and Tempkin model assumes linear decrease in the heat of adsorption with surface coverage.

The experimental data obtained for 4-NP and 2,6-DNP were analyzed by means of the equations describing these models. The linear regression and other parameters of these equations are presented in Table 9.

Table 9. Parameters of Langmuir, Freundlich and Tempkin adsorption isotherm equations for nitrophenolic compounds at 25°C [238].

Nitrophenol	**Langmuir parameters**		**Freundlich parameters**		**Tempkin parameters**	
	q_{max} (mg/g)	**b (L/mg)**	**K_F (mg/g)**	**1/n**	**k_1 (L/mg)**	**k_2**
4-NF	277.77	86.44	2.04	1.52	33.6	0.59
2,6-DNF	41.15	0.63	2.01	2.30	5.40	36.39

A better criteria to test the correlation between experimental data (to) and one of the three isotherm equations (Langmuir, Freundlich and Tempkin), is a parameter known as normalized percent deviation [250], or percent relative deviation modulus, *P*, [257,259]. It is generally accepted that when the *P* value is less than 5, the fit is considered to be good [262]. The values for percent relative deviation modules, *P*, calculated for all three models and both nitrophenol derivatives are presented in Table 3. It can be seen that, in the case of 4-nitrophenol the value of *P* is less than 5 only for Freundlich model (P=0.9), and in the case of 2,6-dinitrophenols for the Langmuir model (P=2). The efficiency of adsorption process can be predicted by the dimensionless equilibrium parameter R_L. Isotherm is considered to be unfavorable when $R_L>1$, linear when $R_L=1$, favorable when $0<R_L<1$ and irreversible when $R_L=0$ [259,263,264].Since all the R_L valuescalculated for the treated nitrophenols are between 0 and 1, the adsorption processes are favorable in all cases.

Conclusions

In this study different adsorbant materials were used (granular activated carbon, powder activated carbon, graphit powder) for the adsorbtion of 4-NP and 2,6-DNP adsorption, in order to assess their applicability to remove nitrophenols derivatives from aqueous solutions. The experiments were carried out in single adsorber, keeping the operating parameters constant (temperature, speed of agitation and so on). The experiments were conducted in a batch mode at constant working conditions. The equilibrium adsorption data were described by the Langmuir, Freundlich and Tempkin mathematical models. The values of the constants and correlation coefficients in all three models were calculated. Based on the correlation coefficients both Langmuir and Freundlich models are suitable for the study (the squares of correlation coefficients are all >0.97). According to the analysis of the results, and the values of normalized percent deviation *P*, the experimental data for 4-NP adsorption onto granular activated carbon type NORIT GAC1240W were correlated reasonably by the Freundlich model, while those for 2,6-DNP adsorption were correlated by the Langmuir model.

From the liniarized Langmuir equation, the calculated adsorption capacity values ($q_{\max}$) are: 277.77 mg g^{-1} for 4-NP, respective 41.15 mg g^{-1} for 2, 6-DNP adsorption on granular activated carbon type NORIT GAC 1240W.

Because the equilibrium parameter $R_L$values are close to 0 ($10^{-5}\div10^{-6}$) for 2, 6-dinitrophenol at higher concentrations ($5\cdot10^{-4}\div10^{-3}$ mol L^{-1}), it is possible to presume that, in this conditions, the adsorption is irreversible. Future experiments are necessary to study the reversibility of the nitrophenol compounds adsorption onto activated carbon.

Chapter II.5. The combined procedure adsorption on activated carbon with electrochemical reduction for the elimination of nitrophenols from aqueous solutions

5.3. Electrochemical removal of nitrophenols on fixed bed of activated carbon cathod

After the determination of the experimental conditions using electrochemical reduction, adsorbtion and the combined procedure electrochemical reduction with adsorption in in simple batch reactor, experiments were performed using monocompartment electrochemical reactor (experimental setup B, page 73), with fixed bed of activated carbon type NORIT ROW 0.8 mm pellets, employed as the cathode, while Ti plates were used as the anode. Figure 49 (A) and 49 (B) present the variation of absorbance over time in 4-NP and 2,6-DNP removal, respectively. Results were obtained with the combined (Ads+ER) procedure. The removal of nitrophenols from the solutions was completed very fast. Hence, within the initial 20 min 94% of 4-NP 92% and of 2,6-DNP, respectively, was removed.

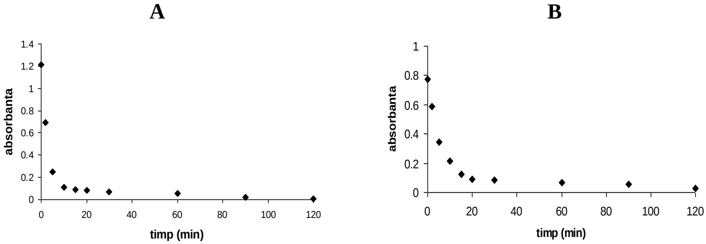


Figure 49. Absorbance evolution during the electrochemical reduction of: 4-NP (λ=317 nm) **(A)** and 2,6 DNP (λ=430 nm) **(B)** on stainless steel cathode in the presence of a fixed bed of granular activated carbon (experimental setup B) [249]. (10^{-3} M of 4-NP and 2,4-DNP solutions in phosphate buffer pH 4; i: 20 mA cm^{-2}).

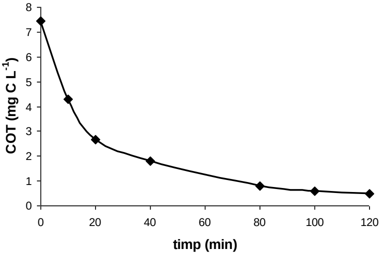


Figure 50. TOC variation during the electrochemical reduction of 10^{-3} M 2,6-DNP solution in phosphate buffer solution (0.1 M; pH 4), on stainless steel cathode in the presence of granular activated carbon.
(i: 20 mA cm^{-2}; spectrophotometre TOC-DR2800 HACH-LANGE; TOC test cuves LCK 385, 0÷30 mg L^{-1}).

This procedure combines the electrochemical transformation of nitrophenol derivatives with their adsorption and their transformation products on the activated carbon surface in an electrochemical reactor.

The results obtained by spectrophotometric determination (Figure 49), were confirmed by the total organic carbon determination. Figure 50 presents the TOC values variation in time for 2,4-DNP removal by electrochemical reduction on stainless steel cathode in the presence of a fixed bed of granular activated carbon type NORIT ROW 0.8 mm pellets.

Conclusions

Preliminary tests were made using a simple glass reactor for the removal of nitrophenol derivatives from aqueous solutions using a combined procedure of electrochemical reduction with adsorption on granular activated carbon. The obtained data were compared with those obtained using the two methods individually, under the same experimental conditions, showing that the combined process is far more reliable (it increases the overall removal process nitrophenol in solution by reducing the time required). The synthetic 4-NP and 2,6-DNP solutions concentrationswere reducedunder the maximum values allowed by environmental international legislation (0.33 mg L^{-1}and 0.5 mg L^{-1} respectively).

The methods used for the combined process evaluation were: cyclic voltammetry, UV-Vis spectrophotometry and TOC measurements. After determining the optimal experimental conditions using the electrochemical reduction tests, the adsorption on active carbon and the combined process adsorption with electrochemical reduction the monocompartment electrochemical reactor was used (experimental setup B), in which the cathode consists of a granular activated carbon NORIT ROW pellets fixed bed. TOC determinations confirms the results obtained by using UV-VIS spectrophotometry regarding the 4-NP and 2,6-DNP removal from synthetic solutions. The goal of using the combined process was achieved, namely: increased efficiency of the removal of nitrophenol derivatives and possible *in situ* regeneration of activated carbon in order to minimize consumption.

Chapter II.6. The removal of nitrophenolic pesticides from aqueous solutions

6.1. Introduction

Nitrophenolic herbicides, as DINOSAM ((*RS*)-2-(1-methyl butyl)-4,6-dinitrophenol), DINOSEB ((*RS*)-2-*sec*-butyl-4,6-dinitrophenol), DINOTERB (2-*tert*-butyl-4,6-dinitrophenol), DNOC (4,6-dinitro-o-crezol), etc., are very toxic and biorefractory substances and they are classified as priority pollutants, dangerous for the environment, in USA and E.U., where production, merchandising and utilization is banned or restricted. Nitrophenolic herbicides yield 2,4-DNP and 4-NP as initially and major degradation product, which are both toxic and biorefractory compounds. They will be used as model molecules in experimental determinations. The obtained results shall be subsequently applied to some dinitrophenolic herbicides.

From the data presented in the previous chapters it can observed that, in the case of electrochemical transformation of 4-NP and 2,4-DNP, the efficiency of their removal process from the solution depends on many factors, such as: the method of removal (electrochemical oxidation or reduction), electrochemical reactor type, electrode material, supporting electrolyte etc. The best results were obtained using the electrochemical reduction process on stainless steel cathode in the presence of granular activated carbon (4-NP removal rate of 99% and 2,4-DNF at 96% after 2 hours of electrolysis). The results were promising for the electrochemical oxidation processes the anode of Ti/Pt-Ir, where the removal of the 4-NP occurs at a rate of 96% after 2 hours of electrolysis.

6.2.1. Electrochemical reduction of DNOC and DINOSEB on stainless steel cathode in the presence of granular activated carbon

Electrochemical reduction experiments were performed in monocompartment electrochemical reactor with electrolyte recirculation (experimental setup B, pag. 73), on stainless steel cathode in the presence of granular activated carbon type NORIT ROW 0.8 mm pellets (fixed bed) and Ti anode. Figures 52 (A) and 52 (B) present the absorbance variation during DINOSEB and DNOC dinitrophenolic pesticides removal processes.

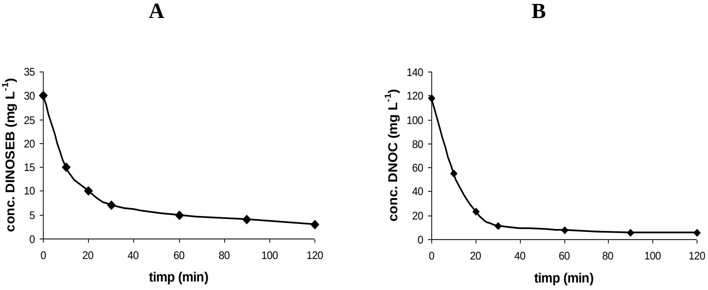


Figure 52. The concentration variation during the electrochemical reduction of 100 mL of 30 mg L^{-1} DINOSEB **(A)** and 118 mg L^{-1} DNOC **(B)** solutions in Britton-Robinson buffer (pH 2.11) on stainless steel cathode in the presence of a fixed bed of granular activated carbon.
(**data included in the final Report for the research grant for young researcher type II, financed by BEnA–Titan SA, 2009)**

The initial concentration in phosphate buffer solution of pH 4 was 30 mg L^{-1} for DINOSEB and 118 mg L^{-1} for DNOC. After 120 minutes of electrolyze the DINOSEB removal was about 90%, while DNOC concentration has decreased by 95%. This is probable due to the effect of different groups which are present on the aromatic nucleus and the mutual position of these groups. The determination of the process evolution for the herbicides DINOSEB and DNOC electrochemical reduction was performed using UV-Vis spectroscopy by following the absorbance evolution over time at a wavelength of 375 nm. The concentration was calculated by interpolating the corresponding calibration equation.

6.2.2. Electrochemical oxidation of DNOC

A volume of 100 mL of 118 mg $L^{-1}$of DNOC solution (corresponding to 50 mg L^{-1} of TOC) in Britton-Robinson buffer solution (pH 2.11) was electrolyzed at 0.450 A to test its comparative degradation by anodic oxidation with Pt and Ti/Pt-Ir anodes. In both cases, the starting yellow solution became dark-brown after 5 min of treatment due to the formation of some soluble aromatic products. At longer electrolysis times, the intensity of the color gradually decreased to a colorless solution after 100 min of electrolysis, when such aromatic species were completely destroyed by the hydroxyl radicals adsorbed on the surface of each anode.

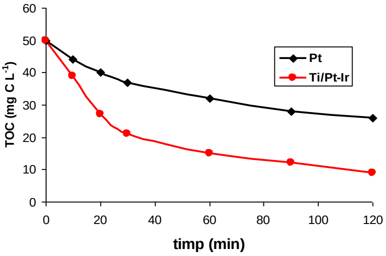


Figure 53. TOC variation during the electrochemical oxidation of 100 mL of118 mg L^{-1}DNOC solution in Britton-Robinson buffer solution (pH 2.11) on Pt and Ti/Pt-Ir anodes and graphite cathode.
(I=0,450 A; TOC-DR2800 HACH-LANGE spectrophotometer; LCK 385 test cuvets, 0÷30 mg $L^{-1}$and LCK 386, 30÷300 mg L^{-1}, respectively;(**data included in the final Report for the research grant for young researcher type II, financed by BEnA–Titan SA, 2009)**).

Figure 53 shows the change in the TOC value with applied current for both experiments. A very fast TOC abatement can be observed for Ti/Pt-Ir, leading to overall mineralization (85% TOC decay after 2 h). In contrast, this parameter drops very slowly for Pt, only being reduced by 48% after 2h. The use of a Pt anode under comparable conditions leads to quite poor depollution, because it produces smaller amounts of highly reactive hydroxyl radicals on its surface which destroy organics.

6.2.3. Electrochemical oxidation of DINOSEB

A volume of 100 mL of 30 mg L^{-1} DINOSEB solution in phosphate buffer solution (0.1 M; pH 2.11) was electrolyzed at 0.450 A to test its comparative degradation by anodic oxidation with Pt and Ti/Pt-Ir.

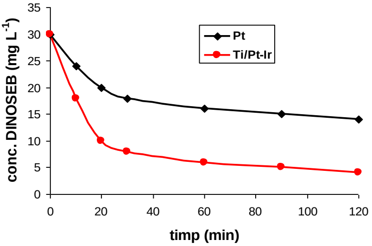


Figure 54. DINOSEB concentration abatement with time in electrochemical oxidation processes on Pt respective Ti/Pt-Ir anodes and graphite cathode. (Experimental conditions: DINOSEB initial concentration: 30 mg L^{-1} in phosphate buffer solution (0.1 M; pH 2.11);(I=0.45 A); **data included in the final Report for the research grant for young researcher type II, financed by BEnA–Titan SA, 2009).**

Figure 54 presents the abatement of the concentration of DINOSEB solution during electrochemical oxidation processes (based on spectrophotometric data). A very fast decrease in the concentration of DINOSEB can be observed for Ti/Pt-Ir (87% after 2h, while on Pt anode the decreasing of the concentration is only 55% in the same experimental conditions).

Conclusions

All the presented procedures proved to be effective in the removal of mono and dinitrophenol derivatives, and can be also applied to synthetic solutions of dinitrophenol herbicides. At the end of the experiments, the concentration of these toxic compounds reached a value below the maximum limits set out in European and American environmental standards. Voltammetry, UV-Vis spectrophotometry, high performance liquid chromatography and total organic carbon determinations are effective methods for controlling thenitrophenol derivatives removing processes.

Chapter II.7. The development of a biosensor for phenol derivatives detection

7.1. Introduction

In this chapter a comparative study for electrodes modified with polymeric films doped with different CNT using cyclic voltammetry and electrochemical impedance spectroscopy (EIS), which had as its main objective the development of a nanocomposite material suitable for the development of a biosensor based on horseradish peroxidase (HRP) for the detection of phenolic compounds is presented, . The method used to immobilize HRP on the surface of the transducer (SPE or GCE) is the incorporation of SWCNT and MWCNT in conducting polymers films (PEI and PPy). The steps involved in the development of sensors based on glassy carbon electrode modified with SWCNT and PEI are presented in Figure 55.

The obtained configurations were used to monitor the signal produced by the electrochemical reduction of the enzymatically generated electroactive oxidized species of acetaminophen (N-acetylbenzoquinoneimine—NAPQI) in the presence of hydrogen peroxide by amperometry, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) [155,165] (Scheme 3).

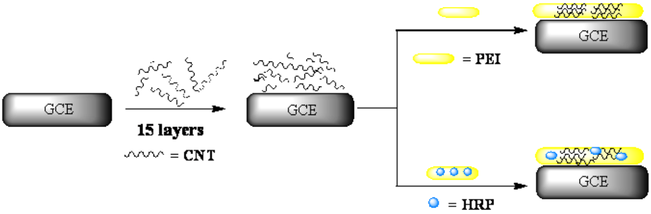
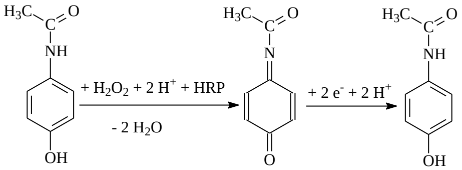


Figure 55. The schematic representation of the steps involved in the biosensor fabrication.

A comparative study between different types of CNTs for doping the polymeric films was performed. The biosensors were applied to the assay to determinate acetaminophen in various drug formulations (mono and bicomponent) by the standard addition method [155].



Scheme 3. The enzymatic and electrochemical transformation of acetaminophen in aqueous medium in the presence of H_2O_2.

7.2. Electrochemical behavior of the glassy carbon electrodes modified with CNTs, conducting polymer and HRP

7.2.1. Voltammetric behavior of the modified glassy carbon electrodes [155]

Five different types of CNTs were tested to determine the optimum electrochemical behavior and optimal material for biosensor development. The electrochemical behavior of the CNTs modified electrodes is influenced by the size of each type of tested nanotubes (diameter and length).as shown in Figure 56.

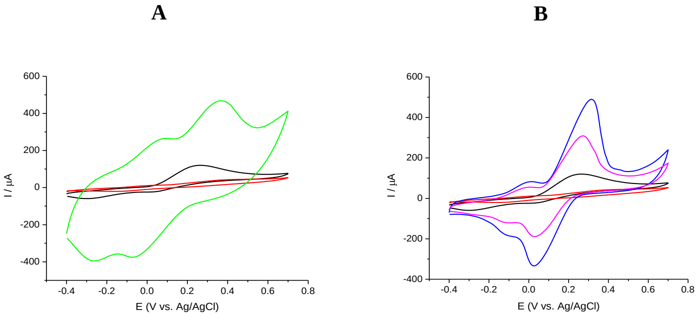


Figure 56. The electrochemical behavior of 10^{-4} M acetaminophen in PBS (pH 7.4; 0.1 M) at: (**A**) SWCNT/PEI (green) and (**B**) MWCNT (type 1)/PEI (blue); MWCNT (type 3)/PEI (magenta); compared with GCE simple (black) and GCE modified with PEI (red). [155].

The best results in terms of electrochemical oxidation of acetaminophen, observed from the CV measurements were obtained on single-wall carbon nanotubes and multi-wall carbon nanotubes type 4. For the nanocomposite composed of CNT and polypyrrole deposited on GCE, the electrochemical behavior was investigated using CV in 0.1 M aqueous solution of $LiClO_4$

7.2.2. Chronoamperometric behavior of the modified glassy carbon electrodes [155]

Chronoamperometry is based on measuring the resulting current in an electrochemical oxidation or reduction reaction, of an electroactive species. It is usually achieved by maintaining a constant potential of an electrode relative to the reference electrode [285]. Reference electrodes are generally Ag/AgCl type, while the working electrodes are typically modified with magnetic particles, carbon nanotubes or polymers [286,287]. The resulting current is directly correlated to the concentration of electroactive species or their production or consumption in a biocatalytic layer [287]. Biocatalytic reaction rates are often chosen in such a way that they depend linearly on the concentration of the analyte which results in a linear dependence of current on the concentration of the analyte [287]. A great advantage of this technique is the ability to record amperometric electrochemical response in hydrodynamic environments [286].

The incorporation of HRP in the polymeric film allow the use of the obtained nanocomposite biosensor in chronoamperometry studies, in the presence of hydrogen peroxide and with the addition of successive volumes of a acetaminophen solution.

Chronoamperometry studies show that the best results are obtained onGCEmodified withSWCNT/Ppy, and the nanocomposite obtained by modifying the electrode with carbon nanotubes and HRP immobilized in polypyrrole film (MWCNT+Ppy+HRP) has better stability compared to that obtained with polyethyleneimine (MWCNT+PEI+HRP).

To improve the reproducibility, sensitivity and mechanical stability of the developed biosensors, the layer-by-layer method was used for the deposition of CNTs on electrode surface (instead of drop drop coating). For this purpose successive layers of SWCNT in THF suspension were deposited on the surface of the glassy carbon working electrode (diameter of 1 and 4 mm) and on the planar graphite based screen-printed electrode. Chronoamperometry determinations were also carried out in order to compare the nanocomposite systems. It could be observed that SWCNT/PPy and SWCNT/PEI have higher electrochemical behavior compared with that obtained using MWCNT. This behavior can be attributed to the simple structure of the SWCNT that can facilitate electron transfer between the enzyme and the electrode surface modified with conductive polymer.

For the enzyme immobilization by layer-by-layer method, SWCNTs with a diameter of 1.5 nm and a length of 1-5µm has been used, using PEI as polymer. This technique involves placing the 15 layers of dispersed SWCNT in THF on the surface of the electrode and drying the solvent by evaporation after each deposition.

Cyclic voltammetry (CV) graphs of bare and CNT entrapped in polyethyleneimine coated GCE were performed in 10 mM $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ solution prepared in phosphate buffer (0.1 M; pH 7.4). The results are presented in Figure 60. As it can be observed for all investigated carbon nanotubes (single and multi-walled) the current peak intensity is significant increased compared to the bare GCE. The SWCNT+PEI modified GCE (red) presented the largest area enclosed by CV curve while the lowest value is obtain with the MWCNT (type 1, green) modified electrode which has the smallest length and diameter (L=30±10 µm and d=1-5 µm). In the case of the MWCNT (type 4, magenta) modified GCE, which has the largest length and diameter (L=30±15 µm and d=5-20 µm) the current peak intensity is almost the same as for SWCNT modified GCE. The behavior of these CNTs modified electrodes was expected to be distinct due to the different amounts of deposited material on the electrodes from various suspensions and different particle dimensions.

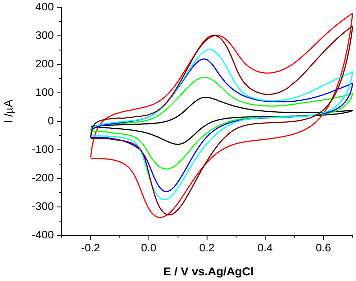


Figure 60. The cyclic voltammograms obtained for: bare GCE (black) and GCE+CNT: SWCNT (red); MWCNT type 1 (verde); MWCNT type 2 (blue); MWCNT type 3 (cian); MWCNT type 4 (wine) using $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ 10 mM in phosphate buffer solution (0.1 M; pH 7.4); scan rate: 0,1 Vs^{-1}; 5 cycles [233].

As seen in Figure 60, the current intensity increased at the both ends of the scanning range only in the cases the modified electrodes, suggesting that while on the bare glassy carbon electrode no adsorption and desorption phenomena are observed, in all other cases this does occure and is more or less pronounced depending on the morphostructural characteristics of the used carbon nanotubes [233].

7.2.3. The characterization of the biosensor by Electrochemical Impedance Spectroscopy experiments

Electrochemical impedance spectroscopy measurements have been carried out to characterize the changes at the surface of the electrodes which occurred after the immobilization of the CNTs and HRP in the PEI film. Different behaviours of the studied CNTs have been observed, due to the differences in their structural characteristics.

Figure 61 (A) shows the Nyquist plots of CNTs modified GCE in the entire range of tested frequencies from 0.01 Hz to 100 kHz. The zoomed Figure 61 (B) emphasizes the high frequency zone. Figure 61 (C) shows the Nyquist plots of CNTs+PEI+HRP modified GC electrodes in the entire range of tested frequencies from 0.01 Hz to 100 kHz. The zoomed Figure 61 (D) presents the high frequency zone (data not shown in abstract).

On these plots, the diameter of the semicircle is referred as charge transfer resistance R_{ct} while the high frequency intercept on the Z_{real} axis represents the solution resistance (R_{sol}). It could be observed that the results obtained with cyclic voltammetry are confirmed by EIS experiments. The SWCNT and MWCNT type 4 present the lowest R_{ct} values, and the best voltammetric and impedance response. In the light of these results and due to the fact that the use of SWCNTs offers some advantages over MWCNT, SWCNTs were chosen for further experiments. Some of the advantages of SWCNT are: the formed networks are multi-fold, the SWCNTs do not require further purification compared to MWCNT, the active surface is expanded, the SWCNT network format is well defined and of high uniformity, providing a known surface coverage, the charge transfer between SWCNT and active center of the enzyme is facilitated.

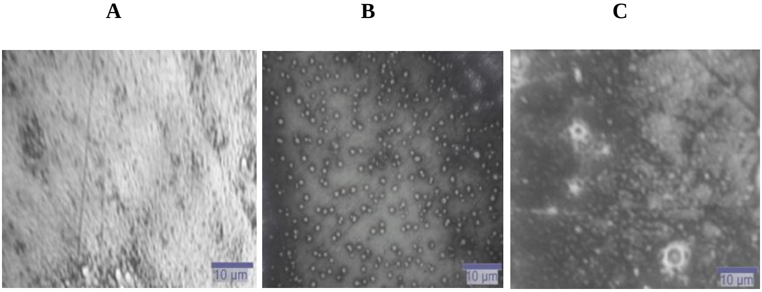


Figure 62. Microscopy image of: **(A)** SWCNT modified GCE; **(B)** SWCNT+PEI modified GCE and **(C)** SWCNT+PEI+HRP modified GCE [233].

It could be noticed the formation of a non homogene thick film at the surface of the electrode in the case of: GCE modified with: SWCNT (Figure 62 (A)), SWCNT+PEI (0.1 mg/ml) (Figure 62 (B)) and SWCNT+PEI+HRP(0.3 mg/ml) (Figure 62 (C)). Comparing the images of the electrode modified with CNTs (Figure 62 (A)) with the one of the electrode modified with CNTs and PEI film (Figure 62 (B)) it can be noticed an improvement in the homegeinity of the surface after adding the PEI film.

In order to acquire information from electrochemical impedance spectroscopy, the data must be fitted to an equivalent circuit.

An equivalent circuit was modeled for the unmodified GCE in contact with the redox probe. This circuit is: R1(Q1[R2W1]), (see the inset of Figure 64 (A)), and contains the solution resistance (R_{sol}), the constant phase element (CPE=Q1), the charge transfer resistance (R_{ct}) and the Warburg impedance (W). Here the conventional C_{dl} is replaced by CPE. Replacing C_{dl} with CPE represents the non uniform behavior of adsorbed species on irregular geometry and small electrodic surface. The reaction seems to occur in a single step and a combination of kinetic and diffusion processes through a layer of infinite thickness describes the whole process.

The Nyquist plot of bare GCE shows a semicircle portion at higher frequencies (lower Z values), corresponding to the electron transfer limited process and a linear part in the lower frequency range (higher Z values) for the diffusion-limited process.

An equivalent circuit may be modeled for the modified glassy carbon electrodes in contact with the redox probe. For this purpose the ladder type (eg. R1(C1[R2(R3C2)(C3[R4W1])])) and Voight type equivalent circuits were tested. Given the fact that the Voight type circuit showed better results with respect to Iadder type circuits, the former was chosen for fitting further experimental data (see the insets in Figure 64 (B), 64 (C) and 64 (D)).

When the GCEs are modified with layers of SWCNT, SWCNT+PEI and SWCNT+PEI+HRP, the proposed circuit is: R1(R2C1)(R3C2)(C3[R4Q1]), as seen in the insets in Figures 64 (B),64 (C) and 64 (D). The model used to describe the behavior of those films consists of a solution resistance ($R1=R_{sol}$), in series with a parallel combination of the adsorption process capacitance ($C2=C_{ads}$) and the adsorption process resistance ($R3=R_{ads}$). This is again in series with the double layer capacitance ($C3=C_{dl}$) connected in parallel with a series combination of the charge transfer resistance ($R4=R_{ct}$) and the constant phase element (CPE), which together represent the diffusion process. In this case, the Warburg impedance is replaced by CPE, an element from the equivalent circuit that describes either the capacitance of the film/GCE interface or the film/solution interface, modeled as a constant-phase. The terms R_{ads} and C_{ads} which probably refer to the relaxation after the adsorption process, were included in the equivalent circuit in order to obtain adequate fits. The last combination from the series equivalent circuit was added in order to fit the high frequency domain. This term is probably caused by the reference electrode and contain a parallel combination of a resistance ($R2=R_{ref}$) and a capacitance ($C1=C_{ref}$).

The same suggestion was done earlier by Nijhuis et al. [290] which obtained the same shape for the Nyquist plots (with two arches) in the case of a water-soluble supramolecular assemblies of redox-active ferrocenyl-decorated poly(propylene imine) dendrimers and *β*-cyclodextrin adsorbed at self-assembled monolayers on gold electrode.

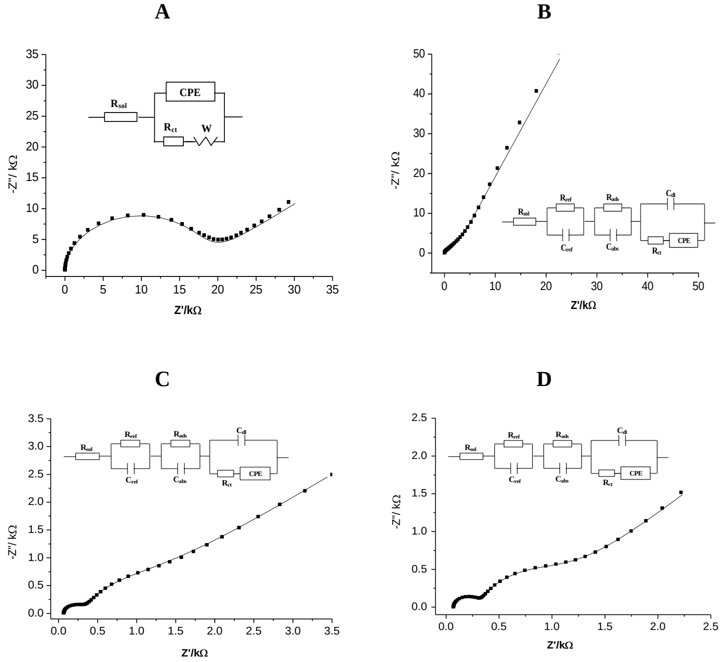


Figura 64. Corelation between Nyquist plots for: **(A)** bare GCE; **(B)** SWCNT modified GCE; **(C)** SWCNT+PEI modified GCE and **(D)** SWCNT+PEI+HRP modified GCE in 10 mM $K_3[Fe(CN)_6]+K_4[Fe(CN)_6]$as redox probe, in phosphate buffer (0.1 M; pH 7.4). **Inset:** the equivalent circuits used for interface process modeling [233].
(Amplitude: 10 mV, first frequency 100 kHz last frequency 10 mHz (61 frequencies). Fitting results are given by lines.).

The proposed equivalent circuit fits all types of the modified glassy carbon electrodes (with CNTs, CNT+PEI and CNT+PEI+HRP) and in the case of CNT+PEI and CNT+PEI+HRP modified GCE, the Nyquis plots present two semicircles. In the case of CNT modified GCE there is no clear separation betwen the two semicircles, the first one having a very small radius (see for example Figure 64 (B): the Nyquist plot for SWCNT modified GCE).

Especially in the case of SWCNT modified electrodes, the EIS spectra show parts of two arches with very small radius followed by a straight line, showing a sharp increase in the imaginary part of the impedance at lower frequencies, which are representative for ion diffusion dominant processes [289]. This electrode shows its linear EIS performance over a wider range of frequencies. This could be attributed to a poorer accessibility of ions to the pores of the film from the electrodes.The presence of the two semicircles (that correspond to the time constant τ_1 and τ_2) in the Nyquist plots were confirmed by Bode data presentation.

A mechanism was proposed in order to describe the processes that take place at the interface. First step is represented by the transport of the electroactive species ($[Fe(CN)_6]^{4-}$) at the electrode surface, process with a time constant $\tau_1 = R_{ct}C_{dl}$. The electroactive species are then transformed at the surface in ($[Fe(CN)_6]^{3-}$) which is adsorbed. $C2=C_{ads}$ represent the capacitance of the adsorption process and its value is used together with the resistance of the adsorption process ($R_3=R_{ads}$) in the calculation of the second time constant $\tau_2 = R_{ads}C_{ads}$ which is the time constant of the relaxation process.

After the desorption of $[Fe(CN)_6]^{3-}$, the diffusion in bulk solution take place. The proposed mechanism is represented in Scheme 4, which corresponds to a two steps reaction in the presence of diffusion limitations and taking into account the non uniform adsorption:

$$[Fe(CN)_6]^{4-} \xrightarrow{\tau_1} [Fe(CN)_6]^{3-}{}_{ads} + e^-$$

$$[Fe(CN)_6]^{3-}{}_{ads} \xrightarrow{\tau_2} [Fe(CN)_6]^{3-}{}_{sol}$$

$$1/2\ O_2 + H^+ + e^- \longrightarrow H_2O$$

Scheme 4. The proposed mechanism for the interface process [233].

The values for the two time constants describing the process were calculated from the data obtained by fitting and it can be presumed that this process is faster on

SWCNT modified GCE and the charge transfer rate is decreasing after the formation of PEI and PEI+HRP films. Regarding the charge transfer resistance, on SWCNT+PEI modified GCE the values are dramaticaly decreasing at about 656 Ω compared with 3130 Ω in the case of SWCNT modified GCE. One possible explanation is that the polymer layer smoothes the rough surface of the carbon nanotubes deposited on the electrode surface allowing a better charge transfer through the film. Another explanation could be the high adherence of PEI on the electrode surface. The addition of HRP in the PEI film had a small influence on the charge transfer and the R_{ct} obtained values are comparable.

The second time constant, describing the relaxation after the adsorption on the electrode surface indicates that this process takes place with medium rate on SWCNT modified GCE, with high rate on SWCNT+PEI modified GCE and with low rate if the HRP enzyme is entraped in the polymeric film with SWCNT deposited on GCE.

7.2.4. Biosensor optimization [155]

Different numbers of layers were tested in order to optimize the response of the biosensors. The best results were obtained for 15 cycles. By decreasing the numbers of cycles the stability of the biosensors is lower and the sensitivity also decreases. Increasing the number of layers didn't improve the sensitivity of the sensors.

The influence of the enzyme loading in the nanocomposite was also investigated by varying the quantity of the entrapped enzyme (0.3 and 0.6 $mg{\cdot}mL^{-1}$ of HRP polymer solution). A two-fold increase in the enzyme concentration did not double the sensitivity, so for testing the biosensor on real samples the concentration of 0.3 $mg{\cdot}mL^{-1}$ of HRP in the polymer solution was used.

The apparent Michaelis-Menten constant was calculated using the Michaelis-Menten equation and the value obtained that the biosensor possesses high biological affinity to acetaminophen and are in agreement with the earlier reports [296]. The detection limit for five configurations was calculated for a signal to noise ratio of 3 and the lowest limit of detection was obtained for the 0.3 $mg{\cdot}mL^{-1}$ HRP/SWCNT/PEI modified GCE (d=1 mm).

The reproducibility and stability are the two important parameters for the evaluation of the performance of the sensor. The reproducibility of the biosensors was examined at 10 μM acetaminophen solution. The relative standard deviation was between 1.31% and 2.56% for HRP/SWCNT/PEI at GCE (d=4 mm) configuration and 1.78% for HRP/SWCNT/PEI at graphite based SPE, respectively (n=5). Comparing the modified SPEs with the modified GCEs it could be underlined that the RSD and the recovery are better in the case of GCE as working electrode. This could be explained by the roughness of the SPE that lead to a non uniform distribution of the SWCNT and HRP at their surfaces. The results are in agreement with other previous results obtained on Zr alkoxide/HRP/PEI/ SPEs and Zr alkoxide/HRP/PEI/ GCEs [152,187].

In order to demonstrate the stability, the response of the acetaminophen was measured every day for 10 days. All electrodes, when not in use, were stored under semi-dry conditions and were stable for at least 10 days. 65% of the original biosensor response remained after 10 days. The decrease in current response may be due to the denaturation of the enzymes in long-time keeping in semidry conditions and the removing of the nanocomposite from the electrode surface due to its partial water-solubility in the case of PEI. The stability of the sensors and their sensitivity can be attributed to the excellent biocompatibility of the CNTs for preserving the activity of HRP and to the strong covalent interaction between CNTs, conductive polymers (polypyrrole) and the enzyme.

7.3. Real samples analysis [155]

The obtained biosensors were applied for the assay of acetaminophen in a couple of commercial drug formulations: Panadol® (GlaxoSmithKline) and Panadol Extra®(containing acetaminophen and caffeine, GlaxoSmithKline). The results obtained are consistent with those reported by the manufacturer (500 mg/tablet), see Table 18.

It could be assumed that the presence of other active compounds affect the enzymatic activity of the HRP immobilized in the nanocomposite CNT-conductive polymer in the amperometric experiments, therefore the degree of recovery values obtained for multicomponent pharmaceutical compounds are weaker.

In order to compare the electroanalytical results, acetaminophen solutions (standard and obtained with the investigated pharmaceuticals) were analysed by spectrophotometry at 243 nm, the official analytical method recommended by the Romanian Pharmacopoeia (Table 19).

Table 18. Acetaminophen electrochemical determination from monocomponent commercial pharmaceuticals. [155].

Electrode	Analyte	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
GCE(d=4mm)/SWCNT/PEI+ HRP (0.3 mg/mL)	Acetaminophen	10 20	10.01 20.08	100.01 100.40	0.98 1.06
	Acetaminophen /Panadol® GlaxoSmith Kline	- 10 20	10.04 20.88 31.09	100.40 104.40 103.64	1.67 1.31 2.04
	Acetaminophen /Sanador® Laropharm	- 10 20	9.94 19.77 28.97	99.44 98.85 96.60	2.35 2.54 2.09
SPE graphit/SWCNT/PEI+HRP (0.3 mg/mL)	Acetaminophen	10 20	9.98 20.03	99.80 100.15	1.42 1.86
	Acetaminophen /Panadol® GlaxoSmith line	- 10	9.91 20.53	99.10 102.65	2.81 2.93

Table 19. Determination of 4-(acetilamino)phenolby spectrophotometry[155].

Compound	Absorbanță (λ=243 nm)		Reccovery (%)	RSD (%)
	theoretical	practical		
Acetaminophen	0.645	0.649	100.71	0.05
Acetaminophen/Panadol® GlaxoSmithKline		0.655	101.62	0.11
Acetaminophen /PanadolExtra® GlaxoSmithKline		0.607	94.17	0.18

The obtained results are in good agreement with those obtained by electrochemical method using the new biosensors, both for pharmaceutical products containing acetaminophen only and those with two active compounds. Regarding the declared content of active substance in tablets, Romanian Pharmacopoeia requests a deviation of ±5% [39].

Conclusions

A comparative study between different types of carbon nanotubes (single and multi-layer) deposited on glassy carbon electrode surfaces immobilized in polyethyleneimine films together with HRP was performed using the CV and EIS measurements. The main aim of this comparative study is to optimize the nanocomposite electrode material for applications in the biosernsors field. After the optimization of experimental conditions, a mechanism was proposed to describe the processes occurring at the interface modified electrodes and the time constants were also calculated for the two processes that take place at the modified electrodes. The equivalent circuits were proposed and the Kramer-Kronig tests were performed in order to prove their reliability.

The performance of graphite-based and modified SPE and GCE were tested in order to obtain new devices for the detection and determination of acetaminophen. The optimized biosensor configuration (GCE+SWCNT (15 layers) + PEI (1mg/ml) + HRP (0.3 mg/mL)) was successfully used for amperometric detection of acetaminophen in synthetic solutions and commercial pharmaceutical with good sensitivity, selectivity, detection limits, relative standard deviation and recovery levels. The recovery data obtained by using the new implemented biosensors are in accordance with the results obtained using the officinal method listed in Romanian Pharmacopoeia (UV-Vis spectroscopy).

The used strategy offers exciting opportunities for developing other biosensors for environmental and pharmaceutical analysis, taking into account the simplicity of electrode surface modification method, the cost which is significant reduced compared to other methods used in practice. The developed biosensor allows the analytical applications without requiring highly skilled operating personnel and expensive equipment, is fast, selective and presents precise analytical response.

GENERAL CONCLUSIONS

The main goal of this thesis was finding applicable electrochemical reduction or combined processes for the phenolic compounds elimination from the synthetic aqueous solutions of these derivatives with a content exceeding the maximum concentration allowed by European regulations and international standards. It also aimed to achieve a biosensor for determination and quantification of phenolic compounds from synthetic samples and pharmaceutical products with application in environmental analysis.

During the research, the study of the redox behavior of the nitrophenols and their derivatives has been carried out, both in the direct and mediated charge transfer processes (electrochemical characterization) and mechanisms have been proposed for electrochemical reduction and oxidation of 4-nitrophenol on different electrode materials. Some voltammetric methods have been used (cyclic voltammetry, square wave voltammetry) and the areas of applicability of these methods for the determination of nitrophenol derivatives and their limits of detection in aqueous medium have been determined. Electrochemical, spectral and chromatographic methods and the determination of the total organic carbon have been used to control the process of removing phenolic compounds from solution.

Some direct and mediated electrochemical transformation experiments (oxidation and/or electrochemical reduction processes) and adsorption on different adsorbent materialsfor nitrophenols were carried out and a comparative study of these processes has been conducted. Using this studies a combined process of adsorption on activated carbon with electrochemical reduction was implemented for removing nitrophenol from aqueous solutions and the obtainedresults were significantly improved compared to those obtained in the simple processes.The results obtained in the case of model molecule of nitrophenols were extended to more complex molecules containing residues of nitrophenols and capable of generating nitrophenols by decomposition (dinitrophenol pesticides such as dinoseb ((RS)-2-*sec*-Butyl-4,6-dinitrophenol), DINOTREB (2-*tert*-butyl-4,6-dinitrophenol) and DNOC (4,6-dinitro-*o*-cresol)). It can observe that all experimental procedures used are effective in the removal of mono and dinitrophenol derivatives, and can be applied aslo for synthetic solutions of dinitrophenol herbicides. At the end of all experiments, the concentration of these toxic compounds reached a level below the maximum limits set out in European and American standards and environmental legislations.

As a continuation of this research an amperometric biosensor for determination of phenolic compounds from aqueous solutions has been developed. This biosensor is based on glassy carbon electrodes modified with a nanocomposite film of carbon nanotubes attached to a polymer film incorporating the enzyme horseradish peroxidase (HRP), and was tested and optimized for the determination of 4-(acetylamino)phenol (acetaminophen) from aqueous solutions of synthetic and real samples (pharmaceutical mono and multicomponent). In the case of acetaminophen the obtained limits of detection are in the range of 1.36 and 8.09 μM, and the dynamic range over which the sensors are applicable are between 4.08 and 79.01 μM, or 24.27 and 118.06 μM, depending on the electrode type. Recovery levels between 96.6% and 104.4% obtained for real sample analysis using the developed biosensors are consistent with those obtained using the officinal method provided by Romanian Pharmacopoeia: the UV-Vis spectrophotometry.

As future perspectives it is desired to be able to apply the developed biosensor for determination of phenolic compounds in real samples (surface water or groundwater from areas where such compounds were used) and to immobilize other enzymes (eg poliphenoloxidase) on the surface of the sensor, with future applications on other phenolic compunds and even nitrophenols.

ORIGINALITY

The originality of this thesis consists in the implementation of a combined process: adsorption on granular activated carbon with electrochemical transformation, method that was successfully applied for the removal of mono and dinitrophenol and of some nitrophenol pesticides from aqueous solutions. This method provides both removal of the reagents in solution, and the organic products formed during the electrochemical transformations that take place at the anode and cathode of the electrochemical reactor.

Another element of originality and novelty is the design and optimization of a biosensor based on glassy carbon electrode modified withcarbon nanotubes deposited by layer-by-layer method with the subsequent immobilization of HRP through PEI polymer. This type of biosensor was used for the first time in the investigations of the thesis for determining 4-(acetylamino)phenol at μM level, from both synthetic solutions and real samples (pharmaceutical products). The stability of this type of support for the construction of the sensor (carbon nanotubes deposited on a conductive surface and trapped in the polymer film) makes it suitable for different applications giving it versatility. Therefore, if other enzymes are immobilized in the polymer film, the biosensor can become selectively applicable to various types of compunds other than aminophenols.

The results of the research have been the subject of 10 research papers, of which: 5 original research articles published in international and Romanian ISI journals; 1 original research article submitted for publication in an international ISI journal; 1 original article published in a Romanian journal category A^+ CNCSIS; 1 original research article published in a Romanian BDI journal category B^+ CNCSIS; 2 papers published in the proceedings type journal of international conferences.

The obtained data were presented at many national and international conferences as poster and oral presentation.

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ANNEXE 1. List with abbreviations

2-NP	2-nitrophenol
3-NP	3- nitrophenol
4-NP	4- nitrophenol
2,4-DNP	2,4- dinitrophenol
2,6-DNP	2,6-dinitrophenol
DINOSAM	(*RS*)-2-(1-methyl butyl)-4,6-dinitrophenol
DINOSEB	((*RS*)-2-*sec*-butyl-4,6-dinitrophenol
DINOTERB	2-*tert*-butil-4,6-dinitrophenol
DNOC	4,6-dinitro-o-crezol
ETINOFEN	α-etoxi-4,6-dinitro-*o*-cresol
MEDINOTERB	6-*tert*-butil-2,4-dinitro-*m*-crezol
DINOCAP	(*RS*)-2,6-dinitro-4-octilfenil crotonat + (*RS*)-2,4-dinitro-6-octilfenil crotonat
HRP	Horse readish peroxidase
LD_{50}	amount of substance that has lethal toxic effect for 50% of adult animals in the tested group
DMF	N,N-dimethylformamide
DMSO	dimethyilsulfoxid
CV	Cyclic voltammetry
FSCV	Fast scan cyclic voltammetry
DPV	Diferential pulse voltammetry
SWV	Square wave voltammetry
BDD	Boron dopped diamant
LSV	Liniar Voltammetry
DV	DerivativeVoltammetry
HV	Hydrodynamic voltammetry with rotating disc electrode
EIS	Electrochemical Impedance Spectroscopy
THF	Tetrahidrofuran
DMF	Dimethylformamide
PEI	Poliethilen imine
Ppy	Polypyrrol
CNT	Carbon nanotubes
SWCNT	Single wall carbon nanotubes
MWCNT	Multywalled carbon nanotubes
GCE	Glassy carbon electrode
SPE	Screen-printed electrode
TBR	Britton-Robinson buffer solution
TOC	Total Organic Carbon

ANNEXE 2. Scientific activity

I. Research paper published and submitted for publication from the doctoral thesis

1. **M. Tertiş**, A. Florea, B. Feier, I.O. Marian, L. Silaghi-Dumitrescu, A. Cristea, R. Săndulescu, C. Cristea, Electrochemical impedance studies on single and multi wall carbon nanotubes–polymer nanocomposites for biosensors development, *Journal of Nanoscience and Nanotechnology*, **2013**, *submitted* (**IF 1,149; AIS 0,74**).
2. **M. Tertis**, A. Florea, R. Săndulescu, C. Cristea, Carbon based electrodes modified with horseradish peroxidase immobilized in conducting polymers for acetaminophen analysis, *Sensors*, **2013**,*13*, 4841-4854 **(IF 1,953; AIS 1,4)**.
3. **M-C. Tertis**, M. Jitaru, L. Silaghi-Dumitrescu, Combined procedure for nitrophenols removal, *Revista de Chimie (Bucuresti)*, **2010**, *61*, 360-363 **(IF 0,693; AIS 0,15)**.
4. V.K. Tchieda, I.K. Tonle,**M-C. Tertiş**, E. Ngameni, M. Jitaru, Adsorption of 2,4-dinitrophenol and 2,6-dinitrophenol onto organoclays and inorganic pillared clays, *Environmental Engineering and Management Journal*, **2010**, *9*, 953-960 **ISSN 1582-9596 (IF 1,435; AIS 0,142)**.
5. **M-C. Tertiş**, M. Jitaru, V. Coman, M. Filip, D.A. Lowy, Electrochemical reduction-adsorption procedure for the removal of nitrophenol contaminants from aqueous media, *Studia Universitatis Babes-Bolyai Chemia*, **2009**, *Special issue 1*, 151-162 **(IF 0,086; AIS 0,063)**.
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7. **M-C. Tertiş**, M. Jitaru, A. Trifoi, V. Coman, Electrochemical treatment of wastewater containing different nitrophenols, *Proceedings of the 2nd International Conference on Advanced Materials and Systems,* Editura Certex, Bucuresti, **2008**, 397-403**, ISBN 978-973-1716-39-8**.
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10. M. Ihos, **M. Tertiş**, M. Radaelli, M. Jitaru, L. Szpyrkowicz, Removal of 2,6-dinitrophenol from wastewaters by electrochemical and adsorptive processes, *Proceedings of the International Symposium "The Environment and Industry",* Vol. I, Bucharest, Ed. Estfalia, 25-27 October, **2007**, 100-105, **ISSN 1843-5831**.

II. Research paper published and submitted for publication out of the doctoral thesis

1. A. Maghear, **M. Tertiş**, L. Fritea, I.O. Marian, E. Indrea, A. Walcarius, R. Săndulescu, Tetrabutylammonium-modified Clay Film Electrodes: Characterization and Application to the Detection of Metal Ions, *Talanta*,**2013**, *submitted*.
2. **L. Fritea,** M. Tertiş, C. Cristea, R. Săndulescu, New β-Cyclodextrin Entrapped in Polyethyleneimine film Modified Electrodes for Pharmaceutical Compounds Determination, *Sensors***,** 2013, *13, accepted for publications*.
3. A. Florea, **M. Tertis**, R. Sandulescu, A. Cristea, *Designing polymer-based immunosensing platforms for cancer biomarker detection, Proceedings of the 4th* IEEE International Conference on e-Health and Bioengineering, *EHB, Iasi, Romania, 21-23 November,* **2013,** (EHB 2013 will be indexed in IEEE Xplore®, Conference Proceedings citation Index (ISI-Proceedings), SCOPUS and INSPEC (IET) data bases.), *accepted for publication*.
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12. V.K. Tchieda, I.K. Tonle, **M-C. Tertiş**, E. Ngameni, M. Jitaru, D.A. Lowy, Organoclays and inorganic-organic pillared clays. Preparation, characterisation and potential use as electrode modifier, *Studia Universitatis Babes-Bolyai Chemia*, **2009**, Special issue 1 (this issue contains papers based on the comunications presented at „14éme Édition des Journées d'Électrochimie, 6-10 Juillet, 2009, Sinaia, Roumanie"), 163-174**,**ISSN 1224-7154 **(IF 0.086)**.

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II. Scientific activity

1. B. Feier, **M. Tertis,** R.Săndulescu, A. Cristea, C. Cristea, Carbon based electrodes as a platform for the development of novel sensors, Third Workshop Specific Methods for food safety and quality, 27 September **2012**, Vinca Institute of Nuclear Sciences, Belgrade, Serbia **(presented by Assoc.Prof. Dr. C. Cristea)**.
2. **M. Tertis,** A. Campean, R.Săndulescu, Détermination voltampérométrique de certains alcaloides en utilisant une électrode de carbone vitreux activée par voie électrochimique, Third Regional Symposium on Electrochemistry of South-East Europe (RSE-SEE 3), Atelier scientifique international francophone NOMARES, 13-17 may **2012**, Bucharest, Romania **(presented by Prof. Dr. R. Săndulescu).**
3. **M. Tertis,** A. Ilioaia, R.Săndulescu, Modified electrodes for biosensors used in pharmaceutical and environmental analysis, Second Regional Symposium on Electrochemistry of South-East Europe (RSE-SEE 2), 6-10 June **2010**, Belgrade, Serbia **(presented by Prof. Dr. R. Săndulescu).**
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5. **M-C. Tertis**, M. Jitaru, A. Trifoi, V. Coman, Electrochemical treatment of westewater containing different nitrophenols, The International Conference ICAMS: The 2nd International Conference advanced materials and systems, Bucharest, Romania, 23-24.10. **2008**.
6. **M-C. Tertis**, M. Jitaru, L. Szpyrkowicz, Contribution to the reduction of nitrophenols concentration from wastewaters by electrochemical ways and by adsorption, The International Conference BIOREMED 2007: Chemical and Biochemical techniques to improve the Environmental Chemistry, Cluj-Napoca, 19-20.09.**2007**.
7. **M-C. Tertis**, M. Jitaru, L. Szpyrkowicz, Contribution a la diminution de la concentration des nitrophenols des eaux residuelles par moyens electrochimiques et par adsorbtion, The International Conference B.E.N.A.-ICAI: Sustainable Development in the Balkan Area: Vision and Reality, Alba-Iulia, 18-20. 07. **2007**.
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Poster presentations (28; 14 from thesis)

1. **M. Tertiş**, L. Silaghi-Dumitrescu, R. Săndulescu, Contrôle et elimination des pollutants nitrophénoliques des eaux résiduelles synthétiques, International conference Journées d'Electrochimie, Paris, France, 8-12 Juillet **2013**.
2. C. Cristea, **M.Tertiş**, R. Săndulescu, Modified carbon based electrodes as platform for the development of novel sensors, Fourth Regional Symposium on

Electrochemistry of South-East Europe (RSE-SEE 4), Liuljana, Slovenia, 26-30 May **2013**.

3. **M. Tertiş**, L. Silaghi-Dumitrescu, R.Săndulescu, Nitrophenolic compounds control and elimination from wastewaters, Fourth Regional Symposium on Electrochemistry of South-East Europe (RSE-SEE 4), Liuljana, Slovenia, 26-30 May **2013**.
4. C. Cristea, **M.Tertiş,** A. Florea, R.Săndulescu, Screen-printed electrodes modified with carbon nanotubes for the quantification of acetaminophen, „63rd Annual Meeting of the International Society of Electrochemistry”, Prague, Czech Republic, 19-24 August, **2012**.
5. **M.Tertiş**, A. Florea, R.Săndulescu, C. Cristea,Noi Biosenzori cu nanotuburi de carbon si HRP pentru detectia si cuantificarea acetaminofenului, Zilele UMF „Iuliu Haţieganu” Cluj-Napoca, România, 3- 7 decembrie, **2012**.
6. C. Cristea, **M.Tertiş**, A. Florea, R.Săndulescu, Carbon nanotubes modified screen printed electrodes for the acetaminophen detection, 12th Symposium and summer school on bioanalysis, organized in the frame of CEEPUS CIII-HU-0010-06-1112 network, Cluj-Napoca, Romania, 1- 31 July **2012**.
7. **M-C. Tertiş**, M. Jitaru, L. Silaghi-Dumitrescu, Transformation électrochimique des dérivés nitrophénoliques–facteurs d’influence, International conference Journées d’Electrochimie, Sinaia, Romania, 06-10. 07. **2009**.
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9. M. Jitaru, M. Toma, **M-C.Tertiş**, Combined adsorption and electrochemical oxidation of phenols on zeolites modified electrodes, International conference „59th Annual Meeting of the International Society of Electrochemistry”, Seville, Spain, 06-13. 09. **2008**.
10. **M-C. Tertiş**, M. Jitaru, M. Toma, Traitement électrochimique des eaux residuelles contenant nitrophenols, Conferința internațională „Le Quinqième Colloque Franco-Roumain de Chimie Appliquee, CoFrRoCA”, Bacău, Romania, 25-29. 06. **2008**.
11. **M-C. Tertiş**, M. Jitaru, Removal of nitrophenols from wastewater by electrochemical way, International conference „1stRegional Symposium on Electrochemistry for South-East Europe”, Rovinj, Croatia, 04-08. 05. **2008**.
12. **M. Tertiş**, M. Jitaru, R. Jitaru, Traitement electrochimique des eaux residuelles synthetiques contenant nitrophenols, Conference witn international participation „5 ème Rencontre Nationale d’Electrochimie RNE 05”, Agadir, Maroc, 28-29. 03. **2008**.
13. L. Szpyrkovicz, **M-C. Tertiş**, M. Jitaru, Contributions à la diminution de la concentration des nitrophénols des eaux résiduelles par le processus combiné électrochimique-adsorption, International conference International conference Journées d’Electrochimie, Lyon, France, 02-06.07.**2007.**
14. **M-C. Tertiş**, M. Jitaru, Contributions to electrochemical synthesis of aromatic amines, 4th Europeean Summer School on Electrochemical Engineering, Palić, Serbia and Montenegro, 17–22. 09. **2006**.

III. Research grants (nationals (8) and (1) one proiecte internaționale)):

National grants (8):

1. EurElDep, CEEX Module III, grant 47/2006-2007 - UBB Cluj-Napoca, **Conf. CSI Dr. M. Jitaru**.
2. NANELPOL, CEEX MATNANTECH, grant 68/2006-2008 - UBB Cluj-Napoca, **Conf. CSI Dr. M. Jitaru**.
3. CEEX BIOTECH, grant 141/2006-2008 - UBB Cluj-Napoca, **Conf. CSI Dr. M. Jitaru**.
4. Contract 43 GR/23.05.2006-2008, cod CNCSIS 1323 - UBB Cluj-Napoca, **Conf. CSI Dr. Maria Jitaru**.
5. ELCAT-CO_2, PN II, grant 32114/2008 - UBB Cluj-Napoca, **Conf. CSI Dr. M. Jitaru**.
6. PN II-71-067/2007 - UBB Cluj-Napoca, **Conf. CSI Dr. M. Jitaru**.
7. Imunosenzori amperometrici pentru biomarkeri în cancerul ovarian şi uterin Project PN-II-ID-PCE-2011-3-0355 - UMF Cluj-Napoca, **Prof. Dr. R. Săndulescu**
8. Școala de vară: Electrochemistry for Environmental and Biomedical Applications 2013 PN-II-ID-SSA-2012-2-027 - UMF Cluj-Napoca, **Prof. Dr. R. Săndulescu**.

International grants (1):

1. Pol de excelență regional: Metode electrochimice pentru controlul şi îndepărtarea poluanților organici şi anorganici; Grant 2700PL309 - UBB Cluj-Napoca, **Conf. CSI Dr. M. Jitaru**.