Scientific report

regarding the project implementation in the period January 2014-December 2014

During 2014, the third year from the project, were carried out the activities included in the additional contract signed in 2014 for the achievement of general objective of the project, an electronic system based on chemical sensors and biosensors for the analysis of biogenic amines. In the following pages will be described the activities performed, objectives achieved and dissemination activities carried out in the period January - December 2014.

The objective this year was **Testing of sensitive performance of sensors and biosensors;** for this purpose were performed the activities necessary for a better characterization of sensors and biosensors developed in previous years of the project.

Some of the most important characteristics for use in practice of sensors and biosensors are related to their life time (reproducibility, stability and durability), to the selectivity for the target compound and to the limit of detection. Thus, all types of sensors and biosensors developed in previous activities of the project were tested in order to select the best ones in the terms of sensitive performances for applications on real samples, that will be carried out in 2015.

1.1 Study of sensors and biosensors selectivities

In studies regarding selectivity were used sensors and biosensors fabricated and characterized in previous stages of the project. The results will be presented below.

Sensors based on polypyrrole doped with different doping agents and **the sensors based on phthalocyanine** detect biogenic amines in samples on the basis of their influence on the characteristic peaks of modifying materials or present their own peaks due redox reactions at the electrode surface. Thus, in the case of dopamine, epinephrine, histamine and trimethylamine characteristic peaks are obtained when cyclic voltammetry or square wave voltammetry were used as detection methods.

For determination of the selectivity of responses provided by **sensors based on carbon paste modified with phthalocyanines** voltammetric curves were recorded by square wave voltammetry in aqueous solutions of KCl, phosphate buffer solutions of pH 6, 7 and 8, $KClO_4$ and others. It was observed that when sensors are immersed in different electrolyte solutions, recorded voltammograms are stable, depending on the nature and concentration of the electrolyte. These peaks are influenced by the presence of interfering substances. Therefore, if the solutions of electrolytes are added electroactive substances can be observed changes of peak potentials and their currents (related to the redox processes of phthalocyanines, of polypyrrole, etc.) but also the appearance of peaks due electroactive substances present in solution. Voltammograms obtained become more complex with the increasing of number of active compounds present in the sample. Therefore, the sensors are useful in studies of monitoring the freshness of food; voltammetric signals are processed using multivariate data analysis methods in order to select useful information for this type of analysis.

In the case of solutions containing two or more electroactive amines, to increase the resolution of the peaks, square wave voltammetry was used. In these conditions are obtained voltammograms with well separated peaks that are useful in quantitative determinations based on regression curves. For example, the sensor based on carbon paste (CPE) modified with $LuPc_2$ can detect and quantify dopamine and histamine in a complex sample containing besides the two amines and other interfering compounds such as tyramine, tyrosine, cysteine and various ions (Na^+, Ca^{2+}, Cl^-).

It were studied the interferences from successive exposure of **sensors based on Langmuir-Blodgett films (LB) of phthalocyanine** at different electrolyte solutions. For this purpose were prepared ITO based electrodes coated with LB films of $LuPc_2$, $GdPc_2$ and $DyPc_2$. Each sensor was immersed in potassium chloride solution. After recording of five consecutive cyclic voltammograms, the electrodes were removed from the solution, washed with bidistilled water and let to dry. Subsequently, the sensors were immersed in phosphate buffer solution pH 7 or biogenic amines and cyclic voltammograms were recorded. Finally, the electrodes were immersed in a solution of KCl and cycled six times in order to eliminate potential interference species adsorbed on LB film. After this measurement protocol it was observed that oxidation and reduction peaks corresponding phthalocyanine are modified and new peaks were observed that do not exist in voltammograms of sensor recorded in 0.1 M KCl solution. Therefore, the original signal of the sensor is significantly altered and cannot be recovered the initial response. The results showed that some chemical species present in the solution are adsorbed on LB film during cycling of and remain in LB film causing a "memory" effect, in other words there is an irreversible contamination of the electrode.

Similar studies were performed for **sensors based on carbon paste modified with phthalocyanine**. In this case, the differences observed between voltammograms recorded in 0.1 M KCl solution with a new sensor and voltammograms same sensor in 0.1 M KCl solution used in the analysis of biogenic amines are much lower than in the case of LB sensors, the sensors can be recovered and reused by polishing or cycling in KCl solution.

In the case of **sensors based on polypyrrole** doped with different doping agents, modifying the sensor signal after measurement of solutions of biogenic amines is reduced, and the voltammetric signal is recovered through cycling (5-6 cycles) in a solution of KCl or phosphate buffer solution of pH 7.

Study of interference in the case of biosensors were performed according to the enzyme used and the potential interference that may be present in real samples analysis. Detection method used was amperommetry in the optimal operating conditions of biosensors (applied potential, pH, ionic strength, temperature, continuous stirring).

In the case of biosensor based screen printing electrodes (SPE) modified with carbon nanotubes functionalized with carboxyl groups and tyrosinase (**Ty/SWCNT-COOH/SPE**) was determined the interferences of different compounds from the sample on analytical signal corresponding to the analyte. The result was expressed as the limit of tolerance, which is the maximum concentration of foreign substances that cause a relative error of about ± 5% in the determination of tyramine. Concentrations of interfering substances were 0.01 M Ca^{2+} and Cl^-; 0.001 M putrescine, histamine, tyrosine and glutathione. Tolerance ratios to interfering substances in determination of 100 μM concentration of tyramine were 2500 for Ca^{2+} and Cl^-; 300 for putrescine, histamine and glutathione; 20 for tyrosine. Therefore, this biosensor is useful for the selective determination of tyramine in complex samples containing biogenic amines. The absence of peak currents modifications in the presence of interfering chemical species demonstrated that Ty/SWCNT-COOH/SPE biosensor is a very good biosensor for the detection and quantification of tyramine in complex samples. Use of this biosensor in detecting of tyramine in fish products has the advantage of high selectivity because other biogenic amines do not interfere. Detection of tyramine is related to presence of phenolic group in the chemical structure of tyramine.

In the case of biosensors based on electrodes of Pt modified with polypyrrole doped with phosphate ions and containing enzyme tyrosinase immobilized (Ty/PO_4-PPy/Pt), the studies on the selectivity were conducted in this way. It was studied the influence of different compounds that could interfere in the determination of 50 μM tyramine in optimal experimental conditions. The result was expressed as the limit of tolerance. The concentration of interfering substances were 0.1 M Mg^{2+}, Ca^{2+} and Cl^-; 0.01 M histamine, putrescine, tryptophan, spermine, phenylalanine and glutathione. The tolerance ratios to a interfering compounds in the detection of 50 μM tyramine were 2000 for Mg^{2+}, Ca^{2+} and Cl^-; 200 for histamine, putrescine, spermine and glutathione; 100 for tryptophan; 20 for phenylalanine. Selectivity in case of interference caused by some phenolic compounds was studied by cyclic voltammetry. Cyclic

voltammograms of catechol solution containing 50 μM or 50 μM phenol added to a solution of tyramine 50 μM present two cathodic peaks, one at -0.05 V (related with phenol or catechol) and one at -0.250 V (related with tyramine). In the case of amperometric detection of tyramine at -0.250 V was determined that phenol and catechol cause an additive error in determining tyramine, but less than 2%. However, this biosensor is useful for the selective determination of tyramine in complex samples containing biogenic amines. The absence of significant changes in peak currents recorded in the presence of interfering species demonstrates that the biosensor Ty/PO_4-PPy/Pt can be considered an optimal biosensor for the detection and quantification of tyramine in complex samples.

The use of this biosensor for the detection and quantification of tyramine in fermented foods has the advantage of a high selectivity for other amino compounds that do not interfere. This is due to the specific action of the tyrosinase with tyramine, which is a double functional compound which contains an amino group and a phenolic group.

Also, for the biosensor based on vitreous carbon (GCE) modified with single wall carbon nanotubes (SWCNT) and diamine oxidase (DAO) (**DAO/SWCNT-GCE**) was studied the influence of interfering substances on detection of 50 μM epinephrine for the biosensor based on glassy carbon modified with single wall carbon nanotubes and diamine oxidase. Concentrations of interfering substances were 0.01 M Na^+ and $S_2O_5^{2-}$; 0.001 M urea, uric acid, and citric acid. Tolerance ratios to interfering substances on detection of 50 μM epinephrine were 2400 for Na^+ and $S_2O_5^{2-}$; 800 for urea, 180 for uric acid and 180 for citric acid. Glucose and glycine in concentrations of 0.001 M have no influence on the biosensor response on the detection of 50 μM epinephrine. These results demonstrate that the biosensor DAO/SWCNT-GCE is a biosensor with optimal characteristics for the determination and quantification of epinephrine.

Similar values of tolerance ratios were obtained for **biosensors based on diamine oxidase** (DAO) immobilized on **different carbonaceous materials** (graphite, carbon nanotubes, graphene and carbon nanofibres - the support is carbon paste or vitreous carbon electrodes) **polypyrrole doped with phosphate ions** by the casting method followed by cross-linking with glutaraldehyde on determination of 50 μM putrescine. High selectivity is related to specific biocatalytic action of the enzyme.

Biosensors fabricated by immobilizing **monoamine oxidase (MAO) in mixed LB film of arachidic acid (AA) and dysprosium (III) bisphthalocyanine ($DyPc_2$)** present tolerance ratios of 240-280 to interfering substances (glucose, ascorbic acid, uric acid, cysteine) on detection of histamine50 μM. High selectivity is due to specific biocatalytic action of the enzyme and mediator effect of phthalocyanine on electrons transfer.

1.2 Study of the sensors and biosensors stability

To assess the stability of **sensors based on Langmuir-Blodgett thin films (LB) of phthalocyanine**, there were recorded several consecutive cycles in 0.1 M KCl solution. The first cyclic voltammograms is slightly different from the second one. Then successive voltammograms are highly reproducible. For example, an LB film of $GdPc_2$ immersed in 0.1 M KCl aqueous solution shows a very high reproducibility, so after 100 successive scans what is seen is only a slightly decreases the peak intensity below 3.5% for all sensors studied. When the measurements were stopped and the sensor based on the LB film is removed from the solution (even for a few minutes), significant changes in cyclic voltammograms recorded were observed. Irreproducibility caused by removing the electrode from solution and storage were assessed following the procedure described below: after the registration of three cycles in 0.1 M KCl solution the electrodes are removed from the solution and washed with ultrapure water. Then the electrodes are kept using three methods. One of the electrodes is kept in bidistilled water, the second in a solution of 0.1 M KCl, and the third is held in the air. Periodically (from 15 to 15 minutes), the sensors are re-immersed in KCl solution and three cyclic voltammograms were recorded. Cyclic voltammograms recorded after storage differ significantly from the first voltammograms recorded (when the electrode is new), highlighting the loss of signal repeatability regardless the condition of electrode storage. The changes observed differ from one

sensitive material to another and from an electrolyte to another, and are primarily related to lowering of peak intensity, peak shifting to higher potential values and the appearance of new peaks or "shoulders" of the initial peaks. This effect can be attributed to the changing of LB films structure (the degree of order) after immersion in solutions of electrolytes and performing of voltammetric measurements. Although, LB technique allows to obtain very high ratio electrode surface/volume, which facilitates the electrochemical processes at the sensors surfaces, and the lack of stability and reproducibility of the measurement are the main disadvantages of electrodes based on LB films However, the LB technique is very reproducible, allowing the fabrication of practically identical sensors. Differences between sensors prepared in the same conditions are less than 0.5% in terms of response obtained by cyclic voltammetry. This type of sensors were used as disposable sensors.

In order to test the viability of sensors based on **carbon paste (CPE) modified with phthalocyanines ($LnPc_2$)** were conducted a series of systematic experiments to investigate the electrochemical behavior in time and utilization capacity. It should be emphasized that in all cases, the sensors need a stabilization phase, which consists in recording of five cyclic voltammograms in order to establish a equilibrium between the sensor and the solution analyzed. To determine the stability of responses provided by sensors based on carbon paste modified with phthalocyanines, voltammetric curves were recorded by square wave voltammetry in aqueous solutions of KCl and biogenic amines (in phosphate buffer pH 7), removing the sensor from the analyzed solution after each measurement. It is noticed that the peak potentials are almost constant (% RSD <1%) and the variations of peaks intensities with increasing number of repetitions are very low (% RSD <3%) for 100 repetitions. Variation of entire curve measured as % RSD of the 10 kernel coefficients is <5% for all this type of sensors ($GdPc_2$, $LuPc_2$, $DyPc_2$). From these data it can be stated that the voltammetric sensors based CPE modified with phthalocyanines shows good stability and can be used in routine measurements. In addition, the sensor surface can be recovered by polishing the electrode surface with a filter paper and cycling in 0.1 M KCl solution.

Regarding the repeatability of sensors based on **polypyrrole doped with different doping agents** should be mentioned that the first five cycles recorded are slightly different from those recorded later, but after this this stabilization step the voltammetric cycles are highly reproducible. After 100 cycles was observed only a slight and progressive decrease in peak intensities (there is an overall decrease in signal intensity about 3%). On the other hand, when cycling is stopped and the sensors are removed from the solution and stored in air or in ultrapure water for several minutes, and then reintroduced into solution, no changes are observed in electrochemical signals obtained (variations are less than 1% for all studied sensors). These results show the good short-term stability and repeatability of the response of sensor based on polypyrrole.

In the case of biosensors, at this stage was studied the biosensor based on **polypyrrole and tyrosinase** in order to determine the stability of the biosensor response when it is used intensively. Thus, there was recorded a total of 50 cycles in 50 μM dopamine solution (electrolyte solution concentration is 0.01 M phosphate buffer, pH 7.0) finding that there is a decrease in cathodic current of 2%. Biosensors are stable and can be used to analyze a large number of samples without a significant loss of sensitivity. In all quantitative determinations, quantification must be carried out comparing with a reference sample.

Under optimal conditions of pH, ionic strength and temperature the biosensor based on screen-printed electrodes (SPE) modified with single wall carbon nanotubes (SWCNT) and tyrosinase **(Ty-SWCNT/SPE**) has a good stability, at least two hours of continuous use. Note that cyclic voltammograms recorded in 100 μM tyramine solution (electrolyte solution is phosphate buffer 0.01 M, pH 7.0) slightly decrease in intensity after two hours of continuous use. Cathodic peak current shows a decrease of 3.75% after two hours of continuous use. Therefore, the biosensor has a very good stability.

The stability of the Ty-SWCNT/SPE biosensor signal was confirmed by recording a large number of cyclic voltammograms (100 cycles) in 50 μM serotonin solution (electrolyte solution is phosphate buffer 0.01 M,

pH 7.0) . Cathodic peak current decreases with 3.5% confirming the stability of Ty-SWCNT/SPE biosensor to intensive use.

Biosensor **DAO/SWCNT-GCE** shows high stability for continuous use in 50 μM epinephrine solution. The recording of 50 cycles in the potential range 0.5 - 0.5V causes a decrease of voltammetric signal of 2.5%. Similar decreases of voltammetric signal were obtained and for biosensors based diamine oxidase (DAO) immobilized on different carbonaceous materials (graphite, carbon nanotubes, graphene and carbon nanofibers) to cycling in 50 μM putrescine, showing a good stability of biosensors.

Biosensors based on monoamine oxidase (MAO) immobilized on mixed LB film of AA and $DyPc_2$ shows a very goog stability during cycling, voltammetric signal decreasing 8.5% after 30 cycles. This type of biosensors will be used as disposable biosensors. LB method allows obtaining biosensors with a good reproducibility.

Sensors and biosensors based on SPE shows good stability when were used in the analysis of biogenic amines. Registering a large number of cycles do not lead to significant drops of voltammetric signal. After removing of biosensors and sensors based on SPE are retired from the sample solution and keep for a few hours and re-immersed in the solution to be analyzed, a modification of the signal is observed. It was found that this change is related to degradation of the reference electrode (Ag screen-printed). To ensure accurate measurements, sensors and biosensors based SPE should be used as disposable sensors and biosensors.

1.3 Study of sensors and biosensors reproducibility

Reproducibility of fabrication of **sensors and biosensors based on LB films** is very good, the differences between responses obtained by voltammetry is less than 1%. The differences between the responses obtained by amperometric are less than 0.8%. This is the main advantage of using the LB technique in the deposition of sensitive layers for applications in sensors and biosensors. This technique is optimal for the fabrication of disposable sensors and biosensors.

Reproducibility of fabrication of sensors and biosensors based of CPE modified with phthalocyanines and enzymes is good, the differences between the signals is less than 4% for sensors and biosensors prepared in the same conditions. Despite the fact that the preparation of these sensors and biosensors is handling, the strict compliance of protocol manufacturing provide sensors and biosensors of good quality.

Reproducibility of fabrication of **sensors and biosensors based on polypyrrole** depends on the reproducibility of electrosynthesis, the stage when the polypyrrole is deposited on the electrode by using chronoamperometry (CA). In order to test the reproducibility of sensors fabrication by chronamperometry was calculated the charges consumed on the deposition of polypyrrole in the presence of different doping agents. Five sensors were fabricated for each doping agent. Relative standard deviation (% RSD) is lower than 6% for all doping agents used in electrosynthesis (Table 1).

Table 1. Reproducibility data using CA techniques for preparation of PPy doped with different doping agents

Sensor	Doping agent	%RSD
PPy/SO_4	Sulfuric acid	1.83
PPy/DSA	Sodium Dodecilsulfonate	1.35
PPy/FCN	Potassium ferrocyanide	4.34
PPy/AQS	Disodium salt of antrachinonsulfonic acid	1.43
PPy/PWA	Fosfotungstic acid	5.70
PPy/TSA	Sodium toluenesulfonat	4.76
Ppy/NiPcTs	Tetrasulfonate nickel	5.9

	phthalocyanine	
Ppy/NP	Sodium nitroprusside	2.05
Ppy/Mo	Sodium molibdate	3.24

These data confirm the high reproducibility of the method used for the preparation of sensors and biosensors based on polypyrrole.

Also, it was studied the reproducibility of fabrication of **biosensors based on enzyme-modified SPE**. For example, the reproducibility of fabrication of Ty-SWCNT-COOH/SPE biosensors (SWCNT-COOH - carbon nanotubes functionalized with carboxyl groups) was studied by using cyclic voltammetry. It was fabricated three biosensors, it were recorded responses and the voltamperometric signals were compared. The results obtained(cathodic peak current) when Ty-SWCNT-COOH/SPE biosensor was immersed in the solution of tyramine 100 μM (in 0.01 M phosphate buffer, pH 7.0), expressed as % RSD were less than 2%, which demonstrates that the method of fabrication the biosensor has a good quality. This test confirmed the reproducibility of the procedure for immobilizing the enzyme on a solid matrix based on carbon nanotubes functionalized with carboxyl groups.

Similar results were obtained for other enzymes (MAO and DAO) immobilized on nanostructured carbon materials (graphene, SWCNT, SWCNT-COOH, SWCNT-NH_2). The rigorous control of the protocol of fabrication and storage conditions of sensitive compounds are essential for reproducible fabrication of sensors and biosensors.

1.4 Study of the lifetime of sensors and biosensors.

As shown in studies on the stability, **sensors based on LB films of phthalocyanine** are disposable sensors and the possibility of their use in practice is fully justified because the LB technique allows the preparation of sensors with identical characteristics. The same consideration is correct for all types of **biosensors manufactured by means of LB techniques**. These sensors and biosensors shows very good analytical properties, reversible behavior and very low detection limits. Therefore they will be used as disposable sensors and biosensors for the analysis of biological samples of interest in medicine.

Durability (long term stability) of sensors based on **carbon paste electrodes modified with phthalocyanines** was tested by recording voltammetric signals for a period of one month. The sensors were stored in tightly closed boxes in a refrigerator at 4°C. Voltammetric curves were recorded for CPE-based sensors and phthalocyanine in aqueous solutions of electrolytes (KCl solution, phosphate buffer solution pH 6, 7, 8, etc.) and biogenic amines. Variation of voltammetric curves were monitored over time.

By quantifying of changes observed in this time were analyzed regarding potentials peaks changes, peak current and whole variation curves (represented by 10 kernel coefficients). For example, phthalocyanines peak potentials after a month of use (five cyclic voltammograms recorded daily) shows a relative standard deviation less than 2.5% for all sensors and electrolytes analyzed. The same study carried out in the case of biogenic amines showed that peak potential of sensor shows a relative standard deviation less than 5% for all sensors and amines analyzed.

Another study were carried out to test the durability of these sensors in terms of peaks potential, peaks current and a voltammetric curves include the study of four solutions biogenic amines (dimethylamine, trimethylamine, histamine, putrescine) with the same electrode ($LuPc_2$-CPE) for one month. After immersion in each solution of amine several voltammetric curves were recorded (five cycles). Between measurements carried out for each amine, the sensors were washed with ultrapure water and were kept in a hermetically sealed box in the fridge.

In Table 2 are presented the values of relative standard deviation of peak potentials and peak currents of $GdPc_2$ in dimethylamine solution (electrolyte was 0.1M KCl).

Table 2. The values of relative standard deviation of peaks potential for Gd bisphthalocyanine in dimethylamine solution (electrolyte is 0.1 M KCl) after one month

$GdPc_2$ peaks	**Potential (% RSD)**	**Current (% RSD)**
Peak I	2.3	2.5
Peak II	2.4	2.9
Peak III	3.1	4.3
Peak IV	4.6	5.0

As can be observed in Table 2, the relative standard deviation values for peak potentials and peak currents of phthalocyanine are lower than 5%.

% RSD values for total voltammetric curve were calculated based on the 10 kernel coefficients. The values calculated were below 7% for all the solutions analyzed. This result demonstrates that the sensors based CPE modified with phthalocyanine shows a good long-term stability. This type of sensor can be used for long periods of time and, in addition, if the sensor response is modified this can be recovered.

One of the most important advantages of these sensors is that it is possible the recovering (cleaning) of active surface by removing the first layer by polishing with a filter paper. Another method is the regeneration of sensor signal using cyclic voltammetry by recording 10 cycles in 0.1 M KCl aqueous solution.

In conclusion, the stability of sensors based CPE depends on the number of measurements and the type of samples in which they are immersed. An electrode stored under appropriate conditions, can be used for at least one month after that the regeneration of the active area is required.

For testing the durability of **polypyrrole-based sensors** cyclic voltammograms were recorded in 0.1 M KCl solution in the successive days, for 5 days. The changes observed corresponding to voltammograms recorded were a slight decrease in peak currents. Values relative standard deviation (% RSD) were calculated from the average values of sensor responses (measured as peak intensities). In Table 3 were presented the values of % RSD calculated for cyclic voltammograms recorded in five consecutive days with a sensor based on Ppy/DSA immersed in 0.1 M KCl solution.

Table 3. Values of % RSD calculated for peak currents of cyclic voltammetry of sensor Ppy/DSA registered in 0.1 M KCl during five consecutive days

		%RSD **day 1**	%RSD **day 2**	%RSD **day 3**	%RSD **day 4**	%RSD **day 5**
Redox system I	I_{pa}	2%	8%	13%	15%	16%
	I_{pc}	4%	4%	7%	10%	11%
Redox system II	I_{pa}	8%	9%	11%	12%	14%
	I_{pc}	3%	10%	13%	18%	20%

These data indicate a good stability for a period of five days to all sensors, % RSD values are lower than 20%.

In case of using integral curve (10 kernel coefficients for a curve) % RSD values are with 2-3% higher than values calculated for peak current.

Instead,% RSD values are less than 7% when are take into account the peaks potentials.

It must be emphasized that sensors can be replaced easily because fabrication of sensors based on polypyrrole is very reproducible.

In another study were analyzed the biosensors based on polypyrrole and tyrosinase with the purpose of determining the sustainability and preservation of enzyme activity at room temperature and in the

refrigerator. The stability of the enzyme was monitored biosensors for measuring the signal in a solution of known concentration of dopamine (50 μM). At room temperature (20°C) enzymatic activity decreases rapidly, observing a decrease of cca. 70% of the initial enzyme activity in 10 days. A much smaller decrease of biosensor response was observed when the electrode is kept at 4°C in refrigerator. It was calculate that the biosensor preserve 85% from the initial activity of the enzyme after a month. After this period, a accelerated decrease of enzymatic activity was observed. The conclusion of this study was that in order to maintain the sensitivity of biosensors for a relatively long period of time, they must be kept in the refrigerator.

Similar results were also obtained when the enzyme immobilized in the carbon matrix was DAO, 77% from the enzyme activity is maintained after one month of storage in refrigerator.

The stability of the Ty-SWCNT/GCE biosensor during storage in a refrigerator (at 4°C) was confirmed using the biosensor every 24 hours for a month. Evolution of biosensor response during time is as follows. After 15 days, the biosensor response began to decrease. However, the biosensor was maintained 89% of the response (cathodic current) after one month of storage. The stability of Ty-SWCNT/GCE biosensor is good, the biosensor being able to use for at least a month with a proper drift correction.

Durability study of biosensors based on SPE and LB was not necessary because their stability is low (few hours).

1.5 Determination of detection limits of sensors and biosensors

For determination of the limit of detection (LOD) were constructed calibration curves of sensors and biosensors. Measurements were carried out in optimal conditions of pH, temperature, electrolyte etc. LOD was calculated using the relationship LOD = 3 × σ / m (σ-relative standard deviation of the biosensor signal corresponding to the lowest concentration from the calibration plot, m- slope of calibration curve). Values of detection limits are between 10^{-5}-10^{-6}M in the case of sensors and 10^{-6}-10^{-8}M for biosensors. In the following tables are presented some results obtained for a series of sensors and biosensors developed and characterized in this research project.

Table 4. LOD values for sensors based on CPE modified with different phthalocyanines for different amine compounds

Sensor	Limit of detection (M)	Limit of detection (M)
$LuPc_2$-CPE	5.0×10^{-5} (dimethylamine)	7.3×10^{-5} (ammonia)
$GdPc_2$-CPE	6.4×10^{-5} (dimethylamine)	8.6×10^{-5} (ammonia)
$DyPc_2$-CPE	7.8×10^{-5} (dimethylamine)	8.8×10^{-5} (ammonia)

Tabelul 5. LOD values for sensors based on polypyrrole doped with different doping agents on detection trimethylamine

Sensor	Limit of detection (M)
Ppy/FCN	2.32×10^{-5}
Ppy/NP	1.58×10^{-5}
Ppy/PWA	8.44×10^{-5}
Ppy/H_2SO_4	4.57×10^{-5}
Ppy/Mo	6.33×10^{-5}
Ppy/AQS	9.21×10^{-5}

Tabelul 6. LOD values for sensors based on polypyrrole doped with different doping agents on detection of ammonia and putrescine

Sensor	Compound	LOD/ μM

Ppy/FCN	Ammonia	0.74
	Putrescine	1.36
Ppy/NP	Ammonia	1.84
	Putrescine	2.44
Ppy/PWA	Ammonia	4.84
	Putrescine	5.32
Ppy/H_2SO_4	Ammonia	2.35
	Putrescine	2.62
Ppy/Mo	Ammonia	1.35
	Putrescine	2.34
Ppy/AQS	Ammonia	4.98
	Putrescine	5.05

Table 7. LOD values of SPE sensors based on carbon nanofibers, carbon nanotubes, and graphene

Sensor	Compound	Limit of detection / μM
CNF-SPE	Dopamine	0.84
	Epinephrine	1.06
CNT-SPE	Dopamine	1.42
	Epinephrine	1.98
GPH-SPE	Dopamine	2.31
	Epinephrine	3.52

Table 8. LOD values for different biosensors developed in this project

Biosensor	Limit of detection
Ty/SWCNT-GCE	2.54 μM (epinephrine)
Ty/CoPc-CPE	0.84 μM (serotonine)
Ty/PO4-Ppy/Pt	5.7×10^{-7} M (tyramine)
Ty/GPH-C/SPE	2.42×10^{-7} (dopamine)
Ty/GPH-C/SPE	6.56×10^{-7} (epinephrine)
Ty/SWCNT–COOH/SPE	0.62 μM (tyramine)
DAO/SWCNT-GCE	2.42×10^{-7} (dopamine)
DAO/SWCNT-GCE	6.56×10^{-7} (epinephrine)
MAO/AA-$DyPc_2$	1.32×10^{-7} (histamine)
MAO/AA-$DyPc_2$	5.46×10^{-7}(histamine)
DAO/SWCNT-SPE	8.72×10^{-8} (histamine)
DAO/SWCNT-SPE	1.56×10^{-7} (putrescine)

These good results are related to the nature and structure of the sensing element containing nanostructured materials, compatible with biogenic amines and using of electron mediators and enzymes that facilitate the transfer of electrons, increasing sensitivity and selectivity.

1.6 Determination of the response time of the sensors and biosensors

The response time of the sensors and biosensors depends on the technique used for measurement. Thus, if the technique used is cyclic voltammetry, the time for recording of the response can vary among 30 seconds and 5 minutes, depending on the scanning speed and potential range used. For the sensors based on carbon modified with phthalocyanines, cyclic voltammograms were recorded with a scan rate of 100 $mV \times s^{-1}$ and a range of potential from -1V to 1.3V. The same conditions were used in the case of LB sensors-based on phthalocyanines. For sensors based on polypyrrole, cyclic voltammograms were recorded with a scan rate of 50 $mV \times s^{-1}$ and a potential range from -1V to 0.5V.
In the case of biosensors, cyclic voltammograms were recorded with a scanning rate of 50 $mV \times s^{-1}$ and a potential range from -0.5V to 0.5V. For amperometric measurements, biosensors response was quantified as the time to the equilibrium current from the initial state to the new steady state equilibrium which is reached by adding a known amount of analyte. Response time values ranging among 3 and 10s in the optimal operating conditions such as applied potential, pH, ionic strength and temperature.
In conclusion, the time required for analysis is very reduced. Therefore, sensors and biosensors developed in this project are able for on-line in-line and real-time measurements of food and biological samples.

1.7 Determination of reversibility and recovery of sensors and biosensors

Recovery of sensors and biosensors after the use in the analysis of samples depended on the type of sample and number of measurements. The sensors based on carbon paste were recovered after using in electrochemical measurements, the modifications are reversible, by using two methods: polishing of surface with a paper filter and by cycling in 0.1M KCl solution. Both methods provide a good recovery of sensors.
Method of cycling in 0.1M KCl solution is the best method for recovery of sensors based on polypyrrole. By this method the contaminants were eliminates from the surface of the sensors, the modification of polypyrrole is one reversible.
In the case of biosensors, the recovery of voltammetric and amperometric signal was not possible after an intensive use. Contamination of biosensor are related to irreversible modification of enzyme conformation and was not possible to recover the initial state. Storage in inappropriate conditions led to the protein denaturation and losing of biocatalytic activity of enzyme.
In conclusion, the research carried during 2014 were dedicated to the development of sensors and biosensors completely characterized in the terms of analytical performances, life time and condition of use. The knowledge of sensors and biosensors behavior will be used for selection of sensors and biosensors for applications on real samples.

Dissemination of results in 2014

By additional contract signed in 2014 it was decided that in this stage will be published minimum 2 ISI papers and participation in at least 2 scientific meetings. Dissemination of research results was carried out through publication of 4 ISI papers, publishing of a chapter in an international monograph, publishing of a chapter in a national monograph (e-Book) and participation in international conferences with 5 papers. Additionally, it was also sent a summary to participate in an international conference and were sent two book chapters for publication in international monographs (chapters under review). Therefore, the objectives of dissemination activity were 100% accomplished.
Dissemination of research results was carried out by publishing ISI papers, publication of chapters in monographs and participation in international conferences.

Publishing of ISI papers

1. I. M. Apetrei, C. V. Popa (Ungureanu), C. Apetrei, D. Tutunaru, Biosensors based on graphene modified screen-printed electrodes for the detection of catecholamines, Romanian Biotechnological Letters 19(5)

(2014) 9801-9809, http://www.rombio.eu/vol19nr5/19.pdf, impact factor (IF) 0.363; relative influence score (RIS) 0.127.
2. I. M. Apetrei, C. Apetrei, Study of Different Carbonaceous Materials as Modifiers of Screen-Printed Electrodes for Detection of Catecholamines, IEEE Sensors Journal, IEEE Sensors Journal, Published online 11 July 2014, http://dx.doi.org/10.1109/JSEN.2014.2335534, IF 1.852; RIS 1.368.
3. I.M. Apetrei, C. Apetrei, Detection of virgin olive oil adulteration using a voltammetric e-tongue, Computers and Electronics in Agriculture 108 (2014) 148-154, http://dx.doi.org/10.1016/j.compag.2014.08.002, IF 1.486; RIS 2.889.
4. I.M. Apetrei, C. Apetrei, The biocomposite screen-printed biosensor based on immobilization of tyrosinase onto the carboxyl functionalised carbon nanotube for assaying tyramine in fish products, Journal of Food Engineering, 149 (2015) 1-8, http://dx.doi.org/10.1016/j.jfoodeng.2014.09.036, IF 2.576; RIS 1.934.

Chapters in monographs
1. I. M. Apetrei, C. Apetrei, Y. El Rayess, *Characterization of Red Wines Polyphenolics Employing Sensors and Biosensors* (Chapter 2), pp. 41-70. in Wine: Phenolic Composition, Classification and Health Benefits, Editor Youssef El Rayess, 2014, ISBN: 978-1-63321-059-2, Nova Publishers, https://www.novapublishers.com/catalog/product_info.php?products_id=50003&osCsid=647a25d9d412d07c8690696cea0ed681
2. I. M. Apetrei, C. Apetrei, *Biosensor Based on Nanostructured Sensitive Material for the Detection of Epinephrine* (Chapter 5), pp. 55-74. in SENSING - MONITORING - TELEDIAGNOSIS FOR LIFE SCIENCES, Vol. II, FOOD AND ENVIRONMENT (e-book), Editors: L. Floroian, M. Badea, M. Moga, 2014, Editura Universitatii Transilvania din Brasov, ISBN: 978-606-19-0388-7 gen, ISBN: 978-606-19-0390-0 Vol. II
3. C. Apetrei, M. Ghasemi-Varnamkhasti, I. M. Apetrei, *Olive oil and combined electronic nose and tongue*, Elsevier, 2015 (*under review*)
4. C. Apetrei, I. M. Apetrei, *Chemical composition of corn oil*, Nova Publishers, 2015 (*under review*)

Participation in international conferences and papers published in the proceedings of conferences
1. I.M. Apetrei, C. Apetrei, Disposable biosensor for the detection of catecholamines in biological samples, International Conference of Applied Sciences, Chemistry and Chemical Engineering (CISA 2014), Bacau, May 7-9, 2014, Oral presentation, http://www.ub.ro/33-romanian/universitate, Abstract published in: Book of Abstracts, page 10, Alma Mater - Bacau, 2014, ISSN 2066-7817
2. C. Apetrei, I. M. Apetrei, Sensors based on carbonaceous materials for detection of biogenic amines, International Conference Chimia 2014 “New Trends In Applied Chemistry”, Constanta, May 23-24, 2014, Oral presentation, Abstract published in: Book of Abstracts, page 40, http://chimia2014.univ-ovidius.ro/images/Book_of_Abstracts_2014.pdf
3. I.M. Apetrei, C. Apetrei, Expert sensory system for the determination of catecholamines in biological samples, Industrial Technologies 2014, Athens, April 9-11, 2014, Poster, Abstract published online: http://www.b2match.eu/industrialtechnologies2014/participants/210
4. I.M. Apetrei, C.V. Popa (Ungureanu), C. Apetrei, Determination of ammonia and putrescine in beef extract powder using voltammetric sensors, New Trends on Sensing- Monitoring - Telediagnosis for Life Sciences, Brasov, Romania - July 24-26, 2014, Oral presentation, http://maternologie.ro/envirpubhealth/index.php?option=com_content&view=article&id=13&Itemid=8, Abstract published in: Book of Abstracts, page 26, Lux Libris Publishing House, 2014, ISBN 978-973-131-280-4.
5. I.M. Apetrei, C. Apetrei, D. Tutunaru, Biosensor based on nanostructured sensitive material for the detection of epinephrine and norepinephrine, New Trends on Sensing- Monitoring - Telediagnosis for Life Sciences, Brasov, Romania - July 24-26, 2014, Poster, http://maternologie.ro/envirpubhealth/index.php?option=com_content&view=article&id=13&Itemid=8,

Award: C. Apetrei - Young Scientist Paper Award, Abstract published in: Book of Abstracts, page 101, Lux Libris Publishing House, 2014, ISBN 978-973-131-280-4.
6. C. Apetrei, I. M. Apetrei, Nanostructured biocomposite materials for biosensing applications, MESIC 2015, Barcelona, 22nd to 24th July 2015, *under review*

Conclusions

The practical results obtained in this research project are appropriate for the objectives proposed, the activities included the work plan were 100% achieved and the dissemination results is superior to those proposed for 2014. In this year were published 4 ISI papers, the sum of relative influence scores being 6.318, one of the paper being published in the journal *Computers and Electronics in Agriculture*, which has relative influence score of 2.889 showing the relevance of the results obtained in this project. The research carried out is interdisciplinary, multidisciplinary and transdisciplinary, which allowed that the work to be published in the first third of the journal ranking in the fields Agriculture - Multidisciplinary, Food Science & Technology and Instruments & Instrumentation. Also, it were published or are under evaluation chapters in prestigious international publishers, which demonstrates competence and international recognition of the research team members.

The research carried out in the project during 2012-2014 are very good, as evidenced by the quality and quantity of results published or presented at international scientific conferences. There are clear premises that application of sensors and biosensors on real samples (foods, pharmaceutical and biological samples) will lead to important results in terms of scientific and practical application taking into account the technical equipment and the experience of project team members in the field of sensors and biosensors.

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