Anexa nr. 6

ACADEMIA ROMÂNĂ

SCOSAAR

HABILITATION THESIS SUMMARY

Title: *"New horizons in the development of electrochemical sensors and biosensors with applications in clinical and environmental analysis"*

Field of Habilitation: Chemistry

Autor: Lete Cecilia

The habilitation thesis entitled ***"New horizons in the development of electrochemical sensors and biosensors with applications in clinical and environmental analysis"*** presents the main scientific results obtained after being awarded the title of Doctor of Chemistry in 2001 at the University of Bucharest, Faculty of Chemistry. Ten representative scientific papers published in international journals in quartile Q1 (nine articles) and quartile Q2 (one article) were selected. The scientific research presented in the habilitation thesis was carried out through national and international collaborations based on research projects such as 2CEEX-CERES-11-43/2006, M-ERA.net 39/04.01.2016, PN-II-ID-PCE-2011-3-0271 and PN-III-P2-2.1-PED-2021-3693, projects funded by the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI).

The habilitation thesis is structured in three parts: the first presents academic, professional, and scientific achievements; the second outlines the directions for the development of research activity; and the third contains bibliographic references.

The first part of the thesis is organized into two distinct chapters. The first chapter is dedicated to academic and professional achievements, presenting the university career and titles obtained, research activity carried out, collaborations with the academic environment, teaching experience as an associate professor, as well as involvement in national and international technical and scientific projects.

Experience in the field of synthesis and spectroelectrochemical characterization of polythiophene and polyazulene polymer layers, as well as the professional skills acquired in this context, were consolidated during research internships carried out between 2003 and 2012 at Åbo Akademi University in Turku (Finland), in the Department of Analytical Chemistry, under the coordination of Professor Dr. Ari Ivaska. In 2012, the research activity continued at the University of Turku, in collaboration with Professor Karita Kvarnström.

Between 2004 and 2005, under the scientific guidance of Professor Giuseppe Palleschi, I participated for 12 months in a European research project on "Advanced technology for monitoring and controlling winemaking processes and quality." During this postdoctoral fellowship, I developed electrochemical biosensors for determining glucose in wines from various regions of Italy and designed an amperometric system for determining ammonia in drinking water at low concentrations.

Between 2017 and 2019, I was the scientific coordinator for a bilateral Romania-Bulgaria project funded by the Romanian Academy. In collaboration with Prof. Vessela Tsakova, from the Rostislav Kaischew Institute of Physical Chemistry in Sofia, electrochemical sensors based on nanocomposite materials were developed for the determination of tryptophan.

I also completed several internships at the University of Cádiz, where, together with Prof. José María Palacios Santander and Prof. Laura Cubillana, we developed electrochemical sensors and biosensors based on sonogel-carbon electrode material for the determination of dopamine, hydroquinone, catechol, and melatonin. Another internship was carried out at the Université de Franche-Comté in Besançon, where, in collaboration with Prof. Jean-Yves Hihn, electrochemical biosensors were developed for the simultaneous multi-analyte determination of neurotransmitters and organic pollutants, dopamine, and catechol.

I participated as a scientific reviewer in the evaluation committees for the following doctoral theses: i) "Development and characterization of amperometric biosensors based on conducting polymers and nanomaterials for their application to the analysis of clinical and agrifood real samples," written by Juan José García Guzmán, University of Cádiz (2018); ii) "Techno-economic optimization of systems consisting of wind farms connected to pumped hydro storage facilities with hydroelectric recovery. Application to the islands of Gran Canaria and El Hierro," written by Celia Y. Bueno, University of Cádiz (2018); ii) "Techno-economic optimization of systems consisting of wind farms connected to pumped hydro storage facilities with hydroelectric recovery. Application to the islands of Gran Canaria and El Hierro," written by Dr. Celia Y. Bueno Vega, University of Las Palmas de Gran Canaria (2003); iii) "Sonogel-Carbon–Conducting Polymers based Composite Materials and their variants: Obtaining Process and Application in the Preparation of Electrochemical (Bio)sensors," by Dr. David López-Iglesias, University of Cádiz (2021).

The second chapter of the first part of the habilitation thesis covers scientific and professional achievements after 2009, based on 10 representative scientific papers. This chapter presents electrochemical sensors and biosensors for the determination of inorganic and organic pollutants, as well as electrochemical sensors for the determination of biologically active compounds. This chapter presents the methods of synthesis of nanocomposite materials used in the development of electrochemical sensors and biosensors. The synthesis methods used are original methods that use alternating currents or voltages (sinusoidal currents – SC, sinusoidal voltages – SV) with defined frequency and amplitude, which are applied over a constant current/potential. The synthesized nanocomposite materials contain an organic constituent represented by poly(3,4-ethylenedioxythiophene), PEDOT, and an inorganic constituent represented by metal nanoparticles. The metal nanoparticles of Au, Pt, and Ag were synthesized in situ using original methods from the corresponding precursors.

The first part of the section, Electrochemical sensors and biosensors for the determination of inorganic and organic pollutants (I.2.1), presents the SnO_2-based electrochemical platform (SnO_2/Pt/Ti/SiO_2/Si) for the determination of nitrite ions in milk, mineral water, tap water, and beer. Studies on the degree of analytical recovery in real samples have shown that the SnO_2/Pt/Ti/SiO_2/Si electrochemical platform has high accuracy in the determination of nitrite ions, opening up new possibilities for its application in the food industry and environmental analysis.

The second part of the section, Electrochemical sensors and biosensors for the determination of inorganic and organic pollutants (I.2.1) describes the preparation of new electrochemical microsensors based on bio composite materials, PEDOT-Tyr, prepared using both SV and SC methods, which were used in the electroanalysis of dopamine, hydroquinone, and catechol. Following the in situ electrodeposition of PEDOT and tyrosinase on the surface of Sonogel-Carbon (SNGC) electrodes, layers with higher porosity were obtained. Sonogel-Carbon electrodes have important advantages over metal electrodes (Au or Pt), such as low cost, ease of modification of chemical composition and/or surface, renewable surface (mechanically or electrochemically), good reproducibility, sensitivity, and very high biocompatibility. The use of alternating currents can influence the porosity and morphology of the deposited film, while improving the stability of the resulting bio composite material, preventing enzyme loss, and maintaining the biocatalytic performance of the enzyme. Careful selection of the frequency and amplitude of the applied sinusoidal current allows for more rigorous control of the porosity, morphology, and even the electrochemical polymerization process itself.

The third part of the section, Electrochemical sensors and biosensors for the determination of inorganic and organic pollutants (I.2.1), describes electrochemical platforms based on Au microelectrode arrays modified with PEDOT-Ty-based bio composite materials used for the determination of dopamine, catechol, and hydroquinone. The following parameters were determined: optimal enzyme concentration, amplitude and frequency of alternating voltage, pH; detection limit, quantification limit, thickness and roughness of PEDOT and PEDOT-Ty material layers, detection potentials, Michaelis-Menten constant, maximum reaction rate, linear response range, sensitivity. Analytical procedures for the determination of dopamine, catechol, and hydroquinone were developed.

The last part of the section, Electrochemical sensors and biosensors for the determination of inorganic and organic pollutants (I.2.1) refers to the synthesis and electrochemical characterization of inorganic-organic hybrid materials, obtained in different architectural configurations that showed superior electrocatalytic activity in the electrochemical reduction of 4-nitrophenol. Both organic materials (polyazulene or polyazulen-vinyl thiophene) and inorganic-organic hybrid materials (Prussian blue - organic compound) showed excellent electrochemical stability and good reproducibility in the analytical determination of 4-nitrophenol. Prussian blue-based materials ($Fe_4III[FeII(CN)_6]_3$) enabled the development of electrochemical platforms that are efficient in the determination of 4-nitrophenol.

The first part of the section, Electrochemical sensors for the determination of biologically active compounds (I.2.2), presents the quantitative determination of caffeic acid using a PEDOT-AgNPs-based electrochemical sensor in pear and orange juices. The total polyphenol content, expressed in caffeic acid (CA) equivalents, was estimated at 71.5 µM (12.9 mg L^{-1}) in pear juice, a value close to those reported in the literature; in orange juice, a total polyphenol content of 320.7 mg L^{-1} was determined. The composite materials were prepared using the SV method. The role of dopant anions, such as nitrate ions (NO_3^-), during the electropolymerization process of the EDOT monomer was also investigated in order to improve the analytical performance of the obtained electrochemical sensor. The Ag-PEDOT-based nanocomposite material, doped with nitrate ions, showed an improved analytical response in the oxidation of caffeic acid. The SV preparation method, together with the use of an electrolyte containing nitrate ions, allowed the improvement of the analytical response of the proposed sensor for the determination of caffeic acid.

The second part of the section, Electrochemical sensors for the determination of biologically active compounds (I.2.2), describes the determination of tryptophan using electrochemical sensors based on PEDOT doped with PSS (sodium polystyrene sulfonate) or

SDS (sodium dodecyl sulfate). It is well known that the properties of PEDOT depend significantly on the synthesis conditions: the electrochemical method (potentiostatic, based on alternating voltages, etc.), the type of solvent, and the nature of the doping ions. Thus, in this study, thin PEDOT layers were obtained using the potentiostatic (PS) method or the SV method. The influence of the nature of the dopant ions (PSS-polystyrene sulfonate vs. SDS-dodecyl sulfate) on the morphology, thickness, and other characteristics of the polymer layer, including the analytical response, was investigated. PSS is a hydrophilic dopant that promotes the formation of a porous PEDOT layer, while SDS is a hydrophobic dopant that causes the formation of compact and rigid layers. PSS is the most commonly used dopant in PEDOT layers. The aim of this research was to evaluate the electroanalytical performance of electrodes modified with PEDOT without any other additional components in the sensitive layer. Both the influence of the nature of the dopant, PSS or SDS, chosen as counterions with effects on structural properties, and the influence of the synthesis method (also chosen as a way to significantly modify the structural properties) were investigated.

The third part of the section, electrochemical sensors for determining biologically active compounds (I.2.2), includes the determination of epinephrine and serotonin using an electrochemical platform based on composite materials (PEDOT–AuNPs) prepared by an innovative procedure (SC method). The material obtained showed high roughness, uniform distribution of metal nanoparticles, and improved charge transfer. The developed electrochemical platform showed high selectivity even in the presence of a high concentration of ascorbic acid as an interfering chemical species. Tests on real samples (urine) confirmed the accuracy, selectivity, and reproducibility of the electrochemical detection platform.

The fourth part of the section, Electrochemical sensors for the determination of biologically active compounds (I.2.2), presents the determination of quercetin with PEDOT-AgNP-modified electrodes. The electrochemical detection platform based on PEDOT-AgNPs composite material showed good analytical performance in the determination of quercetin, as evidenced by low detection and quantification limits (LOD = 2.8 μM, LOQ = 9.5 μM). The use of the AgNP-based electrochemical platform in the analysis of quercetin in real samples confirmed its efficiency, with analytical recovery values obtained within appropriate limits (from 96.46 to 107.27%), thus highlighting the reliability of the composite material synthesis method for applications.

The fifth part of the section, Electrochemical sensors for the determination of biologically active compounds (I.2.2) presents the determination of hydrogen peroxide using electrochemical sensors based on a composite material consisting of PEDOT and Prussian blue (PBNPs), synthesized using the alternating voltage (sinusoidal, SV) method. The amperometric response of the PEDOT-PBNPs composite material-based sensor was linear in the concentration range between 5 µM and 1 mM H_2O_2, with a detection limit of 1.4 µM H_2O_2. The proposed electrochemical sensor, Pt/PEDOT-PBNPs, showed good repeatability, reproducibility, and operational stability, as well as good selectivity for the determination of H_2O_2 in the presence of interfering species such as dopamine (DA), uric acid (UA), KNO_3, glucose (Glu), KNO_3, and ascorbic acid (AA). The electrochemical sensor was also successfully applied in the determination of H_2O_2 in human blood samples, without the phenomenon of electrode surface passivation occurring.

The last part of the section, Electrochemical sensors for the determination of biologically active compounds (I.2.2), describes the materials used in the development of the electrochemical sensor for the determination of melatonin (MEL), materials consisting of sonogel-carbon (SNGC) and SNGC modified with gold nanoparticles (AuNPs). Their electrochemical and electrocatalytic properties were tested in both synthetic and real samples (serum). Gold nanoparticles improved the electric charge transfer capacity by approximately 25% compared to SNGCE. The best analytical performance in melatonin determination was obtained for the SNGCE/AuNP sensor, which showed an extended linear response range, a low detection limit, and high accuracy in the analysis of real samples. The selective determination of melatonin in the presence of interfering species such as uric acid, ascorbic acid, and dopamine was successfully achieved. The SNGCE/AuNP electrochemical sensor showed good operational stability in the presence of interfering species in the analysis of peripheral human serum samples, maintaining an overall analytical performance comparable to that of other sensors for melatonin determination described in the literature. The study of the influence of the matrix in real samples showed an analytical recovery value of 99.1%, demonstrating the high accuracy of the proposed sensor.

The second part of the habilitation thesis outlines the directions for the development of scientific research. One of the main directions will be the development of electrochemical sensors for determining cholesterol on platinum electrodes modified with copper oxide nanoparticles obtained by hydrothermal synthesis under supercritical conditions. The second direction will be the electrochemical determination of neurotransmitters using PEDOT-based nanocomposite materials.

The last part of the thesis contains the bibliographical references that formed the basis of the research.