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Obiectiv specific 6.13 - Creșterea numărului absolvenților de învățământ terțiar universitar și nonuniversitar care își găsesc un loc de muncă urmare a accesului la activități de învățare la un potențial loc de muncă/cercetare/inovare, cu accent pe sectoarele economice cu potențial competitiv, identificate conform SNC, și domeniile de specializare inteligentă, conform SNCDI
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IOSUD – "DUNĂREA DE JOS" UNIVERSITY OF GALAȚI

Doctoral School of Fundamental Sciences and Engineerin

DOCTORAL THESIS

SUMMARY

APPLICATIONS OF CHEMICALLY AND BIOCHEMICALLY MODIFIED ELECTRODES IN THE ANALYSIS OF AMINO ACIDS IN PHARMACEUTICALS

PhD student,

Ancuța DINU (IACOB)

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Series C: Chemistry No. 4

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Asist. Drd. Ancuța Dinu (Iacob)
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CONTENT:

INTRODUCTION

Indispensable for life, proteins are macromolecular organic compounds that participate in all the functions of the living cell. The structure of proteins includes amino acids, micromolecules with multiple functions, which have in their structure one or more carboxyl functional groups and one or more amino functional groups related to carbon atoms, but also a radical attached to the basic structure.

There have been numerous reports in the scientific literature on amino acid investigations in recent years, due to the interest given by the pharmaceutical industry to these compounds, but especially to the growing medical recommendations. Thus, on the pharmaceutical market, there are various drugs and supplements, in different pharmaceutical forms, with different concentrations, coming from different manufacturers. For these reasons, it is estimated that a series of devices are needed to monitor the quality of these products, presenting in this paper studies related to three amino acids, respectively: phenylalanine, tyrosine and tryptophan; select according to their action on the central nervous system.

Phe, or (S)-2-Amino-3-phenylpropanoic acid, an essential AA and precursor of Tyr, is assimilated by the human body through consuming foods like eggs, meat, fish, and milk, or through the administration of medicinal supplements in view of preventing Parkinson's disease, depression, vitiligo, and attention deficit hyperactivity (ADHD) disorder. Special attention should be paid to people who suffer from PKU, which is an inherited disorder caused by excessive accumulation of Phe in the human body. Consequently, these people should avoid consumption of foods or supplements that contain the Phe AA, or they risk developing other disorders or diseases such as mental retardation, high blood pressure, or cerebrovascular accidents. Today, there is a test for the detection of Phe, starting from birth, with sanguine serum: the Guthrie Test for the neonatal detection of PKU. It was created in 1963 by Robert Guthrie. L-Phe, D-Phe, and DL-Phe are the three forms of this AA, namely the natural form, the synthetic form, and the form found in pharmaceutical products, respectively.

Tyr, or L-2-Amino-3-(4-hydroxyphenyl) propanoic acid, a non-essential AA by comparison with Phe and Trypt, is produced naturally in the human body, even from Phe, and through hydroxylation becomes the precursor of two important neurotransmitters of the central nervous system (SNC): adrenaline and noradrenaline. As in the case of the other AAs, the absence of Tyr in the human body can be compensated for by consuming various foods (nuts, oat, beans, meat, fish, and wheat) or pharmaceutical products—supplements that have the role of treating PKU and neurological disorders like depression, ADHD, Alzheimer's disease, and mental retardation. Tyrosinemia and phenylketonuria are diseases that can occur as a result of excess accumulation or an insufficient amount of Tyr in the body. Thus, tyrosinemia is characterized by an abnormally high level in the blood or urine of Tyr. Phenylketonuria is a condition that prevents tyrosine biosynthesis, in the sense that individuals who suffer from this condition cannot properly process Phe AA, as a result of which they cannot obtain the proper amount of Tyr.

Trypt, or 2-amino-3-(1H-indol-3-yl) propionic acid, is also an essential AA that the human body uses to synthesize proteins; its intake is from external sources such as foods and pharmaceutical products. It has two important functions in the human body: on the one hand, it contributes to the biosynthesis of serotonin, and on the other hand, it is involved in the biosynthesis

of melatonin . The values of Trypt sanguine concentration in the human body are situated within the following normal limits: between 10 and 40 millimoles/L—that is, between 2.05 and 5.15 mg/L. In the case of values under the normal limit of Trypt, various forms of depression and insomnia are triggered, and in the case of values above the normal limit of Trypt, SNC disorders appear: manicdepressive psychosis with delirium, and schizophrenia

Studies on the three amino acids bring originality to this thesis, being included both in the theoretical part where extensive documentation has been developed in the literature, and especially in the part of personal contributions. Thus, a series of versatile devices have been developed with the role of qualitative and quantitative analysis of amino acids in various pharmaceuticals, using new generation materials, with excellent properties, as they proved to be: polymers, molecularly imprinted polymers, mediators electroactives, enzymes, screen-printed carbon electrodes. These versatile devices are found in the work as sensors and biosensors, specifying all stages of their preparation, modification, analysis and validation.

The diversity of methods applied for the study of developed devices has made a significant contribution to this research, as the results obtained have exceeded the performance of other sensors and biosensors mentioned in the articles published so far. These methods were chronoamperometry, cyclic voltammetry, electron scanning microscopy, infrared transform spectroscopy, chromatography and the standard addition method.

The real tests performed on the sensors and biosensors were made up of pharmaceuticals which had the mentioned amino acids as active substances. Future research perspectives provide for further studies with these tools on other samples, such as: biological fluids, food samples; thus managing to contribute to the health of mankind.

Therefore, this research thesis entitled APPLICATIONS OF CHEMICALLY AND BIOCHEMICALLY MODIFIED ELECTRODES IN THE ANALYSIS OF AMINO ACIDS IN PHARMACEUTICALS achieves its objectives, but especially the aim to achieve precise, sensitive, selective, low cost devices for the detection and prevention of measuring and checking the concentration of amino acids in medicines and food supplements.

Keywords: phenylalanine, tyrosine, tryptophan, amino acid, sensor, biosensor, cyclic voltammetry, laccase, polypyrrole, polymer conductor, molecularly imprinted polymer.

LIST OF ABBREVIATIONS

α - alpha
β - beta
***β*-CD** - beta-cyclodextrin
γ - gama
δ - delta
5-HTP - 5 - hydroxytryptophan
AA - amino acid
AAs - amino acids
ADHD - attention deficit hyperactivity disorder
Ala - alanine
Arg - arginine
Asn - asparagine
Asp - aspartic acid
AuNPs - gold nanoparticles
CA - chronoamperometry
CD - circular dichroism
CME - chemically modified electrode
CoPc - Cobalt Phthalocyanine
CV - cyclic voltammetry
Cys - cysteine
Cys-Cys - cystine
CPs - conductor polymers
D - dextro
DAAO - D-amino acid oxidase
DPV - differential pulse voltammetry
DTA - differential thermal analysis
$\mathbf{E_{pa}}$ - the potential of the anodic peak
$\mathbf{E_{pc}}$ - cathodic peak potential
EIS - electrochemical impedance spectroscopy
ETM - electron transfer mediators
FeCN sau $\mathbf{K_4[Fe(CN)_6]}$ - potassium hexacyanoferrate
GA - glutaraldehyde
GC-MS - gas chromatography-mass spectrometry
Gln - glutamine
Glu - glutamic acid
Gly - glycine
His - histidine
HPLC - high performance liquid chromatography
$\mathbf{I_{pa}}$ - anodic peak intensity
$\mathbf{I_{pc}}$ - cathodic peak intensity
Ile - isoleucine
IR - infrared
KBr - potassium bromide
KCl - potassium chloride
L- levo
LAAO - L - amino acid oxidase
Lacc - laccase
LC-MS - liquid chromatography - mass spectrometry
Leu - leucine
LOD - limit of detection
L-Phe - levo - phenylalanine
LOQ - limit of quantification
LSV - linear scanning voltammetry
M - molecular weight
MB - Meldola's Blue
Met - methionine
MIPs - molecularly imprinted polymers
MWCNTs - carbon multilayer nanotubes
NADH - nicotinamide - adenine dinucleotide
NP - sodium nitropusate dihydrate
OMS - World Health Organization
OTC - over the counter medication
PAH - phenylalanine hydroxylase
PANi - polyaniline
PB - Prussian Blue
PEA - phenylethylamine
PEDOT - poly (3,4-ethylenedioxythiophene)
Phe - phenylalanine
PKU - phenylchetonurie
PMS - premenstrual syndrome
POC - point of care
PPy - polypyrrole
Pro - proline
Pt - platinum
PTh - polythiophene
$\mathbf{R^2}$ - coefficient of determination

RMN - Nuclear magnetic resonance
rGO - reduced graphene oxide
RSD - relative standard deviation
SARS - CoV - 2 - severe acute coronavirus
SDS - sodium dodecyl sulfate
Ser - Serin
SEM - electron scanning microscopy
SNC - central nervous system
SPCEs - central nervous system
SWV - square wave voltammetry
TEM - transfer electron microscopy
TGA - thermogravimetric analysis
Thr - threonine
TRP - tryptophan
Tyr - tyrosine
UV - ultraviolet
$V^{1/2}$ - the square root of the scan rate
Val - valine
VIS - visible

THEORETICAL PART. The main studies in the field of the doctoral thesis.

CHAPTER I. AMINO ACIDS

I.1. Introduction

Prevention of various hereditary metabolic diseases, such as phenylketonuria (PKU), alkaptonuria, Parkinson's disease, and orientation toward a 'bio' diet and a healthy lifestyle— removing the factors that lead to numerous disorders and forms of depression— represent the reasons why the present study was conducted. Amino acids (AAs), responsible for the equilibrium of the nervous system—especially phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trypt)— were analyzed with a view to detecting their lack or excess and to treating them accordingly, in due time.

AAs have various functions in the body, especially useful for protein synthesis, and their lack in the daily diet can result in decreased protein formation, so the appearance of unwanted diseases. AAs are part of the proteins of the human body, so they are considered the basic constituents of the body that are found in organs, tissues, skin, muscles, hair. Numerous scientific papers have been found in the literature in which various aspects of AAs have been studied.

I.2. Classification of amino acids

AAs are involved in protein synthesis, but not all, only 20 appear in the genetic code of the more than 500 AAs identified. These 20 AAs did not appear at the same time, some of them being incorporated into the subsequent genetic code.

The following AAs classification criteria were identified: by structure, by the position of the amino group (-NH2), by nutritional requirement and by polarity [6].

Thus, by structure, AAs are organic molecules that contain a basic amino group (-NH2), a carboxyl acid group (-COOH) and a unique organic group for each AA (R).

I.3. Physico - chemical properties of amino acids

The multitude of biological and chemical functions that AAs perform for the human body is due to their physico-chemical properties. From a physical point of view, the remarkable properties are related to appearance, solubility, melting point, taste. Thus, AAs are solid in the form of colorless crystals [11]. Most AAs are usually soluble in water and ethanol, but insoluble in organic solvents (benzene, ether). This property depends largely on the temperature, the nature of the solvent and the isoelectric point. Their melting point is high, respectively between 200-300^{0}C, due to their ionic property. Most AAs do not taste good, but some are sweet (such as Gly) and some are bitter (e.g. Arg) [12].

Chemically, AAs participate in decarboxylation, esterification, acylation, salt-forming reactions, which are due to the existence of amino and carboxyl groups. Also, a number of specific reactions, such as the ninhydrin reaction with Sanger's reagent, Edmann's reagent, can quickly identify AAs [13].

I.4. The importance and uses of amino acids

Regardless of the type of AA, the human body needs complex proteins in order to function properly at all times. The important roles that AAs play are the following:

- regulation of gene expression through the transfer of encoded information, participating in biochemical processes of transcription, translation, post-translational changes [22];
- synthesizes and secretes hormones (for example: Tyr is a precursor for the synthesis of epinephrine, norepinephrine, dopamine and thyroid hormones) [23];
- participates in nutrient-specific metabolism and oxidative stress (as Arg is the activator of N-acetylglutamate synthetase) [24];
- role in cellular protein turnover (synthesizes and degrades proteins through a continuous process - Leu is an inhibitor of proteins in skeletal muscle and liver) [25];
- immune function. Intensive studies have been performed on TRP and Pro AAs for immune function [26];
- role in reproduction and lactation (eg male fertility, ovulation; fetal growth and development and lactogenesis) [9];
- obesity, diabetes and metabolic syndrome (Recent articles have shown that dietary supplementation with Arg has reduced plasma levels of glucose, homocysteine and asymmetric risk factors of dimethylarginine for metabolic syndrome) [25]
- endothelial function, blood circulation and normal lymph function [27];
- acid-base balance [28];
- tissue regeneration and remodeling and others [26].

Analyzing all these functions, we conclude that AAs have a remarkable metabolic regulation, with a primary role in the development, growth, reproduction and homeostasis of organisms; also serving as the main precursors for the synthesis of molecules of enormous importance.

I.5. Amino acid based pharmaceuticals. Method of administration, side effects and contraindications

Analyzing all these functions, we conclude that AAs have a remarkable metabolic regulation, with a primary role in the development, growth, reproduction and homeostasis of organisms; also serving as the main precursors for the synthesis of molecules of enormous importance [29].

There are a number of OTCs (over-the-counter) on the pharmaceutical market that contain significant amounts of AAs, each supplement being accompanied by a package leaflet with key information on active substance indications, administration, adverse effects and contraindications. they can produce them in the body [30]. A complete analysis of AAs, both qualitative and quantitative, could be performed by identification and quantification tests of pharmaceuticals with devices that can be developed in the laboratory.

CHAPTER II. ELECTROCHEMICAL METHODS APPLIED FOR THE DETERMINATION OF AMINO ACIDS

II.1. Overview

Over the years, many scientific researchers have developed numerous methods through which these AAs can be detected rapidly and precisely, both in biological and in pharmaceutical products. In this part of the thesis are centralized the methods reported in the literature through which AAs were detected. Among these methods we can mention the chemical methods, but also the instrumental methods, among which the electrochemical methods based on sensors and biosensors were detailed.

Many scientific articles, reviews, book chapters, and volumes about how to detect Phe, Tyr, and Trypt have been published so far. Each scientific paper describes unique methods of AA detection, which, as technology has advanced, highlighted advantages and disadvantages. In compiling the data in Table 6, a series of method performance criteria were in view: precision, selectivity, accuracy, sensitivity, detection limit, cost, and duration, classified according to the intensity of each method.

Table II.1. *Performance criteria of the methods developed for the detection of AAs*

	Precision	**Selectivity**	**Accuracy**	**Detection limit**	**Cost and duration**
High	Electrochemical methods based on achieving sensors and biosensors [32–35]				
Medium	Instrumental (electrical methods[36], optical methods [37], thermal methods [38], magnetic methods, and radiochemical methods[39])				
Low	Chemical methods (volumetry, gravimetry, precipitation methods) [40]				

II.2. Analytical methods for the detection of amino acids

Although very few, due to their being expensive and requiring specific analytical skills, the chemical methods applied to determine Phe, which constitute the basis of the following research, include gravimetric methods (inorganic, organic precipitation agents, electrodeposition) and volumetric methods (acid–base titrations, de precipitation, complexonometry, oxido-reduction)[41].

II.3. Instrumental methods

These are the most numerous according to the research carried out so far. In determining the amino acids were used:

- Colorimetry [43–45], UV and IR spectrophotometry [46], fluorescence [47–51], chemiluminescence [52,53];
- gas chromatography [54], capillary electrophoresis [55–57], HPLC — high performance liquid chromatography [58–62];
- Raman spectroscopy [63,64], laser-assisted spectroscopy [65], UV-Vis spectroscopy [66] și chemometry [66];
- nuclear magnetic resonance (RMN) [67] și circular dichroism (CD) [68];
- mass spectrometry [43,54,69,70], fluorimetry [71].

Although hese methods have proven to be e_cient, a series of disadvantages could be identified: they are costly, time consuming, and require special analysis and equipment. This explains why the electrical and electrochemical methods, i.e., potentiometry, voltammetry and conductometry, which imply lower costs, accessible maneuverability and allow for higher sensitivity have been preferred in AAs determination recently.

II.4. Electrochemical methods. Classification and description

Considering the category of instrumental methods, the electrochemical ones, which have developed in recent years, they are based on the construction of sensors and biosensors and measure one of the following features: electrode potential, current intensity through the cell, the amount of electricity passing through the cell, resistance and the time required for the development of the electrode process [41]. In turn, these electrochemical methods are subclassified into: potentiometrics, amperometrics, coulometry, conductometrics, electrogravimetry, chronoamperometry (CA) and chronopotentiometry, voltamperometric methods.

CV (cyclic voltammetry) is one of the methods must frequently used for characterizing electrochemical systems, because it provides both qualitative and quantitative information about a studied system. The graphical representation of the current recorded by the working electrode according to the applied potential is called the cyclic voltammetry curve. Using this method, a variety of sensors and biosensors may be applied, studied and modified so as to determine the substance to be analyzed [83].

II.5. Analytical parameters of electrochemical methods

Any electrochemical method applied to the study of an analyte is a measurement. Thus, the World Organization for Standardization (ISO) has established and developed a series of rules and guidelines that mention the characteristics, parameters, quality, shape of a product or service in all areas of activity. This is also the case with the application and development of electrochemical methods, in order to have the certainty that the results obtained from the application of the methods have a real meaning, ie they can be applied anywhere in the world and have the same meaning for anyone. The reference analytical parameters for electrochemical methods are: limit of detection (LOD), limit of quantification (LOQ), selectivity, sensitivity, accuracy, precision, repeatability, reproducibility [90].

CHAPTER III. MODIFIED ELECTROCHEMICAL SENSORS AND BIOSENSORS

III.1. Introduction. Compounds and materials used for electrode modification

Sensors and biosensors, high-interest instruments, are used in many research fields: medicine, pharmacy, industry, transport, environmental protection, and automation. Thus, in the future humanity will depend on many of these devices (with people who suffer from diabetes depending on glucometers—devices that detect the glycaemia levels in the body—constructed with the aid of a biosensor) [91]. This category of sensors is used especially in systems for monitoring the environment and health, in food quality control**.**

If the sensor is an analytical instrument that translates physical and chemical data into measurable signals, biosensors play the same role, but are based on a combination of a biological

recognition compound and a physical translator - the recognition element being either and enzyme, an antibody, or a microorganism - which renders it more sensitive for detecting the substance analyzed.

Thus, the stage of selecting sensor construction/manufacturing materials is extremely important, as the materials can contribute to solving various problems related to analyte detection, such as the redox potential of molecules, the deterioration of electrode surfaces—leading to low reproducibility.

III.2. Electroactive organic compounds: Prussian blue, Meldola blue and cobalt phthalocyanine

Modifications of SPCEs with a range of electrochemical and nanomaterial mediators give them properties that make them useful for various applications. Electroactive inorganic agents, such as: Prussian Blue (PB), Meldola Blue (MB), Cobalt Phthalocyanine (CoPc), Manganese Phthalocyanine, Potassium Ferrocyanide, Copper Phthalocyanine, Iron Phthalocyanine; are electrochemical species that give the electrodes the following advantages: electrocatalytic activity, good performance in terms of LOD and the level of interference, allow the oxidation or reduction to low potentials of the species to be analyzed [94–96].

PB comes in two forms: soluble form $KFe^{III}Fe^{II}(CN)_6$ and insoluble form $Fe_4{}^{III}[Fe^{II}(CN)_6]_3$; thus the PB structure has a mixed valence, the iron atoms can have different oxidation states (Fe^{2+}/Fe^{3+}), so the basic structure consists of a three-dimensional polymer network in which the happy ions alternate with the ferrous ions on the cubic network sites [95,130–133]. From an electrochemical point of view, PB is highlighted by two reversible redox reactions in which PB is reduced to Prussian White (PW) and oxidation to Prussian Yellow (PY) by the mixed valence form of Prussian Green (PG). It has been scientifically proven that the use of this compound has resulted in sensors with increased sensitivity and selectivity [134].

MB is another well-studied compound, with researchers finding its applicability and attractiveness in the field of sensor and biosensor development. This reagent was used for the detection of ascorbic acid, hydrogen peroxide, for the oxidation of nicotinamide - adenine dinucleotide (NADH). It is also a good catalyst used in the construction of electrodes with applicability in industrial chemistry, the manufacture of LEDs and for the detection of pharmaceuticals [94,135–140].

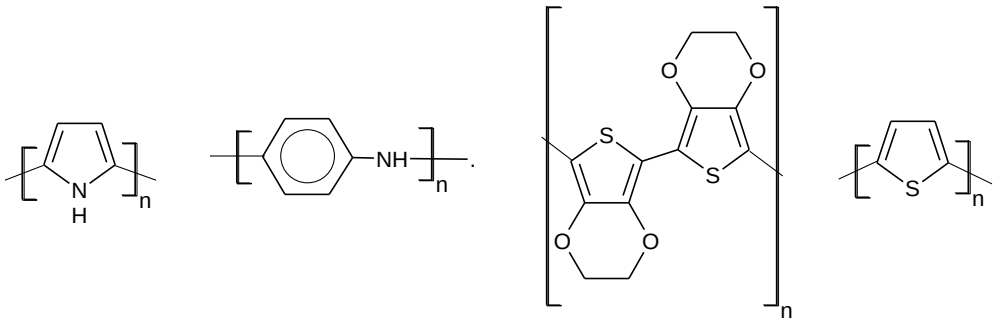
CoPc is a chemical compound, an organic semiconductor, used for the manufacture of many electronic devices (LEDs, photovoltaic panels, sensors and chemical biosensors) due to its advantages, namely sensitivity, thermal and photochemical stability, conductivity, low manufacturing cost [96,141,142].

III.3. Conductor polymers

In the last ten years, numerous research groups have made major contributions to the field of electroanalysis, as well as to the field of materials science, obtaining new classes of materials, such as novel polymers, which have allowed the possibility of a wide range of analytes detection.

Known as macromolecular compounds, polymers may be found in almost all the materials that people use in everyday life. In essence, polymers are made up of several small molecules—called monomers—linked to form long strands [97]. Thus, CPs, also known as “synthetic metals”,

represent a new generation of polymers, electrochemical synthesis being the preferred method of obtaining them since it has the advantage of simplicity and the possibility of achieving polymeric films of various thicknesses and doping levels [144]. The CPs most frequently encountered in scientific research are PPy, PANi, PEDOT, PTh, and polyacetylene, the chemical structures of which are shown in Figure III.6. [144].



a. PPy b. PANi c. PEDOT d. PTh

Figure III.6. The most frequently used conductor polymers: (a) PPy, (b) PANI, (c) PEDOT, and (d) PTs. Adapted from [144]

This category of polymers has drawn the attention of many researchers, particularly because of their main property: electrical conductivity [145].

III.4. Molecularly imprinted polymers

Other polymers involved in numerous studies are MIPs in monomer solutions with template molecules, reticulation agents, or solvents, this being a versatile preparation method that can frequently be used to configure various biomimetic receivers [152].

Since the three AAs are found in biological fluids, implicitly in human blood serum and urine, it is extremely important to monitor their levels in the body, to measure their concentration by means of more sensitive and more selective devices such as sensors and biosensors.

III.5. Enzymes used in the development of biosensors for the detection of amino acids

We demonstrated in the first chapter that AAs are key components in the diet of the individual, so that a quick and accurate detection of them not only in food, but especially in biological fluids and pharmaceuticals have stimulated researchers to develop reliable and sensitive devices. Such devices are biosensors, the manufacture of which requires a modifier, such as enzymes. Typically, the types of enzymes that were used to develop biosensors were oxidoreductases, polyphenol oxidases, peroxidases, or amino oxidases [127].

Other types of enzymes can be used to detect specific AAs, such as laccase (Lacc), tyrosinase, L-glutamate oxidase, L-glutamate dehydrogenase, these enzymes being selected according to the method of analysis. [169].

PERSONAL CONTRIBUTIONS. Sensors and biosensors developed for the analysis of phenylalanine, tyrosine and tryptophan in pharmaceuticals

CHAPTER IV. INTRODUCTION. GENERAL ASPECTS

Metabolic diseases are genetic disorders related to the metabolic pathways of some compounds from food. Such diseases are due to hereditary factors, and these could be diagnosed from birth, and are also related to other diseases occurring during a lifetime, and are identified after the appearance of specific symptoms.

The most common and widespread metabolic disease is PKU, which is due either to the lack of phenylalanine hydroxylase (PAH) or to its low level in the blood. This enzyme is necessary for converting the essential AA Phe into another AA called Tyr aspect, which explains why Phe is one of the most studied AAs. The PKU level may be detected as early as the neonatal period, the detection of PKU markers in biological fluids being performed mainly by using high performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS). However, there are other methods for determining PKU such as: enzymatic activity tests, capillary electrophoresis coupled with laser-induced fluorescence or electrochemical detection, as well as genetic tests.

Many of the methods mentioned above are unsuitable for point-of-care testing (POC), due to their implying numerous processing stages, a long time for analysis, to their being tedious, not portable and rather expensive, and to their requiring highly-specialized personnel. Considering the advantages of electrochemical techniques, namely: their simplicity, excellent potential for miniaturization, easy operation, low cost and high sensitivity, such techniques represent a major research topic.

The need to detect metabolic diseases as quickly as possible has favored the development and application of numerous testing methods, but the differences regarding neonatal screening criteria used in various countries and even in the same country have made diagnosis difficult. Considering the advantages of electrochemical techniques, namely: their simplicity, excellent potential for miniaturization, easy operation, low cost and high sensitivity such techniques represent a major research topic. In fact, the low LOD obtained by using these methods has determined numerous researchers to study AAs. On the other hand, electrochemical methods have a series of limitations on the electrochemical determination of AAs, the facts being observed that electron transfer, sensitivity and reproducibility are low, stability is low for numerous solutions and detection potential is high. Therefore, the use of chemically-modified electrodes (CMEs) for AAs detection could be a feasible alternative.

Wide variety of sensitive surface modifications have been suggested in order to improve the electroanalytical parameters of CME, among which coating with CPs (PPy) or molecularly printed polymers (MIPs). Electrode surface modified were also made with a series of enzymes. There are several enzymes that can be used for the detection of amino acids (AAs), the most common being tyrosinase, but Lacc was selected for this study, as it is easy to use, requiring no co-factors and participating in many oxidation reactions for a wide variety of organic substances (polyamines, polyphenols, diamines, lignins). In addition, the presence of the enzyme led to a biosensor with

much higher performance compared to the previously obtained sensor, i.e., increased stability, conductivity, reproducibility, and sensitivity; easy preparation; and possibility of use at neutral pH.

CHAPTER V. THE MOTIVATION, PURPOSE AND OBJECTIVES OF THE RESEARCH

We live in times with a lot of restrictive measures meant to stop the spread of severe acute respiratory syndrome-coronavirus (SARS-CoV-2), and numerous papers published by experts in psychology as well as medicine draw attention to the fact that the isolation and quarantine have affected everyone and have led to an increase in the number of depression, emotional disorders, anxiety, and sleep issues.

The negative effects that affect the health of the individual caused by the new coronavirus can be partially or completely treated with pharmaceuticals containing a compound, which acts on the central nervous system, namely tyrosine (Tyr), phenylalanine (Phe), tryptophan (TRP), the target compound of this study. Another reason for this thesis is the fact that one of the most common diseases among the population is depression, and in this sense a useful measure could be to monitor the values of AAs, respectively Phe, Tyr, TRP. For the prevention and treatment of mild forms of depression (postpartum, seasonal, premenstrual), on the pharmaceutical market have been developed and are found a variety of medicinal supplements, containing AAs under study, in different concentrations.

The **general objective** of this doctoral thesis was to develop new electrochemical sensors and biosensors by chemical modification with a series of electroactive compounds, CPs and MIPs, as well as by biochemical modification using various enzymes, in order to detect three AAs in pharmaceuticals by electrochemical methods.

The doctoral thesis had a series of specific objectives, which were closely correlated with the activities provided in the individual program of doctoral studies. The proposed objectives were achieved in the first stage by scientific documentation, the preparation of two reviews and two scientific papers, entitled as follows:

- A Review on Electrochemical Sensors and Biosensors Used in Phenylalanine Electroanalysis;
- A Review of Sensors and Biosensors Modified with Conducting Polymers and Molecularly Imprinted Polymers Used in Electrochemical Detection of Amino Acids: Phenylalanine, Tyrosine, and Tryptophan;
- CPs and MIPs. Synthesis, properties, applications;
- Electrochemical methods used in analytical chemistry.

Pornind de la obiectivele generale, au fost stabilite și o serie de **obiective specifice** care au fost atinse, dovadă făcând articolele publicate în reviste de specialitate cu un important factor de impact, după cum urmează:

- *Voltammetric Determination of Phenylalanine Using Chemically Modified Screen-Printed Based Sensors*;
- *Development of Polypyrrole Modified Screen-Printed Carbon Electrode Based Sensors for Determination of L-Tyrosine in Pharmaceutical Products*;

- *Development of a Novel Sensor Based on Polypyrrole Doped with Potassium Hexacyanoferrate (II) for Detection of L-Tryptophan in Pharmaceutics*;
- *Quantification of Tyrosine in Pharmaceuticals with the New Biosensor Based on Laccase-Modified Polypyrrole Polymeric Thin Film*

CHAPTER VI. MATERIALS AND METHODS USED TO DEVELOP NEW SENSORS AND BIOSENSORS FOR AMINO ACIDS DETECTION

VI.1. Compounds analyzed: L-phenylalanine, L-tyrosine and L-tryptophan. Their properties, synthesis and importance for the human body

Of the 11 AAs essential and 9 non-essential for the body, only three were subjected to the present study, namely Phe, TRP (essential AAs) and Tyr (non-essential AA) due to their structural similarity and the role that these AAs have for the human body**.**

VI.2. Materials

VI.2.1. Screen printed electrodes

SPCEs, purchased from Metrohm DropSens (www.dropsens.com) (Oviedo, Spain), were used in all studies. For the voltammetric determination of L-Phe from the studied drugs the working electrodes were modified by the manufacturer with 3 commercial electroactive substances: PB, CoPc and MB**.**

In the second study on the detection of L-TRP in pharmaceuticals, SPCEs DRP-C110 were used, being subjected to electropolymerization by the CA method. These SPCEs were immersed in a pre-prepared monomer / dopant solution of 0.1 M Py - 0.1 M FeCN.

The comparative study for the determination of AA L-Tyr in pharmaceuticals was performed by developing modified SPCEs in the chemistry laboratory, obtaining on the one hand sensors and biosensors on the other.

VI.2.2. Electrochemical equipment and cells

Two potentiostats were used to modify and characterize the sensors: EG&G Potentiostat / Galvanostat Model 263A (Princeton Applied Research, Oak Ridge, TN, USA) controlled by ECHEM software and Biologic SP 150 Potentiostat / Galvanostat (Bio-Logic Science Instruments SAS , France) controlled by EC - Lab Express software. The electrochemical cell was connected to these devices, in which the electrodes were inserted simultaneously, respectively: the reference electrode Ag / AgCl / KCl3M, the auxiliary electrode and the working electrode SPCE.

The Bruker ALPHA FT-IR spectrophotometer (BrukerOptik GmbH, Ettlingen, Germany), controlled by OPUS software (BrukerOptik GmbH, Ettlingen, Germany), was used to study standard samples and real samples containing AAs by the FT-IR method. Elmasonic S10H is the ultrasonic water bath used for dissolving compounds and homogenizing solutions**.**

Important information about the actual samples, about the morphology of the species deposited on the surface of the electrodes, about the crystalline structure and their chemical composition were obtained with the JEOL - JMS-T300 scanning electron microscope.

U-HPLC-Q-Exactive Orbitrap HRMS, was the device used for the chromatographic analysis of the studied AAs (acquisition of Full MS - vDIA), controlled by the software program Xcalibur, version 4.1. With the help of this device, the qualitative determination and identification of AAs from different pharmaceutical preparations was performed.

Also, the analytical balance AS60/220.R2 (SC Partner Corporation SRL, Bucharest, Romania), rated balloons, pipettes and micropipettes were used for weighing solid samples and preparing solutions.

VI.2.3. Solutions and reagents

The reagents used in this study were purchased from Sigma-Aldrich (St. Louis, USA) and used without further purification.

Thus, inactive L-Phe AA detection studies required inactive solutions, such as 0.1 M KCl; 0.001 M L-Phe– 0.1 M KCl, but also electroactive solutions 0.001 M NP - 0.1 M KCl; 0.001 M SDS - 0.1 M KCl; 0.001 $K_4[Fe(CN)_6]$ M - 0.1 M KCl.

Three pharmaceuticals were also analyzed: L-Phenylalanine 500 mg capsule (Solaray), DLPA 500 mg - DL - Phe free form - plant capsules (Solgar) and Amino 75 containing essential AAs in free form (Solgar).) - herbal capsules containing AA L-Phe in a concentration of 75 mg..

For the second study to determine the AA of L-TRP, the following were used: L-TRP (≥ 98%), KCl (≥99.0%), $K_4[Fe(CN)_6]$ (≥ 99.5%), pyrrole (98%). Two pharmaceuticals containing L-TRP were used to validate the results obtained with the modified sensors: Sleep Optimizer SOLARAY (150 mg Tryptophan) and Cebrium NEUROPHARMA (1.02 mg Tryptophan).

In the comparative study between sensors and biosensors developed for the detection of L-Tyr AA, the following substances were involved: L-Tyr (≥ 98%), KCl (≥99.0%), FeCN (≥ 99.5%), SDS (≥ 99.0%), NP (≥ 99%), pyrrole (98%), Lacc, glutaraldehyde (GA). The actual samples were represented by products purchased on the pharmaceutical market. To validate the results obtained by the modified electrodes, three products containing L-Tyr in different concentrations were selected from three manufacturers, one of the products containing several AAs, namely Cebrium. Thus, L-Tyrosine 500 mg (SOLARAY) (500 mg L-Tyr), Thyroid (PARAPHARM) (90 mg Tyr) and Cebrium (EVER NEURO PHARMA) (4.012 mg L-Tyr) were analyzed. In all studies, KCl was used as the supporting electrolyte, with a concentration of 0.1 M.

VI.3. Methods for characterizing electrodes, electroactive mediators and amino acids

VI.3.1. Chronoamperometry analysis

The chronoamperometric technique was used in the studies of this paper in order to analyze the electrochemical activity, the stability of the electrocatalysts, but also for the modification of the electrodes by electropolymerization. With the help of the chronoamperometric experiment it was possible to determine the thickness of the modifying substance film from the surface of the sensors and biosensors, it was possible to study the kinetics of chemical reactions, the study of diffusion processes and absorption.

VI.3.2. Cyclic voltammetry analysis

The detection method that was the basis of this study was CV following the electrochemical behavior of SPCEs modified in the solutions to be analyzed KCl, $K_4[Fe(CN)_6]$, KCl - L-Phe, KCl - L-Tyr, KCl - L-TRP and in solutions obtained from pharmaceuticals. CV is the most attractive and most widely used method by researchers, with the following advantages: it provides qualitative information about electrochemical reactions, it provides information about redox processes, heterogeneous electron transfer reactions and adsorption processes, it offers a fast localization of redox potential of electroactive species [84].

VI.3.3. Fourier transform infrared spectrometric analysis

FT-IR is a simple, easy, fast, non-invasive method, it has proven beneficial for this research, aiming to identify the compounds studied, both in their pure state and especially from the selected pharmaceutical products.

It has been scientifically proven that each test sample has a unique molecular footprint, so the FT-IR method proves to be effective for validating results obtained with other analysis techniques, such as the CV method applied in studies conducted for this research. FT-IR spectrophotometry is also able to identify possible contaminants in a material, to characterize the degree of decomposition and oxidation of molecules, as well as the types of additives in the sample to be analyzed.

VI.3.4. Chromatographic analysis of high performance liquids

The HPLC method used in this research aimed to validate the results obtained by the electrochemical method CV by analyzing compounds, respectively AAs, from samples represented by pharmaceuticals.

VI.3.5. Electron scanning microscopy

Important information reported in this thesis about the external or internal morphology, about the chemical composition and the crystalline structure of the existing compounds in the samples to be analyzed, was obtained with the help of SEM. This device obtains signals resulting from electron-sample interactions, the data being collected from a selected area of the sample surface, the result consisting of a two-dimensional image that displays spatial variations of the properties of the sample to be analyzed**.**

CHAPTER VII: ANALYSIS OF L-PHENYLALANINE, L-TYROSINE AND L-TRYPTOPHAN IN PHARMACEUTICALS WITH ELECTROCHEMICAL SENSORS AND BIOSENSORS

VII.1. Voltammetric determination of phenylalanine using chemically modified screen-printed based sensors

This paper describes the sensitive properties of screen-printed carbon electrodes (SPCE) modified by using three different electroactive chemical compounds: MB, CoPc and PB, respectively. It was demonstrated that the PB modified SPCE presented electrochemical signals with the highest performances in terms of electrochemical process kinetics and sensitivity in all the

solutions analyzed. The sensors were successfully applied to determine the Phe in pharmaceuticals by CV method. The validation of the method was performed by using the FTIR, and by comparing the results obtained by PB-SPCE in the analysis of three pharmaceutical products of different concentrations with those indicated by the producer.

VII.1.1. Electrochemical responses of screen-printed carbon electrodes in 0.1 M KCl solution

The sensors were successfully applied to determine the Phe in pharmaceuticals. The validation of the method was performed by using the FTIR, and by comparing the results obtained by PB-SPCE in the analysis of three pharmaceutical products of different concentrations with those indicated by the producer [236].

The voltammetric responses of the three sensors were recorded in the 0.1 M KCl solution with different scan rate and in various potential areas.. Well-defined signals were obtained, with little background noise when the scan rate was 0.6 V x s^{-1}. The optimal potential range was from -0.4 V to +1.3 V. no clear oxidation or reduction peaks were observed in this potential range, demonstrating that the electrochemical processes of CoPc and MB, respectively, immobilized in electrodes are not favored in this electrolyte and in the potential range used. The values obtained from the cyclic voltammogram of PB-SPCE are included in Table VII.1. The following parameters were determined from the CV was: anodic peak potential (E_{pa}), cathodic peak potential (Epc), anodic peak intensity (Ipa) and cathodic peak intensity (Ipc). $E_{1/2}$, ΔEp and $|I_{pc}/I_{pa}|$ were also calculated

Table VII.1. *Electrochemical parameters obtained from the cyclic voltammogram of PB-SPCE immersed in 0.1 M KCl solution; scan rate 0.6 V × s^{-1}*

Electrode	Peak pair	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ (V) (E_{pa}+E_{pc}/2)	ΔE_p (V) (E_{pa}-E_{pc})	I_{pa} (μA)	I_{pc} (μA)	I_{pc}/I_{pa}
PB-SPCE	I	0,221	-0,009	0,115	0,230	136,1	-186,5	1,36
	II	0,855	0,676	0,765	0,179	64,7	-60,5	1,07

Since KCl is an electroinactive compound, the two pairs of peaks are due to the redox processes of PB immobilized in the screen-printed electrode.

The results correspond to those presented in other specific research studies, the di erences being due to the particularity of the electrode (e.g., surface area, support material) and to the voltammetric technique (potential range, scan rate)

VII.1.2. Electrochemical behavior of PB-SPCE, MB-SPCE and CoPc-SPCE in 0.001 M $K_4[Fe(CN)_6]$ - 0.1 M KCl solution

$K_4[Fe(CN)_6]$ shows redox activity and this aspect may be highlighted by carbon or noble metal electrodes using cyclic voltammetry when an anodic and a cathodic peak are obtained, due to the reversible oxidation of the ferrocyanide ion to ferricyanide [239,240].

As illustrated in Figure VII.3, there is a clear difference between the redox processes seen in the CVs of the three modified SPCEs. More precisely, two peaks, one anodic and one cathodic,

are observed in the case of CoPc-SPCE, and two pairs of redox peaks are observed in the case of MB-SPCE and PB-SPCE. Thus, the redox processes of the electroactive modifier present in the sensitive layer at the level of MB-SPCE and PB-SPCE are observed in addition to the ferrocyanide/ferricyanide redox process.

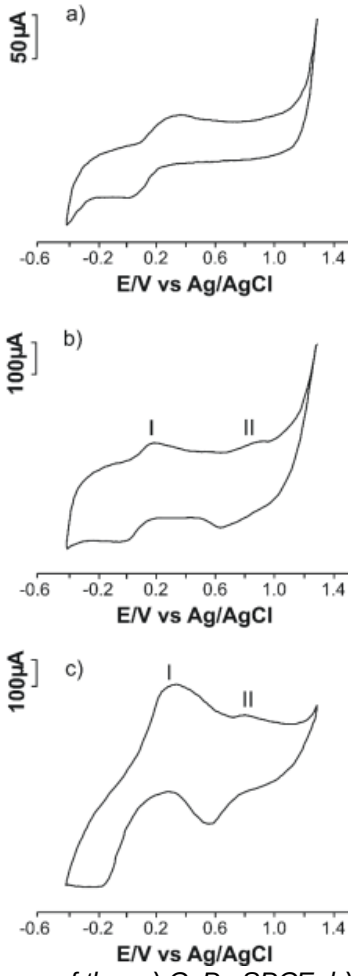


Figure VII.3. *Cyclic voltammograms of the: a) CoPc-SPCE; b) MB-SPCE; c) PB-SPCE immersed in 0.001 M $K_4[Fe(CN)_6]$– 0.1 M KCl solution registered with the scan rate of 0.6 V × s^{-1}*

According to the results obtained from the electrochemical measurements, the fact may be noticed that MB-SPCE and PB-SPCE in Figure VII.3.(b) and VII.3.c have the best responses recorded by CV, where the activity of the MB and PB electroactive compounds with which the sensors were modified is observed in addition to the redox a $K_4[Fe(CN)_6]$ solution.

The Ipc/Ipa ratio is higher or lower than the ideal value 1, but close to it in the case of the PB-SPCE electrode (I_{pc}/I_{pa} is 1.05), proving that the process is quasi-reversible in the case of the PB-modified electrode. Similar results were obtained for other electrodes modified with CoPc, MB or PB [94,96,237].

VII.1.3. Electrochemical responses of SPCEs modified in 0.001 M phenylalanine – 0.1 M KCl solution

The study of the three modified SPCEs was completed by the analysis of the 0.001 M Phe solution dissolved in 0.1 M KCl in the potential range -0.4 V to +1.3 V, at scan rates varying between 0.1–1.0 V × s^{-1}. Figure VII.6. shows the cyclic voltammograms obtained by each modified SPCE immersed in Phe solution, at the optimal scan rate of 0.6 V × s^{-1}.

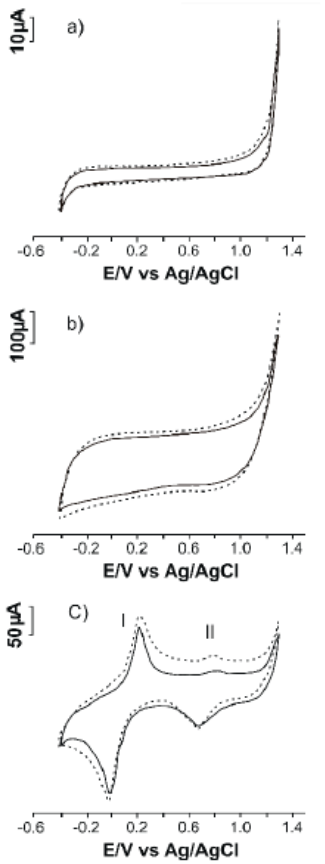


Figure VII.6. *Cyclic voltammograms of the a) CoPc-SPCE; b) MB-SPCE; c) PB-SPCE immersed in 0.1 M KCl solution (dashed line) and 0.001 M L-Phe - 0.1 M KCl (solid line) registered at 0.6 V × s^{-1}*

Therefore, PB-SPCE will be used for Phe detection, being the detection, being the most sensitive of the three electrodes. The peak intensities and potentials obtained by CV for the PB-SPCE sensor are detailed in Table VII.4.

Table VII.4. *Electrochemical parameters obtained from the cyclic voltammogram of PB-SPCE immersed in 0.001 Phe – 0.1 M KCl solution at 0.6 V × s^{-1}*

Sensor	Peak pair	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ (V)	ΔE_p (V)	I_{pa} (µA)	I_{pc} (µA)	I_{pc}/I_{pa}
PB-SPCE	I	0,210	-0,024	0,117	0,234	122,5	-158,2	1,29
	II	0,824	0,697	0,760	0,127	46,3	-43,2	0,93

By comparing the cyclic voltammograms obtained in KCl solution with those obtained in Phe–KCl solution (see Tables VII.1., VII.4. and Figure VII.6.), the fact is observed that the peaks are less intense in the solution containing Phe.

VII.1.4. Influence of phenylalanine concentration on the voltammetric response of PB-SPCE

The concentration of the solutions to be analyzed is essential in the response of an electrochemical sensor. Cyclic voltammograms were recorded in Phe solutions of different concentrations dissolved in 0.1 M KCl solution, between 3.3×10^{-7} M and 2.1×10^{-5} M ($0.33–21 \times 10^{-6}$ M), in order to determine the influence of Phe concentration on PB—SPCE. The electrochemical responses of the sensor recorded by cyclic voltammetry are show in figure VII.8.

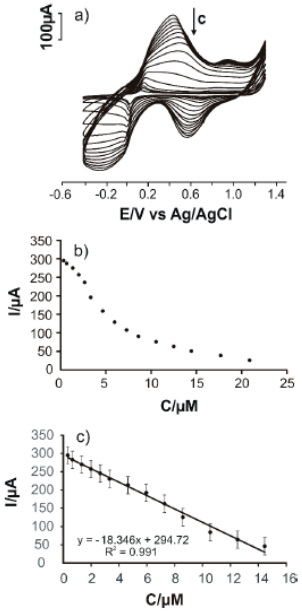


Figure VII.8. *a) Cyclic voltammograms of PB-SPCE immersed in Phe solutions in the range 0.33–21 × 10^{-6} M. b) Dependence between I_{pa} and Phe concentration in the range 0.33–21 × 10^{-6} M. c) Linear dependence between I_{pa} and Phe concentration in the range 0.33–14.5 × 10^{-6} M*

It may be noticed that the higher the concentration (c), the lower the intensity of the peaks. The values obtained for PB-SPCE are LOD = 1.23×10^{-8} M and LOQ = 4.09×10^{-8} M, values comparable with some results reported in the literature. The LOD and LOQ values of the PB-SPCE sensor are acceptable for detection of Phe in real samples. Therefore, this sensor was used to determine Phe in pharmaceuticals.

VII.1.5. Reproducibility, stability, and interference studies

The stability of the PB-SPCE sensorwas evaluated by employing cyclic voltammetry for 50 consecutive scans. The PB-SPCE sensor maintained 96.2% of its initial peak current response when it is immersed in 50×10^{-6} M Phe - 0.1 M KCl solution. Regarding the long-term stability of the

PB-SPCE sensor, it was tested over ten days. The PB-SPCE sensor preserved 94% from the initially response after ten days, with a RSD value of 3.28%. These results demonstrated the good stability of the sensor. Additionally, the reproducibility of the PB-SPCE sensor fabrication was studied by preparing five different sensors.

The fabrication reproducibility tests were carried out in in 50 × 10^{-6} M Phe - 0.1 M KCl solution. The RSD value for the anodic peak current observed for all five sensors was calculated to be 2.5%, demonstrating the good reproducibility of sensor development.

The interference studies of PB-SPCE sensor were carried out in presence of some interfering chemical species using cyclic voltammetry. CV responses were recorded in 50 × 10^{-6} M Phe - 0.1 M KCl solution in the presence of a 10-fold concentration of interfering species, such as glucose, L-valine, L-methionine, L-histidine and ascorbic acid. The results obtained showed that the PB-SPCE sensor retained 93.5% of its activity in the presence of interference chemical species. Therefore, the PB-SPCE sensor could be applied in real sample analysis.

VII.1.6. Quantitative determination of phenylalanine in pharmaceuticals products

In order to validate the sensor in the Phe analysis from real samples, three pharmaceutical products from different manufacturers and containing Phe in different concentrations were selected and analyzed: Amino 75 mg Solgar (L-Phe 75 mg), L-Phenylalanine 500 mg Solaray and DLPA 500 mg Solgar. These pharmaceuticals were analyzed by using two methods: CV (the method developed in this study) and FTIR (standard method) [11].

The purpose of these analyzes was to compare, on the one hand, the results obtained by using the two methods, and the experiment results with the values indicated by the manufacturers in the leaflets of the analyzed pharmaceutical products, on the other. Table VII.5. illustrates the results obtained from the quantitative determination of L-Phe by using the two methods.

Table VII.5. *The amount of L-Phe measured in pharmaceuticals products of different concentrations and different producers.*

Drug	The Amount of Phe Reported by the Producer/mg	The Amount of Phe	
		CV Method/mg	FTIR Method/mg
Amino 75	75	75±2	75±3
L-Phenylalanine 500	500	500±15	498±20
DLPA 500	500	500±14	503±22

Another method by which the L-Phe content of pharmaceuticals was determined was the MSLC in tandem MS (mass spectrometry) / ESI (electrospray ionization) technique using the U-HPLC-Q-Exactive Orbitrap HRMS device. In order to achieve the qualitative determinations for the identification of AA L-Phe in the selected pharmaceutical preparations, the following categories of operational parameters were optimized and subsequently set by UHPLC-MS / ESI: HESI ionization parameters, chromatographic separation parameters, MS operation (Full MS acquisition - vDIA (Independent variable data acquisition)). An example of a chromatogram obtained with the U-HPLC-Q-Exactive Orbitrap HRMS in a real sample is shown in Figure VII.11.

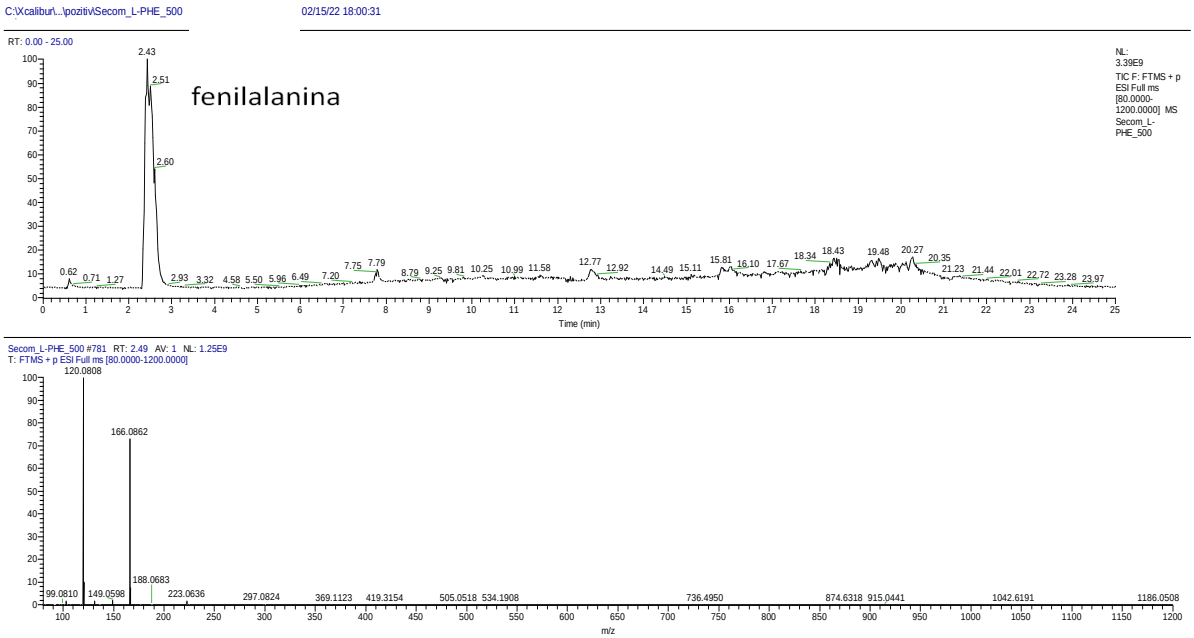


Figure VII.11. *Chromatographic analysis of AA L-Phe in L-Phenylalanine (Solaray)*

According to the data obtained, the amount of L-Phe per capsule in each pharmaceutical product was calculated, the results being centralized in Table VII.8.

Tablel VII.8. *L-Phe content obtained by HPLC method in the analyzed samples*

Drug	HPLC results L-Phe (mg / capsule)
L-Phenylalanine SECOM (500 mg L-Phe)	497,81
Amino 75 SOLGAR (75 mg L-Phe)	74,79
Cebrium EVER NEURO PHARM (4.012 mg L-Phe)	4,12

The data obtained by the chromatographic method confirm the accuracy of the CV and FT-IR methods, proving once again that the prepared sensors are sensitive and selective against L-Phe AA.

VII.1.7. Conclusions

The PB-modified sensor shows electroactivity in all studied environments, which proves that this sensor is also useful for the detection of inactive redox compounds such as Phe.

The use of CV as a detection method allowed for very good analytical performances with applicability in laboratory practice. The PB-SPCE sensor has been demonstrated to have the best analytical performance for the determination of L-Phe in pharmaceuticals. It has a wide linearity range, a high sensitivity and a very low LOD. The results obtained with the PB-SPCE sensor are very close to those obtained by using the standard method, and those indicated by the manufacturer at a 99% confidence level.

VII.2. Development of a novel sensor based on polypyrrole doped with potassium hexacyanoferrate (II) for detection of L-tryptophan in pharmaceutics

This study describes the development of a new sensor with applicability in the determination and quantification of yjr essential AA LL-TRP from pharmaceutical products. The proposed sensor is based on a SPCE modified with the conductor polymer PPy doped with FeCN. For the modification of the SPCE with the PPy doped with FeCN, the CA method was used. For the study of the electrochemical behavior and the sensitive properties of the sensor when detecting L-TRP, the CV method was used. This developed electrode has shown a high sensibility, a low LOD of up to 1.05×10^{-7} M, a LOQ equal to 3.51×10^{-7} M and a wide linearity range between 3.3×10^{-7} M and 1.06×10^{-5} M.

The analytical performances of the device were studied for the detection of AA L-TRP from pharmaceutical products, obtaining excellent results. The validation of the electroanalytical method was performed by using the standard method with good results.

VII.2.1. Chronoamperometric preparation of PB/FeCN/SPCE sensor

For the deposition of PPy on the SPCE, a solution of 0.1 M pyrrole and 0.1 M FeCN was prepared. Then, 15 mL of the solution was introduced in the electrochemical cell, and in the solution, the DRP-C110 electrode was immersed, making connections to the EG&G potentiostat.

The deposition of the PPy thin film in the presence of the doping agent was realized with the help of the CA method, employing the following working parameters: a potential of 0.8 V and a deposition time of 90 s. The obtained chronoamperograms related to the electropolymerization processes are presented in Figure 3 in two forms: the current's dependence on the time (Figure VII.14.A) and the dependence of the electric charge on the time (Figure VII.14.B).

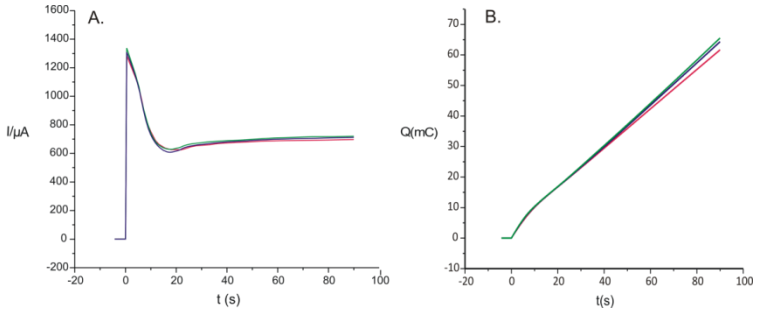


Figure VII.14. *A. Dependence of current (I) versus time (t) of curves registered in the electrosynthesis process of PPy/FeCN films at 0.8 V for 90 s; B. Charge (Q) versus time(t) when PPy underwent electrosynthesis in the presence of FeCN for three replicate sensors*

VII.2.2. The electrochemical behavior of the unmodified DRP - 110 electrode in 0.001 M L- tryptophan - 0.1 M KCl solution

Before the modification, the SPCE was immersed in a solution of 0.1 M KCl – 0.001 M L-TRP, and the cyclic voltamogram was recorded, comparing the results obtained from the unmodified sensor and the results from the modified sensor with PPy/FeCN. Using the electrochemical parameters mentioned above, the voltamogram obtained with the unmodified sensor at a scan rate of $0.1 \text{ V} \times \text{s}^{-1}$ is presented in figure VII.15.

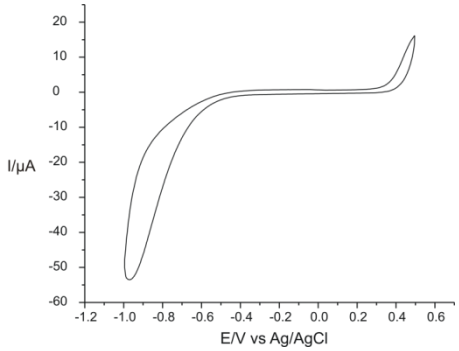


Figure VII.15. *The electrochemical behavior of the unmodified sensor in a double solution of 0.1 M KCl and 0.001 M L-TRP at a scan rate of 0.1 V × s^{-1}*

VII.2.3. The electrochemical response of the modified electrode with PPy/FeCN in a 0.1 M KCl solution and in a 0.001 M L-tryptophan - 0.1 M KCl solution

After the modification with PPy/FeCN, the electrochemical behavior of the sensor was initially analyzed in a 0.1 M KCl solution to observe the redox processes of PPy and the ferrocyanide ion included in the polymer matrix. Figure VII.16. shows the stable signal of the modified sensor immersed in 0.1 M KCl overlayed with the stable signal of PPy/FeCN/SPCE immersed in a double solution of 0.1 M KCl and 0.001 M L-TRP.

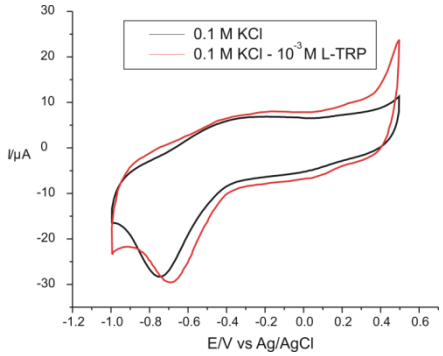


Figure VII.16. *Electrochemical response of PPy/FeCN sensor immersed in a 0.1 M KCl (black line) and in a double solution 0.001 M L-TRP - 0.1 M KCl (red line) at 0.1 V × s^{-1}*

The important difference between the two voltammograms was represented by the presence of AA L-TRP in the second solution, in which the electrode was immersed in the 0.001 M L-TRP solution, having as an electrolyte support 0.1 M KCl.

It was proven by the obtained results that the PPy/FeCN/SPCE sensor could be useful for the detection of L-TRP, similar to reports from other scientific works, with the mention that some characteristics of the electrode (such as the electrode's surface and the modifier material) and some electrochemical parameters (the potential field and the scan rate) were not the same.

VII.2.4. The influence of the scan rate on the PPy/FeCN/SPCE sensor responses immersed in a 0.1 M KCl and 0.001 M L-tryptophan solution

The proposed sensor for L-TRP detection was immersed in the double solution of 0.001 M L-TRP and 0.1 M KCl, recording cyclic voltammograms at 10 different scan rates, and the results are shown in Figure VII.17.A. The scan rates varied between 0.1 and 1.0 V × s^{-1}.

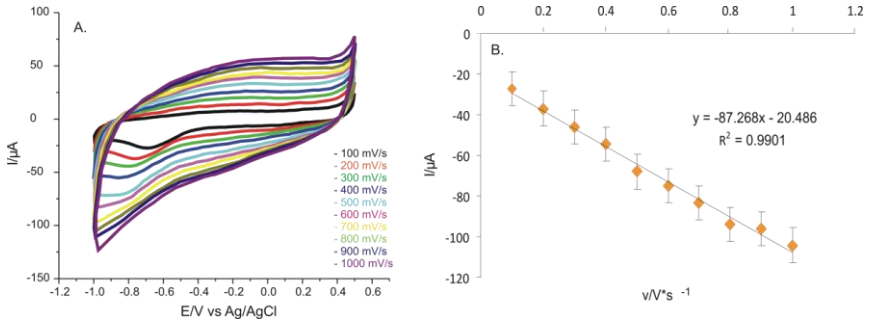


Figure VII.17. *A.Cyclic voltammograms of PPy/FeCN-SPCE sensor immersed in a 0.001 M L-TRP - 0.1 M KCl, solution at scan rates between 0.1 and 1.0 V× s^{-1}; B. The plot of the linear dependence between I_{pc} and the scan rate*

Laviron's equation, allowing the calculation of the degree of coverage on the electrode surface with active centers, that being 1.76 × 10^{-10} mol × cm^{-2}, considering the linear equation between the scan rate and the current of the most intense cathodic peak. This value was close to the results reported in the literature

VII.2.5. Influence of the concentration on responses of the PPy/FeCN/SPCE sensor immersed in a 0.001 M L-tryptophan and 0.1 M KCl solution. Calibration curve

The concentration of the analyzed solution proved to be important for the electrochemical responses of the PPy/FeCN sensor, used in the present study solutions with different concentrations of L-TRP dissolved in a solution of 0.1 M KCl. The concentration range studied was between 3.33 × 10^{-7} M and 2.72 × 10^{-5} M. The linearity range was observed to be between 3.3 × 10^{-7} M and 1.06 × 10^{-5} M, and the calibration equation and the calculated values of the LOD and LOQ are reported in Table VII.10.

Table VII.10. *LOD and LOQ* obtained with the *PPy/FeCN sensor detecting L-TRP*

Sensor	LOD (M)	LOQ (M)
PPy/FeCN-SPCE	1.05 × 10^{-7}	3.51 × 10^{-7}

The LOD obtained with the PPy/FeCN sensor was lower than the sensor's LOD reported in the scientific literature, making it possible to be used for the sensitive detection of L-TRP from pharmaceutical samples.

VII.2.6. Method precision, stability and reproducibility

Precision studies performed for the Ppy/FeCN sensor were performed both interday and intraday, based on solutions with L-TRP contents alongside the concentration of 5×10^{-6}. The interday precision was evaluated on 4 distinct days, and the intraday precision was analyzed in 3 different moments of the day at an interval of 2 h. The relative standard deviation (RSD (%)) presented the following values: 4.2% interday and 3.8% intraday. The good stability of the Ppy/FeCN sensor, both in the short and long term, was demonstrated by the CV method. In the short term, there were 30 consecutive scans recorded with the sensor developed in a double solution of 50×10^{-5} M L-TRP–0.1 M KCl, keeping the intensity of the peaks at 97.8% compared with the initial response. In the long term, there 96% stability out of the initial response was obtained after 5 days, with the RSD representing a value of 95%.

In addition, the sensor's reproductibility was created in a double solution of 50×10^{-5} M L-TRP–0.1 M KCl, preparing three different sensors. The RSD value for the cathodic peak observed in all 3 cases was 3.1%.

VII.2.7. Validation of the modified sensor by quantitative determination of L-tryptophan in pharmaceutical samples

A series of existing products of the pharmaceutical market contains the active compound L-TRP, which is the subject of the present study. Of these, Cebrium and Sleep Optimizer were tested for sensor validation using the FT-IR method, as well as comparing the electroanalytical results with those indicated by the manufacturers. The two pharmaceutical products have different compositions, different concentrations of L-TRP and come from different manufacturers.

Figure VII.19. presents the responses of the sensor immersed in solutions of L-TRP obtained from the analyzed pharmaceutical products. The estimated concentration of L-TRP in the solutions was 5×10^{-6} M.

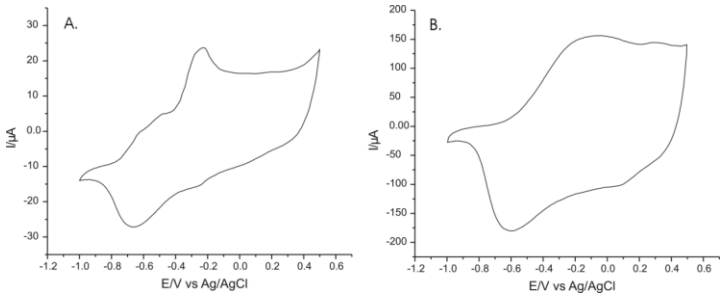


Figure VII.19. *Voltammetric responses of the PPy/FeCN sensors in solutions of: A. Cebrium (EVER NEURO PHARMA); B. Sleep Optimizer (SOLARAY)*

VII.2.8. Conclusions

The CA method proved to be efficient for the PPy doped with FeCN deposition through electropolymerization on an SPCE's surface. The developed sensor in this study, PPy/FeCN/SPCE, presents utility in detecting L-TRP both from model solutions and from pharmaceutical products, showing excellent electroanalytical results, with higher sensibility, precision and good stability. The fast response, low cost and the variety of the fields in which this

new device could be applied for the L-TRP, namely the medicine, pharmaceutical, chemistry and food industries, are important advantages for placement in the commercial market, contributing to the control of pharmaceutical products, monitoring some effects caused by an L-TRP deficiency or excess and food quality control.

VII.3. Comparative study on the experimental identification and quantification of L-tyrosine in pharmaceuticals with the new PPy/FeCN/SPCE sensor and the new PPy/FeCN/Lacc/SPCE biosensor

The present research presents the development of new electrochemical sensors modified with PPy doped with different doping agents such as FeCN, NP, and SDS for a selective and sensitive detection of Tyr.

Also a SPCE was modified with the conductive polymer (CP) PPy doped with FeCN, the polymer having been selected for its excellent properties, namely, permeability, conductivity, and stability. The enzyme Lacc was subsequently immobilized in the polymer matrix and cross-linked with GA, as this enzyme is a thermostable catalyst, greatly improving the performance of the biosensor.

The development and characterization of sensors and biosensors was achieved by the following electrochemical methods: CA, CV, scanning electron microscopy (SEM), FT-IR.

VII.3.1. PPy/FeCN/SPCE sensor and PPy/FeCN/Lacc/SPCE biosensor preparation process

VII.3.2.1. Preparation of the monomer/doping agent solution

A solution of exact concentration obtained from pyrrole, FeCN, and KCl of 0.1 M pyrrole/0.1 M FeCN/0.1 M KCl was used to modify the sensor. The deposition was achieved by connecting the DRP-C110 sensor to the electrochemical cell and introducing the three electrodes into monomer/doping-agent solution, applying a potential of 0.8 V for 90 s at a constant temperature of 25^0C. Subsequently, these prepared sensors were rinsed with ultrapure water. The method to characterize the changes occurring on the sensor working surface was CA.

VII.3.1.2. Manufacture of the sensor by doping the polypyrrole on the electrode surface by chronoamperometry

For the detection of L-Tyr, În prezenta cercetare s-au modificat electrochimic SPCEs în scopul detecției L-Tyr three different solutions of monomer/doping agent (FeCN, SDS and NP) 0.1 M/0.1 M were used. Then, the electrochemical behavior of PPy sensors PPy/FeCN-SPCE, PPy/SDS-SPCE, and PPy/NP-SPCE was analyzed through the CV technique in a solution of 0.1 M of KCl and then in a double solution containing 0.001 M L-Tyr and 0.1 M KCl. In the case of CV, the established parameters were: initial potential 0.0 V, positive vertex potential 0.5 V, negative vertex potential -1.0 V, and the scan rate was between 0.1 and 1.0 V × s^{-1}. The chronoamperograms obtained for six different sensors developed from the same solution are presented in Figure VII.22.

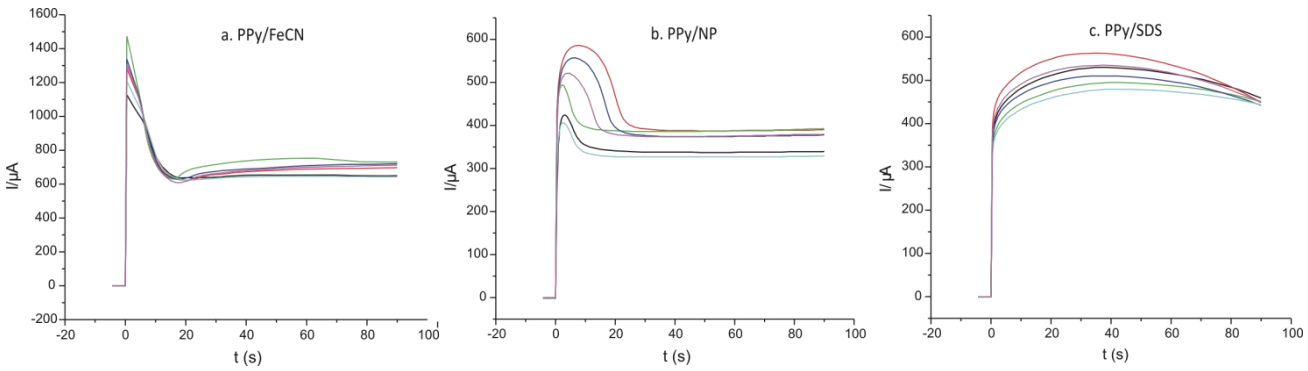


Figure VII.22. *Current versus time curves registered during the electrosynthesis of six different sensors prepared in the same conditions (a) PPy/FeCN, (b) PPy/SDS and (c) PPy/NP*

The doping agents used in this research were selected according to a series of characteristics: electroactivity, multiple charges, and large molecular weight. These characteristics should improve the stability of the sensitive layer as well as the sensitivity and selectivity.

VII.3.1.3. Manufacture of the enzymatically sensor by immobilization of the laccase enzyme

VII.3.2.3.1. Laccase - The enzyme used in the construction of the biosensor

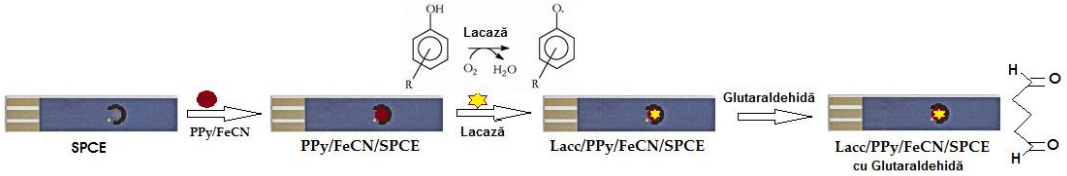
Discovered since the 1980s in the lake tree, Lacc is an enzyme that belongs to the category of copper oxidases that reduce molecular oxygen in water. The enzyme was selected for this study because it has a number of unique catalytic properties, including the property of oxidizing a variety of organic substrates, but also some inorganic compounds. In addition to these properties, Lacc participates in the degradation of polymers, the crosslinking of monomers and the cleavage of the ring of aromatic compounds (as are some of the amino acids).

VII.3.1.3.2. The structure of the laccase

Used in the pharmaceutical, textile, food, chemical industries, because it oxidizes not only toxic but also non-toxic substrates, Lacc is a very specific enzyme and an efficient catalyst. Being the oldest and most studied enzymatic form, Lacc comes in three forms, depending on the copper subunit: Lacc type 1, Lacc type 2 and Lacc type 3, and can be differentiated at UV-Vis.

VII.3.2.3.3. The mechanism of action of the laccase on the surface of the biosensor

The transformation of the PPy/FeCN/SPCE sensor into the PPy/FeCN/Lacc/SPCE biosensor was achieved by droplet pouring the Lacc enzyme onto the working surface of the electrode, equivalent to 10×10^{-6} M enzyme. This stage was followed by cross-linking with GA reagent and drying. The biosensor preparation technique is also called the drop-and-dry technique, illustrated in scheme VII.5**.**



Scheme VII.5. *Schematic diagram of the immobilization of the Lacc enzyme in the construction of the biosensor.*

VII.3.1.4. SEM analysis of the sensor

Since there is no other type of instrument to be applied so widely for the study of solids, the SEM was also used in this research for the purpose of analyzing the modified sensor in the laboratory. The morphology of the polymeric films was studied by scanning electron microscopy and the images obtained are presented in figure VII.27.

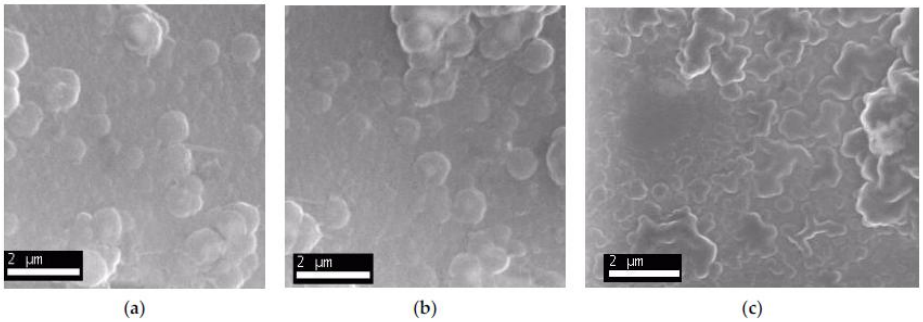


Figure VII.27. *Scanning electron microscopy images of the sensitive element of polypyrrole based sensors doped with: (a) FeCN; (b) NP și (c) SDS*

VII.3.2. Characterization of the sensor and biosensor by cyclic voltammetry

VII.3.2.1. Electrochemical responses of sensor immersed in 0.1 M KCl solution and in 0.001 M L-tyrosine - 0.1 M KCl solution before modification

The electrochemical response of unmodified SPCEs was investigated in two solutions: 0.1 M KCl solution, respectively in 0.1 M KCl–0.001 M L-Tyr solution in the potential range from 1.0 to 0.5 V. This step is important to be able to compare the results obtained with the unmodified sensor with modified electrodes with PPy doped with FeCN, N,P and SDS, but also with the enzyme-modified electrode.

VII.3.2.2. Stable electrochemical responses of chemically and biochemically modified electrodes in 0.1 M KCl solutions and in 0.001 M L-tyrosine - 0.1 M KCl solutions

- in 0.1 M KCl solution

After preparation, the sensors and biosensors were introduced in the 0.1 M KCl solution and the cyclic voltammograms were recorded. peaks. For the stability of this electrodes, six cycles were recorded at a scan rate of 0.1 V × s^{-1}, during which time PPy (in the case of sensors) and Lacc (in the case of biosensors) stabilization in the electrolyte solution took place [254]. This is illustrated in

figure VII.31, according to which it is observed two anodic peaks and two cathodic peaks, which corresponded, on one hand, to the PPy redox process, whereas the II redox process corresponded to the oxidation - reduction process of potassium ferrocynide found in the polymeric matrix.

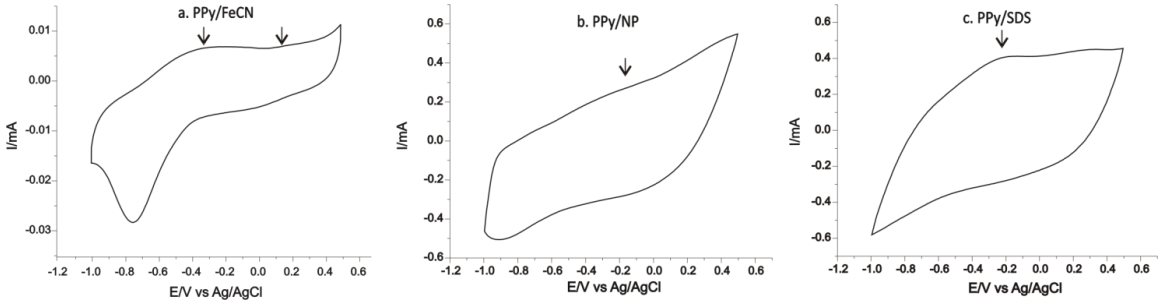


Figura VII.31. *Răspunsurile stabile ale senzorilor pe bază de PPy imersați în soluție de KCl 0,1 M la viteza de scanare de 0,1 V × s^{-1}: (a) PPy/FeCN/SPCE (b) PPy/NP/SPCE (c) PPy/SDS/SPCE*

The sensor PPy/FeCN-SPCE had better defined peaks and a relative reduced background current opposed to the other two sensors, so it underwent further modification, turning it into an enzyme sensor, using the Lacc enzyme. Thus, a comparative study was performed on the stable responses of the non-enzymatic sensor with those of the enzymatic sensor, first in the inactive 0.1 M KCl solution, at the same scan rate.

- in 0.1 M KCl - 0.001 M L-Tyr solution

In the following step, the modified electrodes were immersed into a double solution containing 0.1 M KCl – 0.001 M L-Tyr. On detection of AA L-Tyr, the stable electrochemical responses achieved with the three PPy modified electrodes are presented in Figure VII.33.

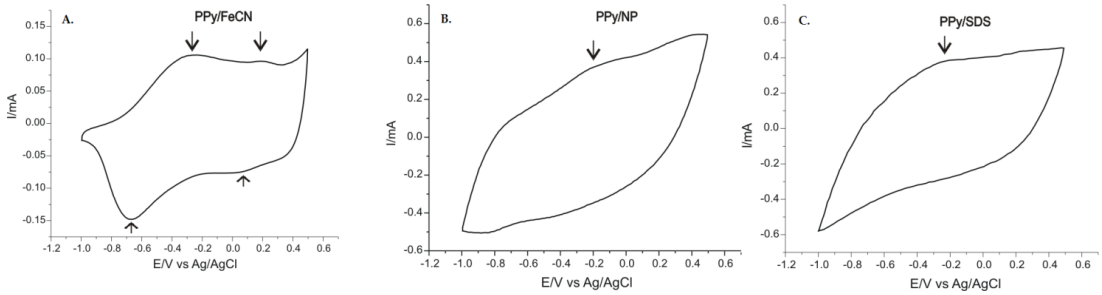


Figure VII.33. *Stable response of polypyrrole based sensors immersed in 0.1 M KCl -0.001 L-Tyr solution at 0.1 V × s^{-1}: (a) PPy /FeCN/SPCE, (b) PPy/NP/SPCE, (c) PPy/SDS/SPCE*

Both peak pairs, related with PPy and FeCN, were influenced by the presence of the L-Tyr by shifting the peak potentials and increasing the peak currents, especially in the anodic scan.

A notable difference is that between the sensor and the biosensor, in which case the peak intensities are increased both in the redox I system, corresponding to PPy, and in the redox II system, corresponding to FeCN included in the polymer matrix on the one hand, on the other Lacc enzyme provides better selectivity and accuracy to the biosensor.

VII.3.2.3. The influence of the scan rate on the responses of the sensor and of the biosensor

It is well known that the scan rate plays an important role in electrochemical measurements as it contributes to bringing out the redox processes and greatly influencing the voltammetric responses of the sensors and biosensors. The voltammograms were recorded at 10 scan rates, from 0.1 to 1.0 V × s^{-1}, in the potential range between -1.0 V and + 0.5 V, thus making possible the study of dynamic characteristics and of sensor signals. The intensity of peaks is directly proportional to the square root of the scan rates, which points out that the redox processes have a diffusion process to determine the kinetics stage. The active surfaces of the unmodified and the three modified sensors were calculated from the linear fitting equations by using the Randles–Sevcik equation, the values achieved for A are presented in table VII.18.

Table VII.18. *Active area surface and roughness factor for the electrodes used in the analysis before and after modification*

Electrode	Solution	Slope ($mA \times s^{1/2} \times V^{-1/2}$)	R^2	Active area (cm^2)	Roughness factor
SPCE	L-Tyr 0,001 M - KCl 0,1 M	0,00005820	0,9946	0,0803	0,63
PPy/FeCN/SPCE		0,00085700	0,9972	1,1824	9,41
PPy/NP/SPCE		0,00027890	0,972	0,3847	3,06
PPy/SDS/SPCE		0,00034100	0,9910	0,4700	3,74

In conclusion, it can be noted that the values of the active area and roughness factor of the PPy/FeCN sensor were the highest, thus this sensor had the highest sensitivity for L-Tyr detection, therefore, the influence of the scanning speed for this sensor was further studied compared to the prepared biosensor, also in the double electrolyte solution 0.001 M L-Tyr - KCl 0.1 M.

VII.3.2.4. Calibration curves and detection limits obtained by PPy/FeCN/SPCE sensor and PPy/FeCN/Lacc/SPCE biosensor respectively

This is an important stage as the equation of the calibration curve can help calculate the limit of detection (LOD) and the limit of quantification (LOQ) of each PPy-modified electrode.

The concentration range researched was between 0.5 – 27× 10^{-6} M for all three electrodes developed in this study. One can note that for all three sensors, there was a linear increase in the anodic intensity peak once the Tyr concentration increased. The sensitivity of the PPy/FeCN-SPCE, PPy/NP-SPCE, PPy/SDS-SPCE, and unmodified SPCE for determination of L-Tyr were: 1.463 A/M, 0.2789 A/M, 0.3412 A/M, and 0.1543 A/M, respectively.

The voltammetric determinations were achieved with all three sensors, and Table VII.20 shows the LOD and LOQ obtained through Tyr detection.

Table VII.20. *Data achieved from the calibration curves for PPy/FeCN, PPy/NP and PPy/SDS sensors at Tyr detection*

Sensor	LOD (M)	LOQ (M)
PPy/FeCN/SPCE	$8{,}20 \times 10^{-8}$	$2{,}73 \times 10^{-7}$
PPy/NP/SPCE	$4{,}30 \times 10^{-7}$	$1{,}43 \times 10^{-6}$
PPy/SDS/SPCE	$3{,}51 \times 10^{-7}$	$1{,}17 \times 10^{-6}$

The lowest LOD and LOQ values were achieved for the PPy/FeCN/SPCE sensor, confirming the superior sensitivity of this sensor. Therefore, the results of the sensor were compared with those of the developed biosensor (Table VII.20.).

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Table VII.21. *Datele obținute pentru curbele de calibrare a senzorului Ppy/FeCN/SPCE și biosenzorului Ppy/FeCN/Lacc/SPCE la detecția L-Tyr*

Electrod	LOD (M)	LOQ (M)
senzor PPy/FeCN/SPCE	$3{,}76 \times 10^{-7}$	$1{,}25 \times 10^{-6}$
biosenzor PPy/FeCN/Lacc/SPCE	$2{,}29 \times 10^{-8}$	$7{,}63 \times 10^{-8}$

VII.3.2.5. Interference studies and studies on the accuracy, repeatability, reproducibility, stability of the sensor and biosensor

Precision, repeatability, reproducibility and stability studies have been performed for both sensor and biosensor. Regarding the electrode stability, this was determined in the same L-Tyr solution during three weeks. After three weeks, it was found that the electrode kept 91% of the initial signal response. The sensors were stored in the refrigerator at a temperature of 4^0C in a closed dry box when they were not used.

The reproducibility of the fabrication process was also studied. Five biosensors were prepared in identical conditions and the responses in 10^{-5} M L-Tyr were registered. As can be observed in Figure VII.40, the differences between the biosensor responses were small, with the relative standard deviation (RSD) being 2.7%.

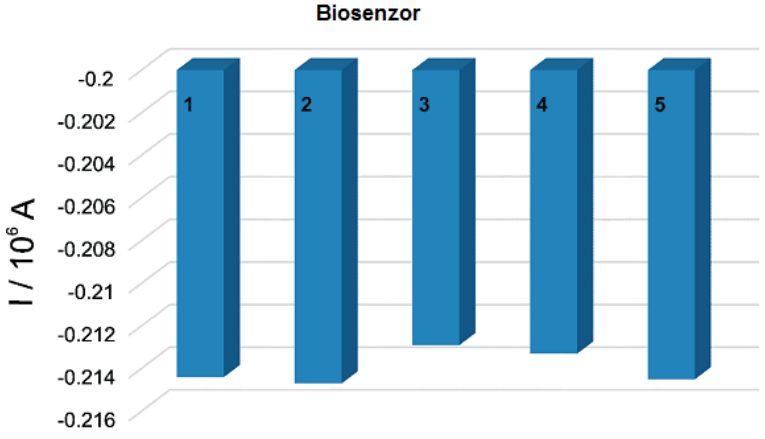

Figure VII.40. *Stability of responses obtained with 5 modified biosensors PPy/FeCN/Lacc/SPCE for L-Tyr concentration 10^{-5} M (RSD = 0.33%)*

VII.3.2.6. Quantitative determination of L-tyrosine with the prepared sensor and biosensor, as well as their validation on real samples

Three pharmaceutical products from three different manufacturers with different Tyr concentrations were selected and analyzed in order to validate the sensors made in this research through AA L-Tyr quantification. The samples were obtained from a local pharmacy. The three pharmaceutical products used were L-tyrosine (Solaray) (L-Tyr 500 mg), Tiroidin (Parapharm) (L-Tyr 90 mg), and Cebrium (Ever Neuro Pharma) (L-Tyr 4.012 mg) and they were analyzed by CV. Therefore, the concentrations of each AA in the solution analyzed were at the similar level.

PPy/FeCN/SPCE was used to detect and quantify L-Tyr in the pharmaceutical products as it had the best analytical performances among the three sensors developed in this research study. The cyclic voltammograms recorded with PPy/FeCN-SPCE for the two pharmaceutical products containing 0.001 M L-Tyr in the analyzed solution are shown in Figure VII.41.

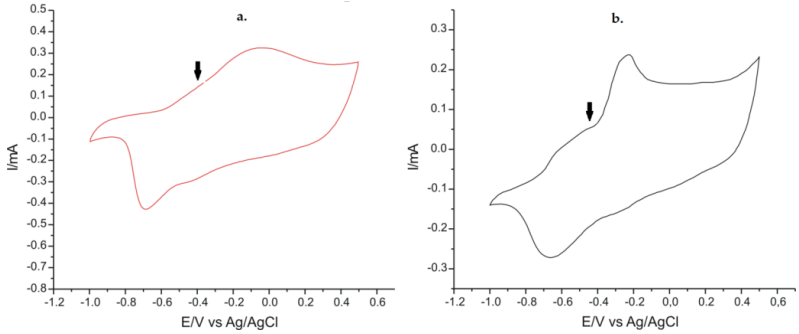

Figure VII.41. *Voltammetric responses of the PPy/FeCN sensor in a solution of: a. L-Tyrosine (Solaray); b. Cebrium (Ever Neuro Pharma).*

Electrodes validation was performed by the FT-IR method. The electroanalytical results for the quantification of L-Tyr obtained by the CV method based on the sensor and biosensor developed in this study were compared both with those obtained by the FT-IR method and with those provided by the producers of the pharmaceutical products under analysis

VII.3.2.7. Conclusions

The PPy films with the ion doping FeCN, NP, and SDS were successfully synthesized through the CA method and deposited on screen-printed carbon electrodes. The sensors were used in order to detect L-Tyr in standard solutions and pharmaceutical products, and it showed that PPy/FeCN-SPCE had the best electroanalytical performances. Good analytical performances were achieved by using CV, which indicates that this sensor can be used in screening analysis. The method based on using PPy/FeCN-SPCE for the quantification of L-Tyr was verified by the standard addition method, which obtained good recovery values. The electroanalytical method has some important advantages when it comes to laboratory practice such as its precision, reliability, simplicity, and low cost. The sensor exhibits fast response, good sensitivity, and stability for the voltamperometric detection of L-tyrosine, also being useful for the selective determination of complex samples containing different AAs. The voltamperometric method can be used when

performing quality control on pharmaceutical products and phytoproducts as well as other samples of interest.

In this study, a new biosensor was developed by immobilizing the laccase enzyme on the surface of an electrode modified with a conductive material with excellent properties, PPy polymer doped with FeCN anion, by chronoamperometry. Characterization of the modified electrode was performed by voltammetric techniques and FT-IR spectroscopy, and the results obtained demonstrated the increased selectivity of the biosensor for the quantitative determination of Tyr compared to those obtained by the unmodified electrode with the Lacc enzyme. In addition, Lacc and CP demonstrated biocompatibility, superior mechanical properties, and a high surface-to-volume ratio for the biosensor. The concentration range in which PPy/FeCN/Lacc/SPCE was tested was in the range of 0.2–6 × 10^{-6} M and the detection limit obtained was 2.29 × 10^{-8} M, a low value compared to the non-enzymatic sensor and other devices reported in the literature. Moreover, this new biosensor demonstrated good stability for one week and acceptable recoveries when tested on real samples, i.e., pharmaceuticals with different Tyr concentrations. The development of this biosensor can prove effective in controlling the quality of pharmaceutical products containing L-Tyr and is a challenge for future research, in the sense of developing a biosensor to detect the level of L-Tyr in food and biological fluids from birth, as such a device can help prevent many diseases.

CHAPTER VIII. THE MAIN VOLTAMMETRIC SENSORS AND BIOSENSORS REPORTED IN THE LITERATURE FOR THE DETECTION OF PHENYLALANINE, TYROSINE AND TRYPTOPHAN

In order to demonstrate that the sensors and biosensors developed in this research thesis have very good analytical performance, we conducted a scientific documentation on what has been developed in the last 5-10 years in this regard. Because the studies focused on both chemically and biochemically modified electrodes, the references in the search engines took into account their modifying elements: electroactive organic compounds, conductive polymers, molecularly printed polymers, enzymes. At the same time, it was intended that these devices be studied for the detection of amino acids (Phe, Tyr, TRP) by electrochemical techniques, such as: CV, CA, DPV, SWV, LSV.

Because the sensors involve different preparation steps compared to biosensors, the results selected from the literature have been structured in two sections: one in which the electrochemical sensors are presented and another with the electrochemical biosensors.

CHAPTER IX. GENERAL CONCLUSIONS

The last two years, in which the global pharmaceutical market has had an increasing evolution compared to that of 2017-2020, have marked the need for the existence of tools and devices to control, measure or verify the quality of medicines. Therefore, the main goal of this paper was to develop easy-to-use, low-cost, accurate, and sensitive devices for the detection of amino acids in pharmaceuticals. The selected amino acids, namely phenylalanine, tyrosine and tryptophan, were the most requested by the population, but also recommended by pharmacists,

due to the consequences of the spread and infection with the new coronavirus, but especially the conditions of panic, fear, stress caused by war. It was also intended that the excess or deficiency of one of the three amino acids could be detected as early as possible to be properly treated. In essence, by means of versatile devices it was possible to establish the assimilation of a correct concentration of phenylalanine, tyrosine or tryptophan, thus ensuring the knowledge of the bioavailability in the body of a substance in a pharmaceutical form (capsule).

I found that:

- Phenylalanine, tyrosine and tryptophan are the most sought after and studied amino acids for the treatment of central nervous system disorders (depression, insomnia, anxiety), but no quick and effective method of quantitatively determining them from biological fluids, pharmaceuticals or from foodstuffs;

- The increased interest of researchers in electrochemical methods is due to their advantages, namely: they save analysis time, costs are lower, response time is faster, the use of reagents in small quantities;

- Conductor polymers and molecularly imprinted polymers are new generation materials, with a wide range of uses, but with limited applications in terms of making electrodes for the detection of amino acids;

- Prussian Blue, a modifier of a screen-printed carbon electrode, is an electroactive organic compound through which inactive redox compounds can be detected, as was the indirect detection of the amino acid phenylalanine in samples prepared with pharmaceuticals;

- Chronoamperometry is a fast electrochemical method of modifying screen-printed carbon electrodes, obtaining a very good reproducibility of the electropolymerization of the sensors prepared in the same experimental conditions;

- The conductor polymer-polypyrrole together with the doping agent, potassium ferrocyanide, are chemical modifiers that have demonstrated improved signals of screen-printed carbon electrodes, detecting the amino acid tryptophan with precision, high detection limit and increased sensitivity; but especially the amino acid tyrosine, when the PPy/FeCN/SPCE signals were compared with those of two other modified electrodes, namely PPy/NP/SPCE and PPy/SDS/SPCE;

- Lacaza is the versatile modifier that transformed the PPy/FeCN/SPCE sensor for tyrosine detection into a biochemical and biosensor sensor, respectively. This enzyme has proven its effectiveness through the drop-and-dry technique, where glutaraldehyde has been used as a crosslinking agent, thus maintaining its enzymatic activity after many uses of the biosensor;

- The most important information and the most intense studies were performed using the cyclic voltammetry method. This method led to original results, being used to study the influence of important electrochemical parameters: scanning rate, analyte concentration, interference studies, accuracy, stability and reproducibility;

- For redox mechanisms, the area of the active surface or the diffusion coefficient was calculated, depending on the parameter that influenced the redox process;

- All the changes that were applied to the electrodes in the laboratory of sensors and biosensors led to results with high performance, proving their usefulness for quality control of pharmaceuticals and other types of samples.

CHAPTER X. FUTURE RESEARCH PERSPECTIVES

The opportunity to carry out complete control, both quantitatively and qualitatively of some drugs or OTCs (over the counter drugs) with a series of versatile devices made in the laboratory of sensors and biosensors can facilitate many activities carried out by inspectors National Medicines Quality Control Agencies. With these devices made in previous studies it has been shown that increased sensitivity and selectivity can make them true rapid testing tools with applicability in various fields: chemistry, biochemistry, medicine, pharmacy, food industry.

Since some of the results obtained with sensors and biosensors for Phe, Tyr and TRP detection, developed and presented in this research thesis are among the best compared to those reported in the literature, and other results are even unique, so as mentioned in Chapter VIII, future studies proposed with these devices focus on the following objectives:

- ❖ testing of sensors and biosensors on biological fluids (urine, saliva, plasma) to detect the three AAs;
- ❖ broadening the spectrum of detection for other AAs of interest with action on the CNS;
- ❖ additional studies for electrode surface changes by methods such as: FT-IR, TEM, SEM
- ❖ broadening the spectrum of electrochemical analysis of sensors and biosensors by other methods DPV, SWV, LSV;
- ❖ modification of the electrodes with other CPs (PANi, Pth, PEDOT) and comparison of the results with those obtained previously;
- ❖ patenting of devices;
- ❖ placing sensors and biosensors on the commercial market, initially by conducting free testing campaigns in medical offices, and later by distributing them on the pharmaceutical and medical market;
- ❖ creating digital applications (installed on smartphone, smartwatch, smart tv) connected to devices made via USB ports to ensure continuous monitoring.

Detection techniques can also be improved by using new, faster and more sensitive techniques, such as ultra-fast CV or by combining detection techniques, such as spectroelectrochemical techniques, which combine voltammetric techniques with UV-Vis or Raman spectroscopy. The input of information of a different nature can bring additional useful information in detection and quantification.

All these activities are future directions of research in a stage of postdoctoral studies, but also for the development of a START-up business plan, as they fall within the CAEN codes 2651 and 3250 which have as field of activity *Manufacture of instruments and devices for measurement , verification, control, navigation*, respectively *Manufacture of medical and dental devices, apparatus and instruments*.

CHAPTER XI. VALORIZATION AND IMPACT OF RESEARCH RESULTS

XI.1. Articles published in ISI listed journals

- **2020**

Dinu, A.; Apetrei, C., A Review on Electrochemical Sensors and Biosensors Used in Phenylalanine Electroanalysis, *Sensors* 2020, 20(9), 2496, https://doi.org/10.3390/s20092496, Factor de impact 3,576, Q1;

Dinu, A.; Apetrei, C., Voltammetric Determination of Phenylalanine Using Chemically Modified Screen-Printed Based Sensors, *Chemosensors* 2020, 8(4), 113, https://doi.org/10.3390/chemosensors8040113, Factor de impact 3,398, Q1.

- **2021**

Dinu, A.; Apetrei, C., Development of Polypyrrole Modified Screen-Printed Carbon Electrode Based Sensors for Determination of L-Tyrosine in Pharmaceutical Products. *International Journal Molecular Sciences* 2021, 22(14), 7528, https://doi.org/10.3390/ijms22147528, Factor de impact 5,924, Q1;

Dinu, A.; Apetrei, C., Development of a Novel Sensor Based on Polypyrrole Doped with Potassium Hexacyanoferrate (II) for Detection of L-Tryptophan in Pharmaceutics, *Inventions* 2021, 6(3), 56, https://doi.org/10.3390/inventions6030056.

- **2022**

Dinu, A.; Apetrei, C., Quantification of Tyrosine in Pharmaceuticals with the New Biosensor Based on Laccase-Modified Polypyrrole Polymeric Thin Film. *Polymers* 2022, 14(3), 441. https://doi.org/10.3390/polym14030441, Factor de impact 4,329, Q1;

Dinu, A.; Apetrei, C., A Review of Sensors and Biosensors Modified with Conducting Polymers and Molecularly Imprinted Polymers Used in Electrochemical Detection of Amino Acids: Phenylalanine, Tyrosine, and Tryptophan, *International Journal Molecular Sciences* 2022, 23(3), 1218. https://doi.org/10.3390/ijms23031218, Factor de impact 5,924, Q1.

Factor de impact cumulat: 22,521 WOS

XI.2. Papers and posters presented at international and national conferences

- **2018**

Ancuţa Dinu, Constantin Apetrei, Development of polyaniline based sensors for the determination of ascorbic acid in pharmaceutical products, The 4th International Conference New Trends on Sensing - Monitoring - Telediagnosis for Life Sciences NT-SMT-LS 2018, Book of abstracts, pp. 39, Brașov, România, August 30 - September 1, 2018, **International Conference - poster**;

Ancuţa Dinu, Constantin Apetrei, Voltammetric Study of Phenylalanine by Means of Sensors Based on Polypyrrole Doped with Different Anions, Scientific Conference of Doctoral Schools SCDS-UDJG 2018 The Sixth Edition, Book of abstracts, pp 83, Galați, România,7th-8th of June 2018, **International Conference - oral presentation**;

Ancuţa Dinu, Constantin Apetrei , Development of the electrochemical sensors for the detection of neurotransmitters, European Conference of Phsyhiatry and Mental Health „Galatia 2018", Abstract will be published in American Journal of Psychiatry and Neuroscience 2018, Galati, Romania, May 9-13, **International Conference** - **poster**;

Ancuţa Dinu, Constantin Apetrei, Development of voltammetric sensors based on conducting polymers for the detection of amino acids, 22nd Conference "New Cryogenic and Isotope Technologies for Energy and Environment" - EnergEn, Book of abstracts, pp 179, Băile Govora, România, October 24 – 26, 2018, **National Conference** - **poster**.

- **2019**

Ancuţa Dinu, Constantin Apetrei, Determination of L-phenylalanine with polypyrrole sensors doped with different anions, Research and Innovation Salon, UGALINVENT, Edition IV, Book of abstracts, pp. 116, Galați, România, 16-18 October 2019, **International Conference** - **poster**.

- **2020**

Ancuţa Dinu, Constantin Apetrei, Voltamperometric Sensors for Detection of the Amino Acid Phenylalanine, National online conference of Byophysics – CNB, Book of abstracts, pp. 66, Brașov, România, June 14 – 16, 2020, **National Conference** - **poster**;

Ancuţa Dinu, Constantin Apetrei, Electrochemical Sensor Modified with Cobalt Phthalocyanine for Voltammetric Determination of Phenylalanine, International online Conference - 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 116, Galați, România, June 18-19, 2020, **International Conference - oral presentation**;

Ancuţa Dinu, Constantin Apetrei, Indirect voltammetric detection of acetylsalicylic acid with carbon paste electrodes, 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 262, Galați, România, June 18-19, 2020, **International Conference** - **poster**;

Ancuţa Dinu, Dorin Dascalescu, Irina Georgiana Munteanu, Alexandra Virginia Bounegru, Ramona-Oana Rosca, Constantin Apetrei, Electrochemical sensors based on nanomaterials employed in water analysis, 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 258, Galați, România, June 18-19, 2020, **International Conference** - **poster**;

Ancuţa Dinu, Constantin Apetrei, Sensitive properties of screen printed carbon electrode modified with Meldola/s Blue for voltammetric detection of Phenylalanine, The 5th International Conference „New Trends on Sensing - Monitoring - Telediagnosis for Life Sciences NT-SMT-LS 2020", Book of abstracts, pp. 103, Braşov (București), România, July 3 – 4, 2020, **International Conference - oral presentation**;

Ancuța Dinu, Constantin Apetrei, Sensitive proprieties of screen-printed carbon electrode modified with polypyrrole and various doppind agents for the voltammetric detection of different amino-acids, UGALMAT, International Conference on Materials Science & Engineering, Book of

abstracts, pp. 25, December 8-9, 2020, Galați, România, **International Conference - oral presentation**.

- **2021**

Ancuța Dinu, Constantin Apetrei, Electrochemical Sensors and Biosensors Modified with Polypyrrole for the Detection of the Amino-acids L-Phenylalanine and L-Tyrosine, 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 111, Galați, România, June 10-11, 2021, **International Conference** - **online - oral presentation**;

Ancuța Dinu, Constantin Apetrei, Development of a Novel Biosensor Based on Laccase/Polypyrrole/Screen-Printed Electrode for Detection of L-Tyrosine in Pharmaceutics, 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 278, Galați, România, June 10-11, 2021, **International Conference** - **online** - **poster**;

Ancuța Dinu, Constantin Apetrei, Dorin Dăscălescu, Irina-Georgiana Munteanu (Bulgaru), Ramona-Oana Roșca (Gunache), Detection of Amino Acids L-Phenylalanine, L-Tyrosine and L-Tryptophan with Biosensors based on Polypyrrole, Exploratory Workshp NeXT-Chem III, Book of abstracts, pp. 11, May 27-28, 2021, București, România, **International Conference** - **online - oral presentation**;

Ancuța Dinu, Constantin Apetrei, Comparative study of two sensors performances regarding the detection of L-Phenylalanine and L-Tyrosine, 16th International Conference on European Integration - Realities and Perspectives, Danubius University , May 14-15, 2021, Galați, România, **International Conference** - **online** - **oral presentation**;

Ancuța Dinu, Constantin Apetrei, Versatile electrochemical devices for L-Tyrosine amino acid detection, 16th International Conference on European Integration - Realities and Perspectives, May 14-15, 2021, Galați, România, **International Conference** - **online** - **oral presentation**;

Ancuța Dinu, Constantin Apetrei, Electrochemical biosensors based on polypyrrole and laccase for the detection of L-Tyrosine in pharmaceutical products, Poster, The 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, July 1-15, 2021, online chemosensors mdpi - 10.3390/CSAC2021-10626 (registering DOI), https://sciforum.net/paper/view/10626, **International Conference** - **online** - **poster**.

- **2022**

Ancuța Dinu, Constantin Apetrei, Molecularly imprinted polymers based electrochemical sensor for quantification of amino acids, International Conference on Contemporary Scientific and Technological Aspects towards an Entrepreneurial Approach, Danubius University, 25 februarie, 2022, Galați, România, **International Conference** - **online** - **oral presentation**;

Ancuța Dinu, Constantin Apetrei, Enzymes involved in the development of electrochemical biosensors for amino acid detection: phenylalanine, tyrosine, tryptophan, International Conference on Contemporary Scientific and Technological Aspects towards an Entrepreneurial Approach, Danubius University, 25 February, 2022, Galați, România, **International Conference** - **online** - **oral presentation**;

Ancuța Dinu, Constantin Apetrei, Modern Alternatives for Depression Monitoring by Measuring the Concentration of Amino Acids in Biological Fluids, European Conference of Psychiatry and Mental Health "Galatia 2022", 23th-27th March, Galați 2022, România, **International Conference - Poster**;

Ancuța Dinu, Constantin Apetrei, Sensitivity, selectivity, precision and accuracy of the new devices designed to prevent post-COVID 19 depression and neuropsychiatric disorders caused by war, International online Conference - 10th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, Galați, România, 9th-10th June, 2022, **International Conference** - **online** - **oral presentation**.

XI.3. Awarding research results

- **2018**

First Prize - Ancuţa Dinu, Constantin Apetrei**,** Dezvoltarea unor senzori pe bază de polianilină pentru determinarea acidului ascorbic din produse farmaceutice, Sesiunea de Comunicări Ştiinţifice Studenţeşti – Section „Chemistry in the Service of Humanity", 17 May 2018, Galaţi, România;

Second Prize - Ancuţa Dinu, Constantin Apetrei, Voltammetric Study of Phenylalanine by Means of Sensors Based on Polypyrrole Doped with Different Anions, Scientific Conference of Doctoral Schools SCDS-UDJG 2018 The Sixth Edition, Book of abstracts, pp 83, Galați, România, June 7th-8th of 2018, **International Conference - oral presentation**.

- **2020**

Third Prize - Ancuţa Dinu, Constantin Apetrei, Electrochemical Sensor Modified with Cobalt Phthalocyanine for Voltammetric Determination of Phenylalanine, International online Conference - 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 116, Galați, România, June 18-19, 2020, **International Conference - oral presentation**.

The most active team of students **Prize-** International online Summer School Food Safety and Healthy Living FSHL – July 05 – 08, Brașov (București), 2020, România

- **2021**

Third Prize - Ancuța Dinu, Constantin Apetrei, Electrochemical Sensors and Biosensors Modified with Polypyrrole for the Detection of the Amino-acids L-Phenylalanine and L-Tyrosine, 8th Edition of Scientific Conference organized by the Doctoral Schools of „Dunărea de Jos" University of Galaţi, Book of abstracts, pp. 111, Galați, România, June 10-11, 2021, **International Conference** - **online - oral presentation**;

Third Prize GALA CEREX IOSUD UDJG (Gala Cercetării de Excelență - Premierea rezultatelor cercetării științifice doctorale - Universitatea „Dunărea de Jos" din Galați) - Dinu, A.; Apetrei, C., Development of Polypyrrole Modified Screen-Printed Carbon Electrode Based Sensors for Determination of L-Tyrosine in Pharmaceutical Products. International Journal Molecular Sciences 2021, 22(14), 7528, https://doi.org/10.3390/ijms22147528, Factor de impact 5.924, **Articol**;

First Mention - Ancuța Dinu, Constantin Apetrei, Dorin Dăscălescu, Irina-Georgiana Munteanu (Bulgaru), Ramona-Oana Roșca (Gunache), Detection of Amino Acids L-Phenylalanine, L-Tyrosine and L-Tryptophan with Biosensors based on Polypyrrole, Exploratory Workshp NeXT-Chem III, Book of abstracts, pp. 11, May 27-28, 2021, București, România, **International Conference** - **online - oral presentation**.

XI.5. Related activities carried out within the individual program of doctoral university studies

- **Participation** in the FSHL International School of Food Safety and Healthy Living - July 5 - 8, Brașov (Bucharest), **2020,** Romania;
- **Participation** in Scientific Communication Sessions for students - **2018;**
- **Support of laboratories in the disciplines:** Chemistry, Catalysis (under the guidance of Professor Dr. Habil Constantin Apetrei), Chemical Pollution and Analysis of Environmental Samples, Pollution and Environmental Protection (under the guidance of Lect.dr. Mihaela Timofti), Analytical Chemistry (under the guidance Professor Maria Cioroi and Associate Professor Simona Ștefan, Therapeutic Chemistry (under the guidance of Professor Oana Dragostin and Associate Professor Elena Lisă) - **2017-2022;**
- **Member of the target group** of the Project *ANTREPRENORDOC, in the framework of Human Resources Development Operational Programme 2014-2020, financed from the European Social Fund under the contract number 36355/23.05.2019 HRD OP /380/6/13 – SMIS Code: 123847* - **2021**
- **Member of the organizing committee** within the Doctoral Schools Conference "Perspectives and challenges in doctoral research", Chemistry - Electrochemistry in Life Sciences Section, Galaţi **- 2020,2021,2022**

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