TURKISH REPUBLIC

ANKARA UNIVERSITY

GRADUATE SCHOOL OF HEALTH SCIENCES

DEVELOPMENT OF ELECTROCHEMICAL SENSOR FOR DETERMINATION OF TYROSINE

Marwah Naser

DEPARTMENT OF ANALYTICAL CHEMISTRY

MASTER'S THESIS

SUPERVISOR

Prof. Dr. Nevin ERK

ANKARA

2023

TURKISH REPUBLIC
ANKARA UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

DEVELOPMENT OF ELECTROCHEMICAL SENSOR FOR DETERMINATION OF TYROSINE

Marwah Naser

DEPARTMENT OF ANALYTICAL CHEMISTRY

MASTER'S THESIS

SUPERVISOR

Prof. Dr. Nevin ERK

ANKARA

2023

Statement

Ankara University

Ankara University Institute of Health Sciences

I hereby declare truthfully that this thesis is my original work; that I followed scientific and ethical principles and rules throughout the stages of preparation, data collection, analysis, and presentation of my work; and that I cited the sources of all data and information obtained within the scope of this study and included these sources in the references section; and that this study has been scanned for plagiarism with Turnitin program used by Ankara University, and that it does not have any plagiarism whatsoever.

Name and surname of student: Marwah Naser

Date: 26/7/2023

Signature:

LIST OF CONTENTS

List of Abbreviations

Tyro	Tyrosine
ClC_4/TiO_2	Chloro-tetracarbon/Titanium dioxide
GCE	Glassy carbon electrode
XRD	X-ray diffraction
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
EDAX	Energy dispersive analysis of X-ray
EIS	Electrochemical impedance spectroscopy
CV	Cyclic voltammetry
DPV	Differential pulse voltammetry
LOD	Limit of detection
HPLC	High-performance liquid chromatography
HCL	Hydrochloric acid
NaOH	Sodium hydroxide
Na_2PO_4	Disodium phosphate
$K_3Fe\ (CN)_6$	Potassium hexacyanoferrate (III)
α	Electron transfers coefficient
B-R	Britton-Robinson buffer
ΔEp	Peak-Peak separation
KCL	Potassium chloride
PBS	Phosphate buffer saline
AAS	Amino acids
AAO	Amino acid oxidase

List of Figures

LIST OF TABLES

1. INTRODUCTION

The monomeric building blocks of proteins, other substances of biological origin, and amino acids are chiral compounds with low molecular weight and have a variety of unique properties that depend on variations in their side chains (Casettari et al., 2012). Therefore, the concept of determining dietary amino acids' bioavailability is essential to the study of nutrition. It is essential to precisely measure the dietary supply of "available" amino acids in relation to dietary requirements for nutrition and also in conditions when dietary amino acid availability can significantly limit protein metabolism (in the case of the elderly, infancy, and immune-compromised patients) (Moughan, 2003). Since amino acids play such vital roles in the healthy organism and play such a significant role in terms of the body's structure and function in both maintaining good health and disease. Such a test might be used to discover aspects about the patient's nutritional and metabolic status and the impacts of stress, trauma, and other therapeutic measures (drugs, etc), as well as nutritional supplementation (Chaitow,1988). The current research was carried out for the removal of the factors of various disorders and forms of depression, as well as for the prevention of various genetic “metabolic diseases” such as "phenylketonuria" (PKU), "alkaptonuria", and "Parkinson's disease". Moreover, the analysis of amino acids (AAs), particularly phenylalanine (Mohamed et al.), Tyrosine (Tyro), and tryptophan (Trypt), which are essential for maintaining the balance of the nervous system, was done in order to detect their deficiency or excess and to treat them appropriately, in due time (Dinu and Apetrei, 2022).

Amino acids are oxidatively converted to α-keto acids that are catalyzed by amino acid oxidase (AAO) which are flavoenzymes that also generate ammonia and hydrogen peroxide (Kasai et al., 2015). Based on the isomer of the amino acid that is utilized as a substrate, it is essential to distinguish between L- and D-amino acid oxidases (Campillo-Brocal et al., 2015). However, the L-amino acid oxidases are of particular interest since amino acid oxidase are extremely specific for L-amino acids, and the best substrates are often hydrophobic amino acids (Tan and Fung, 2008). The ability of the brain neurons to manufacture and utilize a number of neurotransmitters, such as serotonin, acetylcholine,

catecholamine, dopamine and norepinephrine, is dependent upon the concentration of both the amino acid and choline in the blood stream. Serotonin, dopamine, norepinephrine, and epinephrine; are produced in the brain from aromatic amino acids (Fernstrom and Fernstrom, 2007). Tyrosine is an aromatic amino acid that can retain the body's nutritional balance and functions as a precursor for a number of neurotransmitters, including thyroxine, dopamine, noradrenaline, adrenaline, and L-dopa (Feng et al., 2021).

It is described that the abnormal Tyrosine level is significantly associated with several human disorders. Tyrosine deficiency can result in hypochondrium, depression, and albinism alkaptonuria while elevated of Tyro level can cause "dementia" or "Parkinson's disease" and an increase in "sister chromatid exchange" (Tashkhourian et al., 2016). Tyrosine is recently the most effective medication for treating Parkinson's disease which is caused by a decrease in the dopamine action and generation by the brain's dopaminergic neurons (Lütke-Eversloh et al., 2007). Phenylketonuria is a condition characterized by a deficiency in Tyro, and it seems entirely plausible. Tyro supplementation would support individuals with this disorder, as it is thought that supplementation will specifically treat a deficiency of Tyro that restricts neurotransmitter production (Jongkees et al., 2015).

Therefore, an accurate and cost- effective quantitative assessment of Tyro in biological fluids from a validated analytical process is necessary to control its concentration. Electrochemical technique have been widely used to determine drug and amino acid in pharmaceutical dosage forms and biological samples, electrochemical techniques can be used to determine L-Tyrosine because it is an electrochemically active compound (Gu et al., 2015). To date, several analytical approaches for determining Tyro in biological or pharmaceutical samples have been reported, including high-performance liquid chromatography (HPLC) (Neurauter et al., 2013), chemiluminescence (Li et al., 2015), fluorometric method (Guo et al., 2020), "capillary electrophoresis" (Sánchez-López et al., 2016), flow injection (Jarošová et al., 2016), and "gas chromatography" (Deng et al., 2002). For example, Gabriele et al. proposed a sensitive and rapid "high- performance liquid chromatographic" method with "fluorescence detection" for Tyro determination in

pharmaceutical forms and real samples. The result show that a good linearity concentration from 1.25-80μM and sensitive detection limit of 0.3μM. Moreover, Shifeng et al. demonstrated a chemiluminescence technique for determining of Tyrosine concentration and their result demonstrate that low limit of detection of Tyro of 0.0002μM, and satisfactory results. Furthermore, it is essential to remember that the majority of reported methods which use toxic chemicals and expensive analytical instruments, time-consuming, and need well-trained personnel (Noureldeen et al., 2022). Recent studies have shown considerable attraction towards the electrochemical technique due to their effectiveness, simplicity, inexpensive, ability to detect the electroactive molecules and pharmaceutical drugs rapidly, and low reagent consumption ,outstanding precision, higher sensitivity, ease of portability and selectivity (Allahverdiyeva et al., 2021).

1.1. Develop The Electrode

Electrochemical sensors are reported so far used in mostly branches of medical monitoring, industrial and environmental applications, also used to provides better data for measuring pH-value, conductivity (impedance) and the concentration of analyte. To date, many researches have been show that the utilization of glassy carbon (GC) in the application of advanced technology due to its low cost, excellent electrical conductivity, chemical stability, mechanical, and thermal properties, allowing rapid advancements in biomedical, pharmaceutical, electronic, and energy sectors (de Souza Vieira, 2022). According to many researches, there are a number of limitations to using bare electrodes for detection of organic compounds. Therefore, it has recently been proposed to use modified electrodes where several target molecules have been oxidized or reduced using a variety of substances that has been used as electron transfer mediators. Modified electrodes demonstrate significant advantages over traditional electrodes in a variety of applications, such as electrocatalysis and electrochemical methods. Therefore, the motivation of different research for improvement in the analysis using the different directions of chemically modified bare electrodes can benefit analytical applications. In certain situations, when using of glassy carbon electrode (GCE) the electrochemical signal obtained has no well-defined peaks.

Therefore, the surface of bare electrodes is modified to enhance the electrochemical responses of these groups (Kassa and Amare, 2019). Hence, the high active surface area of nanostructured electrode modifiers and their conductivities can improve analyte species adsorption kinetics, which is especially useful in enhancing current density (Sanghavi et al., 2015).

However, metallic nanoparticles (MNPs) have paid more attention to the researcher due to their variety of properties and widely used as modifying agents. These metallic nanoparticle exhibits different types of chemical and physical characteristics because of their size, high thermal and mechanical stability, large active surface area and improve electron transfer rate resulting from molecular recognition events along the entire fiber make these materials good candidates for "sensors" (George et al., 2018; Nishio et al., 2020).

Among all metal oxides, titanium dioxide (TiO_2) is an attractive electro-catalytic substance that has gained significant interest for its study and application over the years due to its low cost, non-toxicity, superior biocompatibility, stabilization of thermal catalysts, and potent adsorptive ability. TiO_2 biocompatibility is critical in medical biology, where the TiO_2 layers on Ti and Ti alloys are contact directly with hip or dental implants in surrounding biological tissue (Macak et al., 2008). Moreover, the semiconducting metal oxides were suitable substances for the conversion of solar energy, as well as TiO_2 is high semi-conductive material that has an excellent optical transmission in the visible and adjacent infrared ranges (Mehmandoust et al., 2022). Additionally, TiO_2 has demonstrated excellent activity as a catalyst for the reduction of some small "organic compounds" because it is stable in both "acidic and alkaline solutions" (He and Hu, 2011). To date, TiO_2 nanoparticles were used in wide potential windows and in the preparation of sensors, as well as improve the electrode stability and repeatability (Mao et al., 2015). The capacity of TiO_2 nanoparticles improves the electron transport on modified electrodes surfaces (Mauruto de Oliveira et al., 2017). As a result, it is a commonly utilized material to electrode modification for "electrochemical sensing" applications (Mohamed et al., 2017; Reddy et

al., 2021). Modifying the electrode surface is beneficial to achieve rapid detection with improved sensitivity and selectivity (Sohouli et al., 2020).

Tetracarbon is a synthetic form of carbon with polymeric structure that resemble biological tissues in many characteristics. "Tetracarbon" has applications in microelectronics and medicine due to its high biocompatibility. Tetracarbon is a biocompatible substrate coating that is produced by depositing short linear chains of carbon stores to the substrate's surface. The carbon chains are non-turbostratic, perpendicular to the substrate surface, and densely packed parallel to one another in hexagonal patterns. In Tetracarbon, van der Waals forces caused the interaction between film's linear carbon chains, resulting in a distance between the chains that range from 4.85 to 5.03 Å. Therefore, tetracarbon layer is similar to an adjacent layer and is irregularly displaced laterally with respect to the other layers. The Tetracarbon structure may "self-organize" in vivo, structurally adjusting to adapt to the structure of a protein molecule developing on and intimately into the Tetracarbon layer as a result of the interaction between the film and the protein's penetration of endogenous ions into the layer (Guseva et al., 2002). Moreover, Chloride is an anion Cl^- that is essential in maintaining the acid/base balance of the body. Chloride can be oxidized but not reduced and is soluble in an aqueous solution.

As a result, the electrochemical approach can be used to detect Tyro. The fundamental purpose of this work is to perform Tyro's "oxidation" and improve a novel "electrochemical" approach for rapid and precise Tyro detection. Recently, we have developed sensitive ClC_4/TiO_2/GCE nanocomposites-based electrochemical sensor toward the voltametric Tyro detection was investigated by DPV approach which achieve wide dynamic linear range and low limit of LOD. The newly developed electrochemical sensor has been used to accurately and sensitively measure the low Tyro concentration in real samples.

1.2. Electrochemical Sensor

Analytical chemistry's developments illustrated that electrochemical sensors offer considerable interest and a rapidly developing class of chemical sensors. A chemical sensor is analytical instrument that can provide detailed information about the environment's chemical composition. However, a chemical sensor's response is directly proportion with the amount of certain chemical species. Hence, all chemical sensors have a signal transducer that converts the analyte's response into signal information on advanced instruments and a chemically selective layer that separates the analyte's response from its environment as shown in figure 1.1.

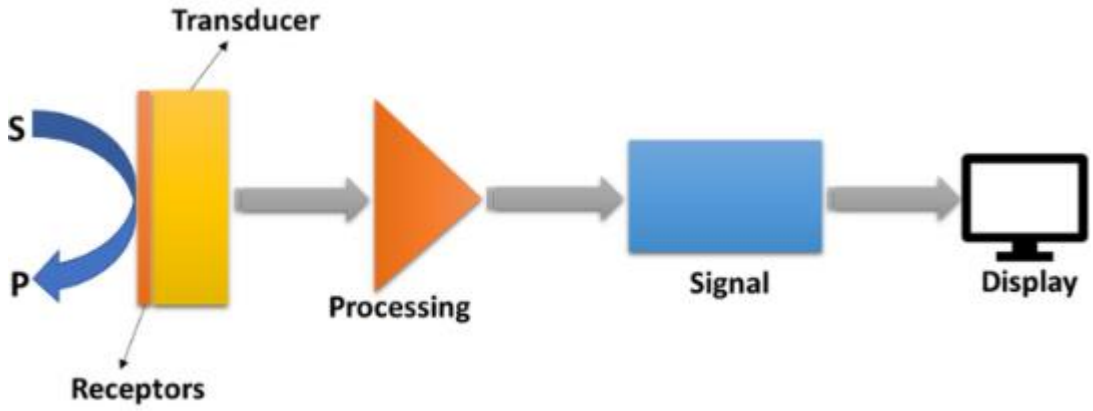

Figure 1.1. A schematic illustration of the main components of a standard sensor (Baranwal et al., 2022).

Electrochemical sensors have been paid more attention due to low cost, their remarkable detectability, and simple operation. The application of electrochemical sensors which offered in a variety of fields including: industrial, environmental, and drug analysis (Stradiotto et al., 2003). The electrochemical techniques are based on the desired analyte and electrode design for use in material detection. They are essential in determining the sensor's sensitivity and the limit of detection (Abo-Hamad et al., 2016). "Electrochemical sensors" are based upon "potentiometric", "amperometric", or "conductivity" measurements.

1.3. Several Electrochemical Approaches

1.3.1. Potentiometric Sensors

Potentiometric sensors are significant class of electrochemical sensor, which determine the relation of the analyte species activity with the potential response with the two-electrode system (Yin and Qin, 2013). Ion-selective electrodes(ISEs) measure the differences in potential between the working electrode (which is usually ion-selective) and reference electrode under experimental conditions without current flow (Isildak and Özbek, 2021).

1.3.2. Amperometric Sensors

Amperometric sensor is an electroanalytical technique consists of a chemical or biological material and the electrode which transfer the analytical signal from the sensor event to an electronic circuit. Moreover, Amperometric sensors' main function is the reporting and monitoring of the electrochemical analytes by measuring the current changes and efficient analyte detection. During the sensing event, signal transduction is achieved by controlling the transducer's potential and monitoring the current as time variable (Dey et al., 2013).

1.3.3. Conductometric Sensor

Conductometric sensor is composed of electrodes separated by a semi-conductive channel. Therefore, the conductivity of the conductometric sensor gradually rises as the environment's relative humidity (%RH) rises. In particular, there are demanding requirement that must be dictated when choosing materials for applications requiring humidity sensing

including hydrophilicity, outstanding porosity, and the difficulty of dissolving sensing layer in water. To date, ultra-sensitive biologically active chemical sensors and conducting polymers based robust have also been established for application in environmental analysis, food and beverage production, and biomedical diagnostics (Akram et al., 2021).

1.3.4. Voltametric Sensors

Voltammetric sensors are easy-to-use, inexpensive, and powerful instruments that have been widely utilized in electrochemical research to examine the characteristics of the electroactive species. There are three essential components of an analytical electrochemistry system: an "electrochemical sensing device", an "electrochemical detecting instrument", and an electrolyte. However, three electrodes were typically used in an electrochemical detection instrument: a "working electrode (WE)", a "reference electrode (RE)", and a "counter electrode (CE)". After surface modification of the working electrode using various materials, they can be utilized for various kinds of metal ions in specific detection. Therefore, distinct types of voltammetry include "differential pulse voltammetry (DPV)", "linear sweep voltammetry (LSV)", "square wave voltammetry (SWV)", and "cyclic voltammetry (CV)". The time waveforms obtained by each function application distinguish these strategies from one another.

1.4. Electrochemical Cell

Three-electrode cells (Fig. 1.2) are widely applied in voltametric experiments with controlled potentials. The cell usually consists of covered 5-50 mL beaker with three electrodes system: "working", "reference", and "auxiliary" electrode that immerse in the sample solution. Therefore, "working electrode" is the electrode where the reaction of interest happens, meanwhile the "reference electrode" produces stable and reproducible potentials which are used to compare the working electrode potentials. This 'buffering'

versus potential changes is accomplished by having a stable configuration of both redox forms, such as Ag/AgCl or Hg/Hg_2Cl_2, is the case with extensively used saturated calomel electrodes and silver chloride electrodes. Hence, the reference electrode can also be isolated from the sample by an intermediary bridge to reduce contamination of the sample solution. Platinum wire or graphite rods are inert conductive substances that used as the current-carrying auxiliary electrode (Wang, 2006).

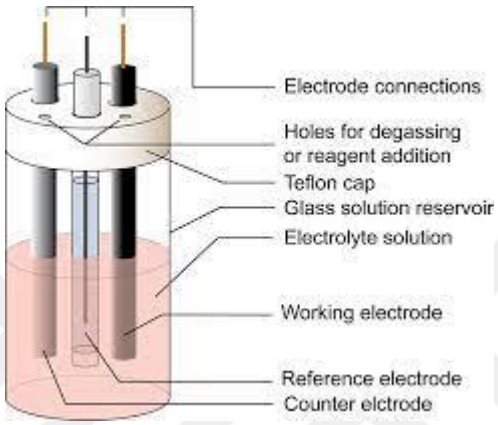

Figure 1.2. Schematic representation of an "electrochemical cell for CV experiments" (Elgrishi et al., 2018).

1.5. Solvents Used in Voltammetry.

Electroanalytical investigations are usually performed in a medium consisting of a support electrolyte and solvent. Herein, choices of the solvents mainly determined by analyte solubility, solvent's redox activity, as well as solvent characteristics include electrochemical activity and electrical conductivity. Therefore, Non-aqueous solvents, such as acetonitrile, dimethyl sulfoxide (DMSO), propylene carbonate, dimethylformamide (DMF), or methanol, have also been used extensively as solvents, Although the water was applied as a solvent more commonly than any other medium. It is essential to use supporting electrolytes in experiments with controlled potentials in order to reduce solution resistance,

and to maintain ionic strength constant. The selectivity of the voltammetric measurements may be affected depending on the electrolyte's composition. The Supporting electrolyte must not oxidize or reduce rapid (Wang, 2006).

1.6. Glassy Carbon Electrode (GCE)

The glassy carbon electrodes (Fig. 1.3) are very popular due to their outstanding electrical and mechanical properties, chemically inert, broad potential window, and relatively reproducible performance. Therefore, to produce repeatable active glassy carbon electrodes surface pretreatment is commonly applied to improve the analytical performance. This pre-treatment is often accomplished by polishing the surface with 0.05 µm alumina particles to obtain shiny appearance and mirror-like surface. After that, the electrode must be washed with deionized water before using it. Performance has also been enhanced by adding activation steps, such as electrochemical, thermal, or laser treatment (Wang, 2006).

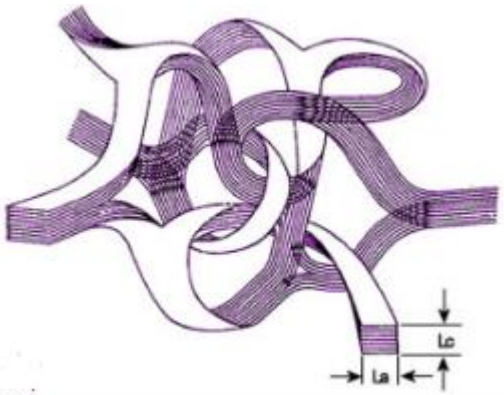

Figure 1.3. The glassy carbon electrode's amorphous structure (Wang, 2006).

2. MATERIAL and METHOD

2.1. Voltammetry

Voltammetry, a family of electroanalytical techniques, is based on electrolytic reduction or oxidation mainly for the analyte detection or investigation of mechanism and kinetics of redox reactions (Bard and Faulkner, 2001). It measures current as a function potential. Initially, Jaroslav Heyrovsky introduced the first voltammetric technique in the early 1920s for which uses only two electrodes. However, modern voltammetry uses a three-electrode system. Results for a voltammetric experiment are presented by a voltammogram which is the based on the current generated by the analyte versus applied potential (Bard and Faulkner, 2001).

2.1.1. "Cyclic Voltammetry"

"Cyclic voltammetry" is the approach that is most frequently utilized to obtain qualitative electrochemical reactions. In many electroanalytical studies, the first experiment is cyclic voltammetry. It allows for a quick determination of "electroactive species" of redox potentials and rapid assessment the influence of media on the "redox process".

In "cyclic voltammetry", the potential of a stationary "working electrode" is linearly scanned with a triangular potential waveform (Fig. 2.1). Single or several cycle might be employed depending on the information desired. Acyclic voltammogram is the current - potential graphic that results.

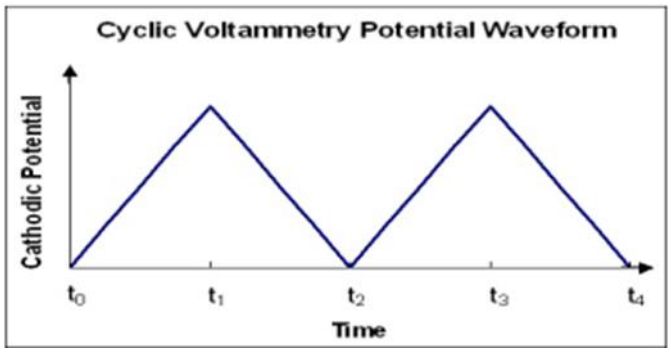

Figure 2.1. Excitation signal at potential time in a cyclic voltammetric experiment (Wang, 2006).

As despite in Fig. 2.2 a "reversible redox" couple predicted reaction throughout a single potential cycle. At first, the oxidized form O is only thought to be detectable. Moreover, during the first half of the cycle, a negative going "potential scan" is picked, starting at a value in which no reductions happen. As a result, the cathodic current increases as the applied voltage approaches the redox process "characteristic E°", and until a peak is attained. The direction of the potential sweep is reversed after t reaching the potential area where the "reduction process (at least 90/n mV beyond the peak)". During the reverse scan, R molecules collected near the surface during the forward half-cycle are re-oxidized back to O, resulting in an "anodic peak" (Wang, 2006).

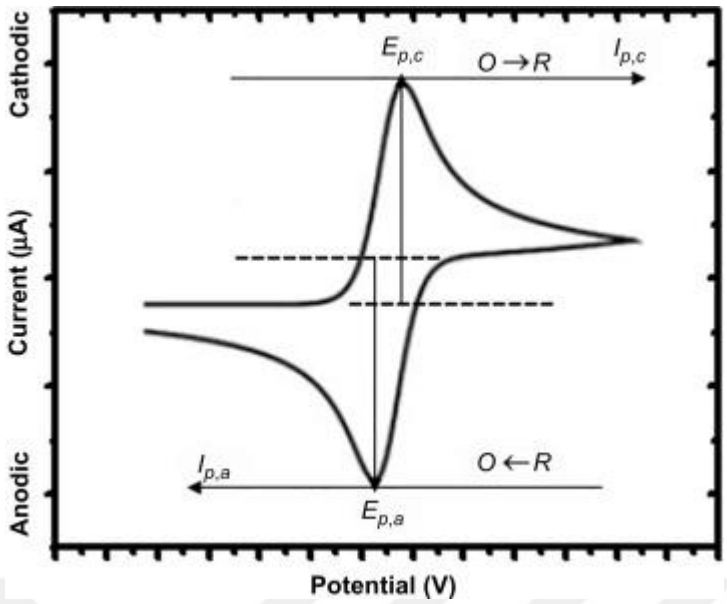

Figure 2.2. A typical "cyclic voltammogram" for a diffusion-controlled, electrochemically "reversible redox process"(Wang, 2006).

2.1.2. "Differential Pulse Voltammetry"

"Differential pulse voltammetry" is a helpful approach for assessing trace amount of various analyte. Fixed magnitude pulses overlaid on "linear potential ramp" are supplied to the "working electrode" right before the end of the drop in differential-pulse voltammetry (Fig. 2.3). The applied potential is plotted against the current difference, which effectively subtracts the first current from the second. The differential pulse voltammogram that result is mainly composed of the current peaks, in which the concentration of aligned analyte is directly proportional to height' of the peak current (Wang, 2006).

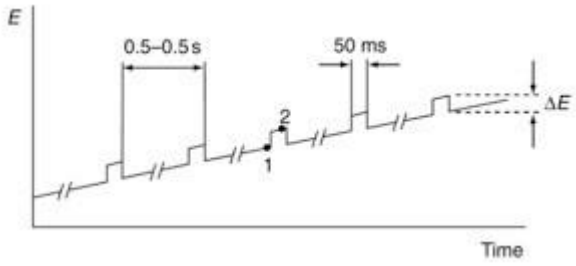

Figure 2.3. Excitation signal for differential -pulse voltammetry (Wang, 2006).

2.1.3. "Impedance Spectroscopy"

"Impedance spectroscopy" is a useful tool for examining the characteristics of chemically "developed electrodes" and determining the rates of electrochemical reaction. When a current runs through a circuit containing resistors, capacitors, and inductors, it encounters impedance, which is a complicated resistance (Fig. 2.4). Equivalent electronic circuits components which match the observed impedance spectra can be used to describe electrochemical processes at the electrode-solution interface. The Randles and Ershler electrical equivalent-circuit model are useful for modeling interfacial phenomena (Wang, 2006).

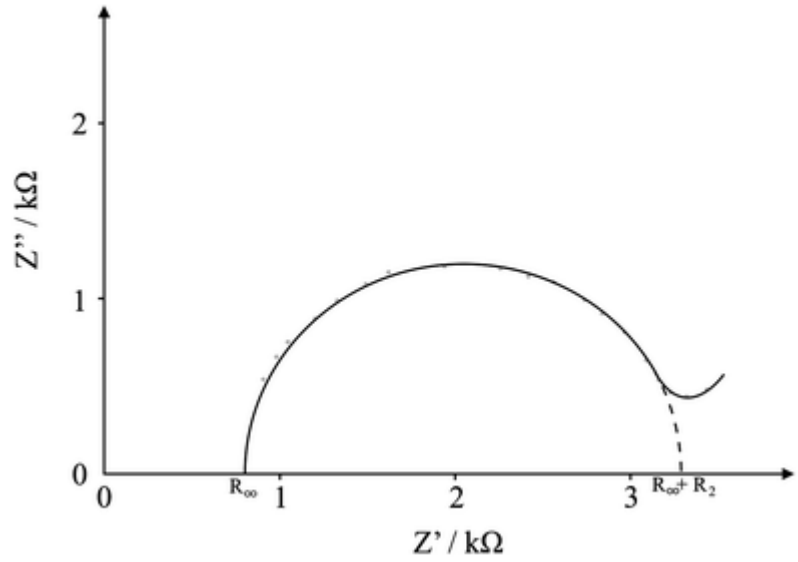

Figure 2.4. The "Nyquist plot" shows the frequency-dependent "impedance characteristics of a Zn (Hg)/Zn^{2+} couple" (Randviir and Banks, 2013).

2.2. Aim of the Thesis

Electrochemical applications are of increasing importance as the modification of electrode surfaces provides more selective and sensitive surfaces in addition to their increased catalytic activation. Electrochemical techniques provide a practical tool for the determination of amino acid Tyrosine in pharmaceutical forms and real samples accomplished by modification of the electrode surface using nanocomposite $ClC_4/TiO2$ under optimized experimental conditions.

3. EXPERIMENTAL and METHODS

Any electrochemical investigation performance is heavily reliant on the working electrode. The electrode material will affect the signal-to-noise ratio, repeatability, redox behavior, charge transfer kinetics, and potential window. Moreover, the shape of the electrode can greatly affect the diffusion of the component on the surface of the electrode.

3.1. Devices

3.1.1. Potentiostat/Galvanostat Device

"Metrohm Autolab potentiostat/galvanostat system (PGSTAT128N, Metrohm, N Herisau, Sweden, 2.1.1 software version)" used to perform the "electrochemical methods" such as "DPV", "CV", and "EIS". The experiments were performed with an electrochemical cell containing glassy carbon electrode.

3.1.2. Characterization Devices

SEM and EDX images were recorded in material microscopy by "Zeiss Gemini SEM 560 at 3.00 KV". "Fourier transform infrared (FT-IR)" spectra were observed on a "Perkin Elmer spectrometer". The "X-ray diffraction" pattern was observed using a "Rigaku smart Laboratory diffractometer (operating at 40 KV, 20 MA)" at a wavelength of 1.540 A^0.

3.1.3. Peripheral Devices

To mix and dissolve the material, an ultrasonic bath (ISOLAB type) was employed. The preparation of the solutions was done using "deionized water (model Sartorius Arium Comfortl-1-UV-T)". For mixing solutions, a "magnetic stirrer with heater (Velp Scientifica type)" was employed. An analytical "electronic sensitive balance (OHAUS model)" was used for the weighing process. The pH of the buffers has been observed using a sensitive "pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA)", and the pH solution was adjusting with 0.1 M NaOH and HCI.

3.2. Chemicals

Tyrosine was purchased from Sigma-Aldrich (Germany). phosphoric acid (H_3PO_4), "hydrochloric acid (HCI)", sodium hydroxide (NaOH), and sodium borohydride ($NaBH_4$) purchased From Merck (Darmstadt, Germany). "Potassium (III) hexacyanoferate ($K_3Fe(CN)_6$), potassium (II) hexacyanoferate ($K_4Fe(CN)_6$) were obtained, L-cysteine, L-arginine, L-alanine, glucose, and uric acid from Sigma-Aldrich (Germany)". Human blood plasma specimens were acquired from Sera-Flex Inc.

3.3. Solution Used

3.3.1. Preparation of Supporting Electrolytes

3.3.1.1. Preparation of Acetate Buffer

First, 136.0 g "sodium acetate" and 77.0 g "ammonium acetate" were dissolved in water and diluted to 1000.0 ml. Then 250.0 mL of glacial acid was added and mixed.

3.3.1.2. Preparation of Phosphate Buffer Saline

8.95 grams of "disodium hydrogen phosphate R" and "3.40 g of potassium dihydrogen phosphate" were dissolved in water and diluted to 1000mL. Buffer pH was adjusted using phosphoric acid.

3.3.1.3. Preparation of Phosphoric acid Buffer

16.6 gr of phosphoric acid was dissolved in 100.0 mL of water. pH value was adjusting using 1.0 M hydrochloride and sodium hydroxide.

3.3.1.4. Preparation of Britton-Robinson (B-R) Buffer

"Boric acid, phosphoric acid, potassium chloride, and acetic acid" solutions were used to make the supporting electrolytes for the Britton-Robinson buffer, and "2.0 M NaOH and 2.0 M HCl" were used to modify the pH levels.

3.3.1.5. Preparation of Potassium Hydrochloride Buffer

0.74 gr of potassium chloride was dissolved in 100.0 mL of water. The buffer's pH was adjusting by using 1.0 M hydrochloride and sodium hydroxide.

3.3.2. Real Samples Preparation

Tyro determinations in human plasma, soybeans, and milk products were accomplished to determine the modified electrode's applicability in real samples. Firstly, fresh milk was purchased from the local market, and then 5.0 mL of liquid milk was combined with certain amount of 0.1 M of "hydrochloric acid" in a vortex mixer for 5 minutes. Afterward, the resultant solution was centrifuged at speed of 5000 rpm for 15 min to obtain the supernatant. Soyabean purchased from local market and grinding to fine powder, then sieved with a sieve of 100–200 mesh. Therefore, 0.1g of soyabean specimens were dissolved in 4 mL "methanol" and extracted by ultrasonic for 30 min at room temperature. After that, the prepared mixture has been "centrifuged for 15 min at speed of 4000 rpm" and the supernatant was obtained and filtered using a 0.45 µm filter membrane. Moreover, for preparation of human plasma, a certain amount of Tyro was added to "1.0 mL of human plasma" that was treated with "1.0 mL of acetonitrile" in order to prepare the plasma sample and to precipitate the proteins. Then, the plasma proteins that have been precipitated were centrifuged for ten minutes at 5,000 rpm. Finally, the prepared human plasma solution was diluted using PBS buffer (pH =3) and used as the sample solution in the next steps.

3.3.3. Preparation of ClC_4/TiO_2/GCE

Firstly, the "GC" electrode surface was rinsed with "alumina slurry" (0.05 µm) to obtain mirrored-like surface, after polishing the electrode surface it was ultrasonicated with ethanol and deionized "water solution 1:1(v/v) for 30 minutes". Then, "1.0 mg of ClC_4/TiO_2 nanocomposite" were dissolved in 1.0 ml of "ultra-pure water" and ultrasonically agitated in water for 30 min. Afterward, modify the clean electrode surface by dropping 6.0 µL of prepared ClC_4/TiO_2 nanocomposite then the surface of modified electrode has been dried at 25 °C at room temperature. Furthermore, non-attached composites were removed from the electrode's surface by rinsing it with ultrapure water.

3.4. Determination Procedure

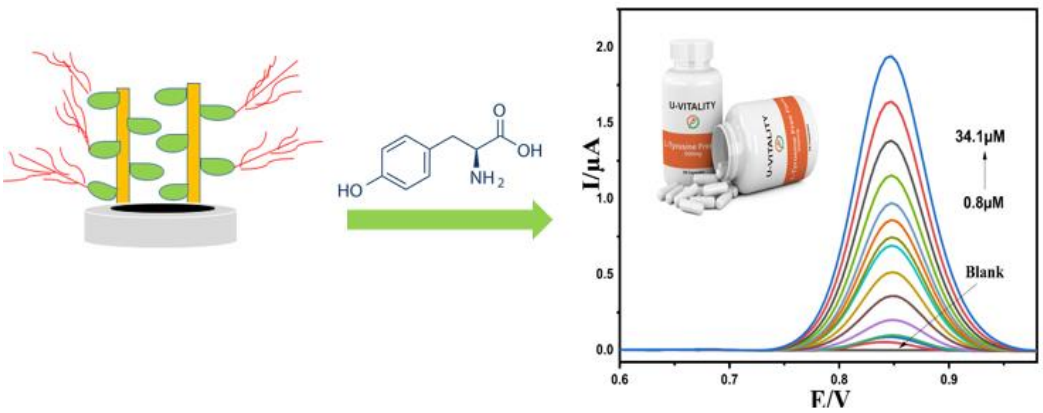

Figure 3.1. The graphical abstract of ClC_4/TiO_2/GCE fabrication.

4. DISCUSSION

Tyrosine is a type of essential amino acid that humans require for the establishment and maintenance of a positive nitrogen balance. It is also an essential constituent of proteins, which are required for human nutrition. Due to its presence in vegetables, it is sometimes incorporated into dietary, food, and medicinal formulations. Therefore, development of a reliable, sensitive and rapid approach for the detection of Tyro in actual samples with maximum therapeutic efficacy and minimal toxicity. To date, various methods have been used for detection of Tyro in therapeutic dosage forms and biological samples. For example, Gabriele et al. proposed a rapid and sensitive "high- performance liquid chromatography" method with "fluorescence detection" for Tyro determination in pharmaceutical form and real samples. The result show that a good linearity concentration from of 1.25-80μM and sensitive detection limit of 0.3μM. Moreover, Shifeng et al. demonstrate a "chemiluminescence technique" for determining of Tyrosine concentration and their result demonstrate that low limit of detection of Tyro of 0.0002μM , and satisfactory results. Furthermore, it is essential to remember that the majority of published methods which use toxic chemicals and expensive analytical instruments, time-consuming, and need well-trained personnel. Recent studies have shown considerable attraction towards the "electrochemical techniques" due to their effectiveness, simplicity, inexpensive, ability to detect the electroactive molecule and pharmaceutical drugs rapidly, low reagent consumption, outstanding precision, higher sensitivity, ease portability and selectivity.

5. RESULTS

5.1. Characterization of ClC_4/TiO_2

5.1.1. SEM Analysis

The structure and morphology of ClC_4/TiO_2 were characterized by "SEM". The "FE-SEM" images of ClC_4/TiO_2 were illustrated in (Fig 5.1). As depicted in Fig 5.1, the ClC_4/TiO_2 composite exhibited a stable, smooth, and homogeneous structure.

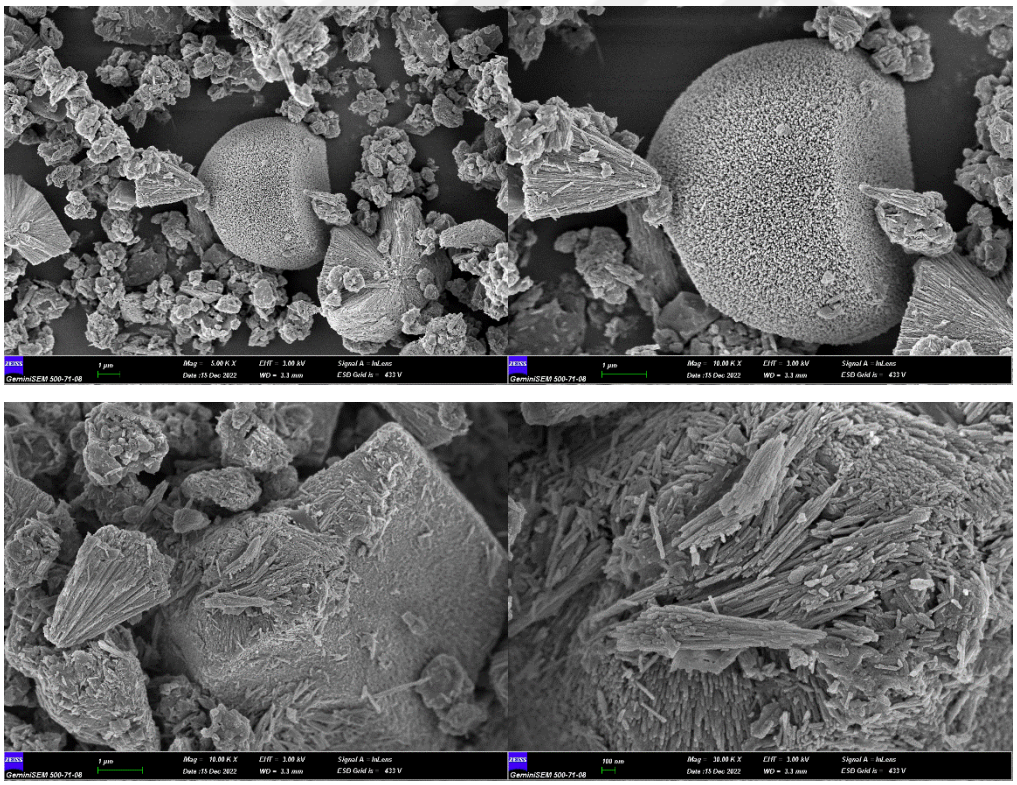

Figure 5.1. SEM images of ClC_4/TiO_2.

5.1.2. EDX Analysis

The successful formation of homogeneous "TiO_2 nanoparticles" on the surface of ClC_4/TiO_2 nanomaterials was confirmed. The weights % of the C, O, Cl, and Ti elements in the "composite materials" were determined via "SEM-EDX" analysis (Fig.5.2). The "EDX analysis of ClC_4/TiO_2" confirmed the presence of related elements.

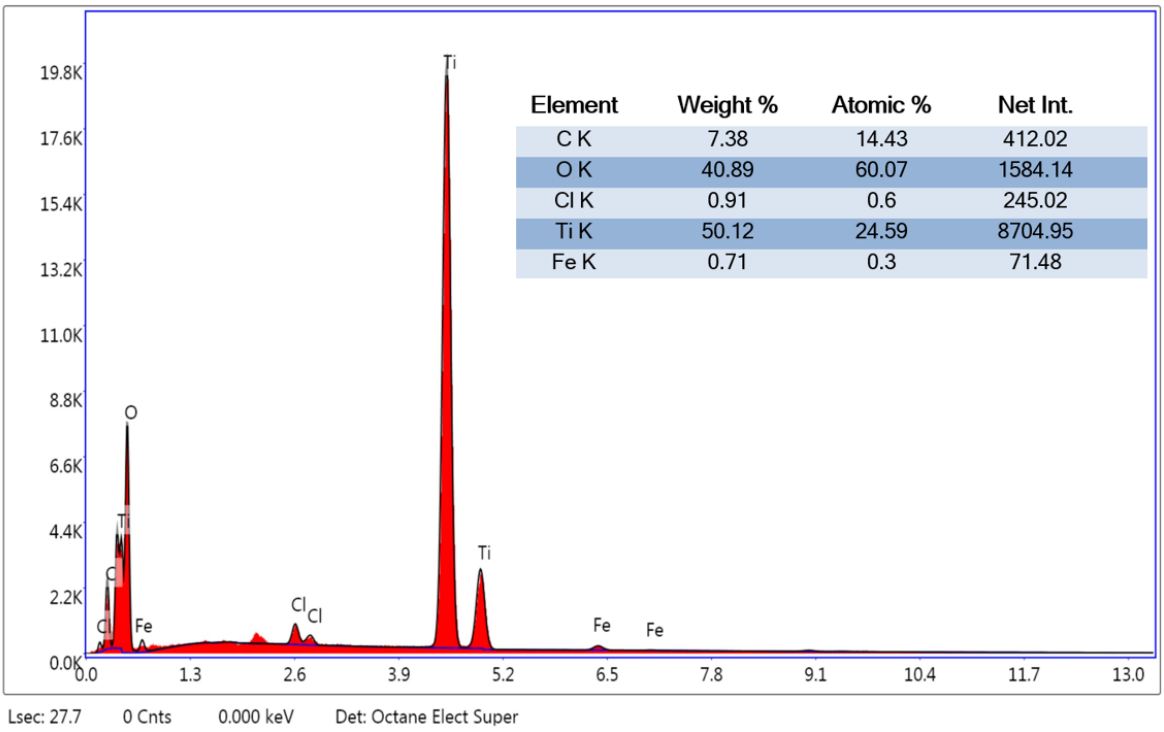

Figure 5.2. EDX of ClC_4/TiO_2.

5.1.3. XRD Analysis

The XRD spectra of synthesized TiO_2 and ClC_4 were illustrated in Fig. 5.3. The "TiO_2 was indexed as hexagonal anatase form" (ICDS: 98–015-4601) (Djerdj and Tonejc, 2006). The peak at approximately "2θ=25° was observed in Titania TA, attributed to TA′s characteristic peaks" (Mehmandoust et al., 2022). Four sharp XRD peaks corresponding to the apparent for Tetra-carbon. The tetracarbon was indexed as tetragonal carbon allotrope (Liu et al., 2018). The XRD spectra of both TiO_2 and ClC_4 confirmed no impurities remained in synthesized nanostructures.

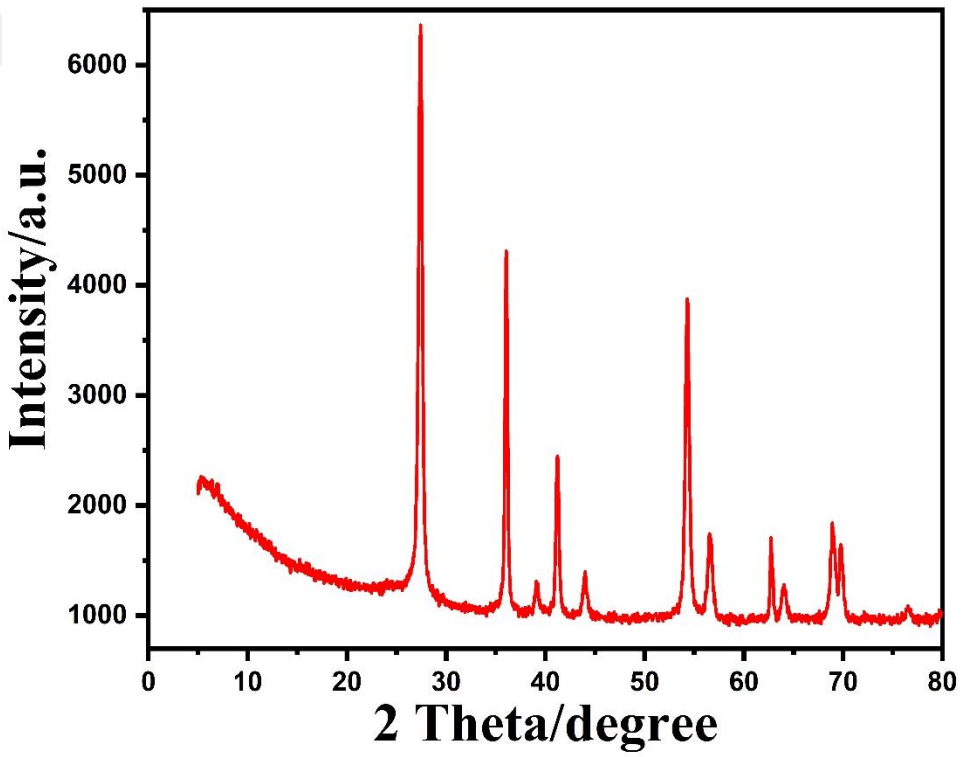

Figure 5.3. XRD patterns of ClC_4/TiO_2.

5.1.4. FTIR Analysis

"Fourier transform Infrared spectroscopy" was utilized to investigate and examine the surface functional groups in the synthesized "ClC_4/TiO_2"**.** As illustrated in Fig, the presence of peak at "748.4 cm^{-1} indicated" the presence of C-Cl. The band located at 1112.3 cm^{-1} attributed to the "C-C stretching vibrations". "Aromatic C=C absorption vibration stretching peaks at 1596.7 cm^{-1} "was observed. Moreover, at 1192.1, 1309.1, 1443.3 bending and stretching peaks of Ti-O-Ti, Ti-O and Ti-OH were detected respectively. An "absorption peak" was observed at "400-800 cm^{-1} (597.2 cm^{-1})" characteristic of TiO_2.

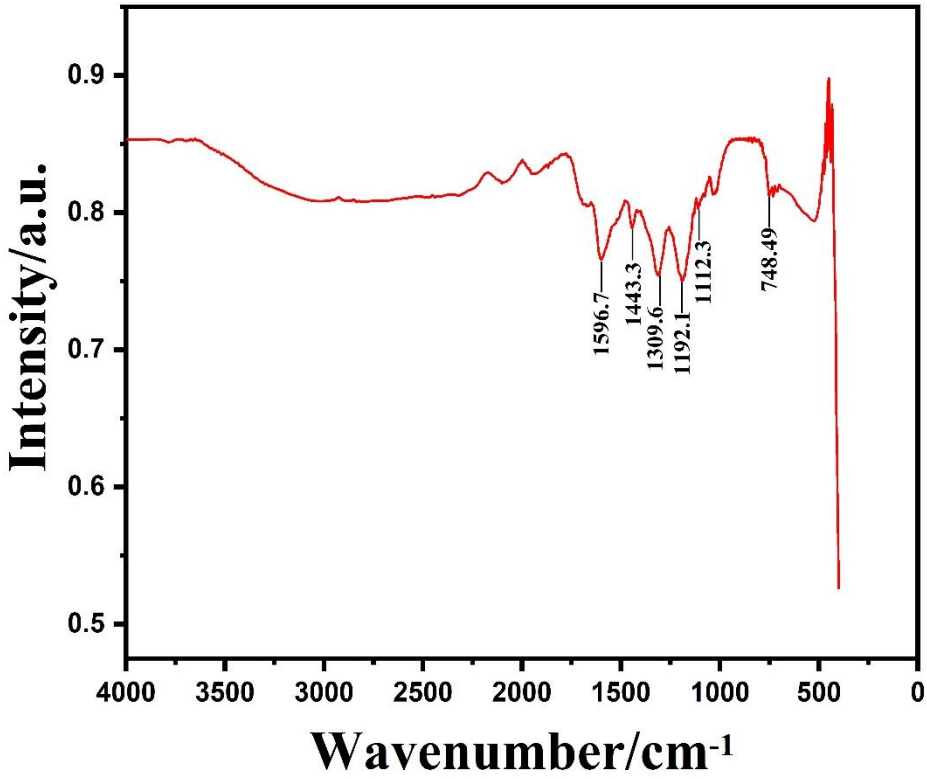

Figure 5.4. FT-IR spectra of ClC_4/TiO_2.

5.2. Electrochemical properties of the developed electrode

To demonstrate the catalytic activity and the electrochemical properties of bare GCE and modified "ClC_4/TiO_2/GCE" have been observed using Cyclic voltammetry (CV) in 0.1 M KCl in 5.0 mM "$[K_3(CN)]_6^{-3/-4}$" at "different scan rates". As presented in (Figure 5.5.A) comparing the CV performances of the bare GCE, and modified ClC_4/TiO_2/GCE at a scan rate of 0.05 Vs^{-1}. The "oxidation peak" current was increased twofold at the "ClC_4/TiO_2/GCE", which ameliorated the sensitivity of "developed sensors" for the "Tyro detection". The "anodic-cathodic peak" separation (ΔEp) value which is critical for electron transfer rate, so that the peak potential separation value (ΔEp) of bare GCE (0.34 V) was larger than modified ClC_4/TiO_2/GCE (0.25 V), demonstrating that rapid electron transfer on the modified electrode surface. Moreover, an improvement in both cathodic and anodic signals indicated higher electro-catalytic performance of the ClC_4/TiO_2 nanocomposite

Furthermore, "Electrochemical impedance spectroscopy" (EIS) is sensitive technique to demonstrate the conductivity of materials and the electron transport characteristics on the electrodes surface. The Nyquist plot of the modified ClC_4/TiO_2/GCE, and bare GCE in the presence of 1.0 mM "$[K_3(CN)]_6^{3-/-4}$ containing" 0.1M KCl at scan rate of 0.05$Vs^{-1}$are illustrated in Fig. 5.5. Therefore, charge transfer resistance (Rct) of $[K_3(CN)]_6^{3-/-4}$ redox probe is shown by the semicircle diameter. Therefore, examined charge transfer resistance (R_{ct}) of bare GCE, and ClC_4/TiO_2/GCE are approximately 6.41 and 3.33kΩ. The results show that ClC_4/TiO_2/GCE has a higher conductivity than GCE, high electron transferability, decrease charge transfer resistance, and enhance the electroactivity of developed electrode due to improve the electroactive surface area.

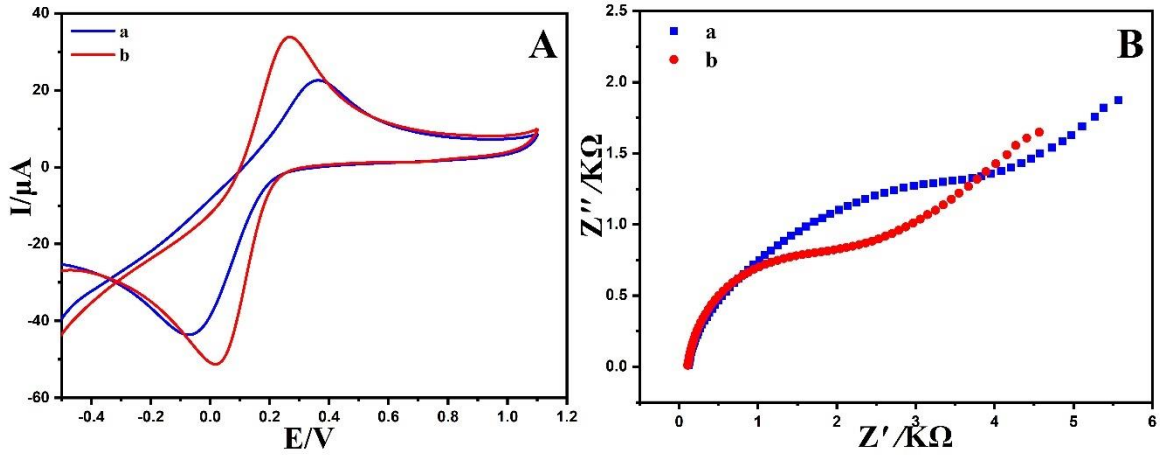

Figure 5.5. (A); Cyclic voltammetry and (B); The Nyquist plots (EIS) of the bare GCE and ClC_4/TiO_2/GCE in the "5.0 mmol L^{-1} $[K_3(CN)]_6^{3-/4-}$ containing 0.1 M KCl" at a scan rate of 50.0 mV s^{-1} a) "bare GCE", b) "modified ClC_4/TiO_2/GCE".

In addition, the "electroactive surface areas" of the modified electrodes were observed using "CV technique" in "PBS buffer (pH= 3)", containing "5.0 mM $[K_3(CN)]_6^{3-/-4}$ in 0.1 M "KCL", this approach is suitable to improve the electrochemical characteristics. However, by plotting the slope of peak current versus "square root scan" to estimate the electroactive surface area using the "Randles–Sevcik equation (Eq. 1)".

$$I_{pa} = (2.69 \times 10^5) n^{3/2} A D^{1/2} v^{1/2} C_0$$

The result show that the active surface area of GCE and ClC_4/TiO_2/GCE were founded to be 0.03and 0.04 cm^2. According to these finding the large electroactive surface area of the modifiedClC_4/TiO_2/GCEcompaired to bare GCE,indicate that the modified electrode has good conductivity and strong electrocatalytic activity due to the high peak current and low peak potential differences.

5.3. Optimization of Developed Electrode

The electrochemical properties of ClC_4/TiO_2 /GCE were investigated using DPV in PBS buffer (pH=3) in the presence 0.1Mm of Tyro. The activity and sensitivity of the developed electrodes effected by optimal experimental conditions. The electrochemical parameter such as composite concentration, stirring, amount of nanocomposite, pH of supporting electrolyte have effect on the intensity of peak current.

The supporting electrolyte is an essential part that impacted on the electrochemical behavior. The impact of different supporting electrolyte including "Phosphate buffer saline" (PBS), "Britton-Robinson buffer" (B-R), "Acetate buffer" (AC), and 0.1 M "KCl" solution, 0.1 M "HCL" solution, "sodium hydroxide (NaOH)" and "phosphoric acid solution" were studied as electrolytes for the determination of the 1.0 µM Tyro at ClC_4/TiO_2/GCE using DPV as seen in Fig. 5.6 A. The Tyro peak current and oxidation potential were significantly dependent on the supporting electrolyte, for this reason the peak current of the PBS is the highest current among the different buffers. Therefore, the PBS buffer was chosen as the buffer for further experiments.

In the next step, the effects of the concentration of ClC_4/TiO_2on the "surface of the electrode" had been studied in the composite concentration range 0.1-2 mg /mL (Fig. 5.6 B). The Tyro peak current was enhanced by increasing the concentration of $ClC_4/TiO2$ more than 0.1 mg mL^{-1}because of high adsorption sites and large surface area, and decreased at concentration more than 1.0 mg mL^{-1}because of overgrowth viable binding sites on the electrode surface and reducing viable adsorbent surface area. Hence, 1.0 mg mL^{-1} of ClC_4/TiO_2 was chosen as the optimal concentration.

Moreover, to observe the impact of the nanocomposite amount on the "surface of the electrode" was achieved by using various amount of composite in the range of 3.0 to 9.0 µLand a suspension of ClC_4/TiO_2 was dripped to the electrode surface and dry at room temperature. Fig. 5.6 C indicated that maximum oxidation peak current was achieved with 6.0 µL of ClC_4/TiO_2. Further increase in the amount of nanocomposite higher than 6.0 µL decrease Tyro peak current due to diminished in the modifier layers' ability to adhere to the electrode's surface and hindered the analyte's diffusion through the dense modifier layer that leads to a noticeable decrease in the modified electrode sensitivity. Hence, 6.0 µL of ClC_4/TiO_2 suspension was chosen as optimal amount for modification of electrode surface.

Study of stirring rate helped to understand the impact of mass transfer on Tyro electro-oxidation, and the best response was at 100 rpm (Fig. 5.6 D).

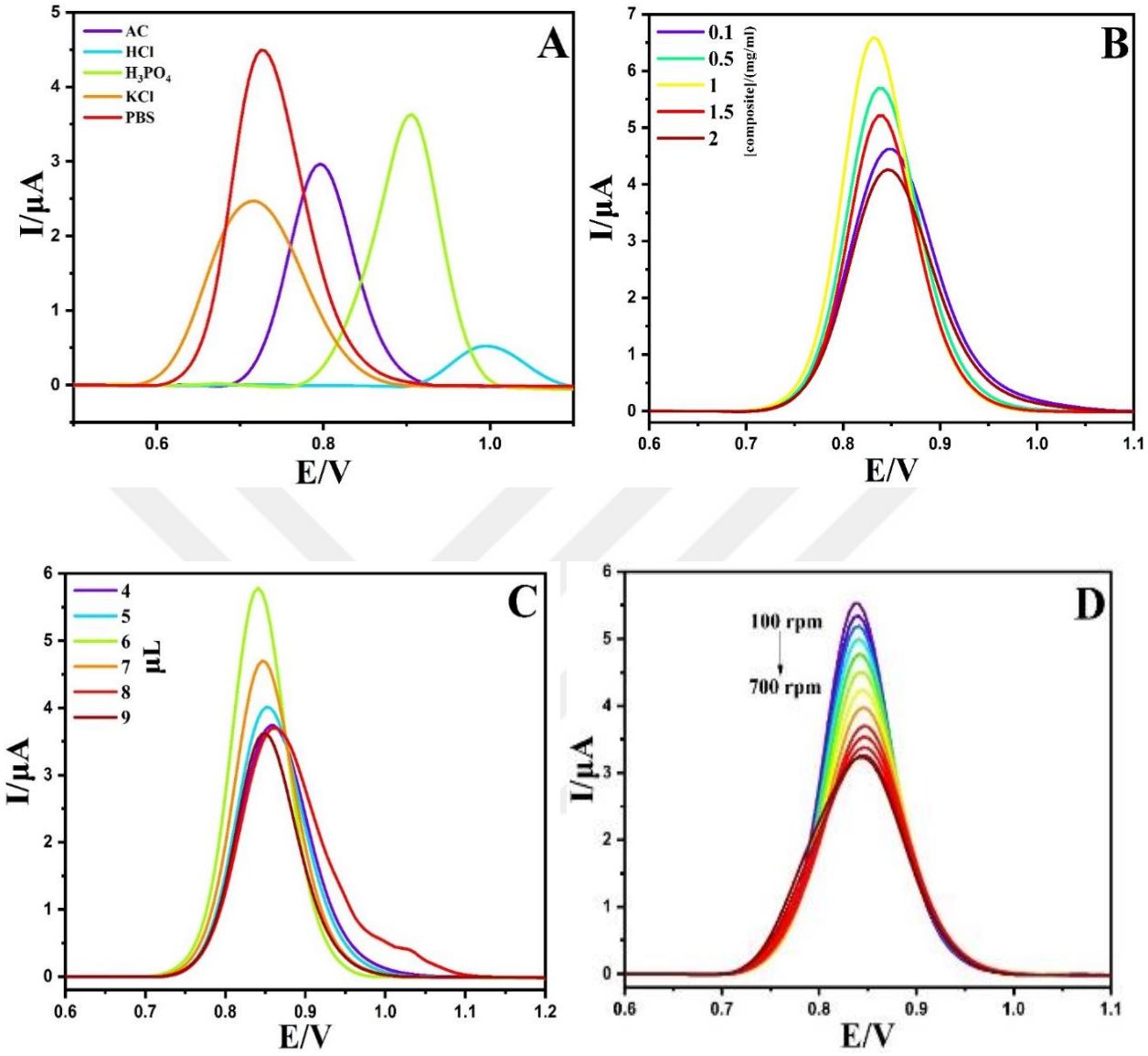

Figure 5.6. (A) DPVs of various supporting electrolytes such as CT, PBS, AC, and B-R, in 0.1mM Tyro (B); DPVs of various concentration modifiers of ClC_4/TiO_2 composite, in 0.1 mM Tyro (C); DPVs of various amount modifiers of ClC_4/TiO_2 composite, in 0.1 mM Tyro (D); DPVs of various stirring rate, in 0.1 mM Tyro.

5.4. The Impact of pH

To demonstrate the impact of the pH of PBS buffer on voltammetric behavior of 0.1mM Tyro has been examined as a critical investigating factor. As shown in fig. 5.8 A the Tyro voltammetric behavior was performed with DPVs in PBS solution at "pH window 3.0 -8.0". The Tyro peak potential and peak current changes when pH solution of supporting electrolyte change. The oxidation peak current was increased at pH 3 and decreased after pH 4.0 to 8.0. The Tyro oxidation peak was changed to the negative potentials with enhancing pH from 3.0 to 8.0, and the redox mechanism of Tyro is irreversible. Furthermore, pH 3.0 was selected as the suitable pH for further investigations of Tyro oxidation. Moreover, Fig. 5.8 B illustrate the plot of pH versus "peak potential" which confirmed by a slope of 0.0544 V/pH (R^2 = 0.9914). Therefore, pH slope value versus oxidation peak is close to the Nernstian quantity 0.059 V/pH, that illustrate the oxidation process of Tyro and equal number of electrons and protons are obtained (Mehmandoust et al., 2023). According to the "Nernst equation ($dE_p/dpH = 0.059\ m/\alpha n$)", the number of proton to electron ratio was obtained as 2:2. Hence, the proposed electro-oxidation mechanism of Tyro at ClC_4/TiO_2/GCE is illustrated in Fig. 5.7 (Oxidation mechanism of Tyrosine).

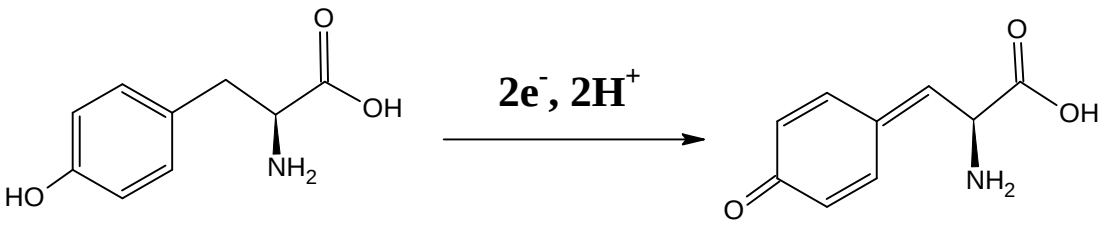

Figure 5.7. Electrochemically oxidation mechanism of Tyrosine towards ClC_4/TiO_2.

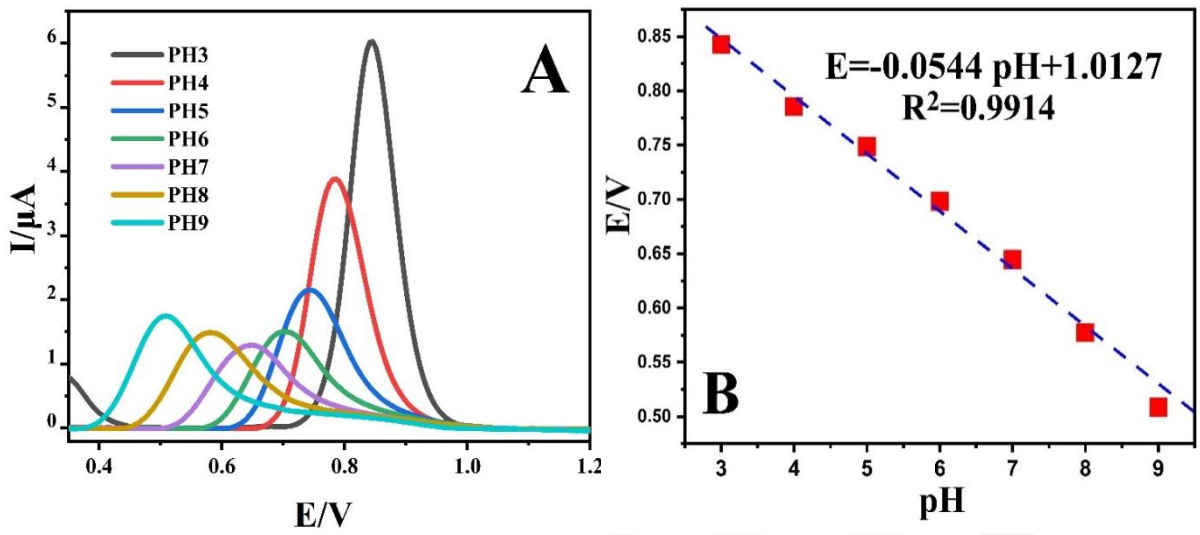

Figure 5.8. (A)DPV for solution containing 1 μM of Tyro in the range of pH from 3-8 on the ClC_4/TiO_2/GCE, (B) Effect of "pH values" on the Tyro "peak current and potential" on the ClC_4/TiO_2/GCE surface containing 0.1 mM Tyro at different pHs.

5.5. Impact of Scan Rate

To investigate the impact of electro- oxidation performance of Tyro on ClC_4/TiO_2/GCE surface in PBS (pH 3.0) in the presence of 0.1 mM Tyro at "various scan rates (10.0-300.0 mV s^{-1})" using "CV technique" (Fig. 5.9). The result observed that the intensity of "anodic peak current" of Tyro was enhanced with the enhancement of scan rate. Fig. 5.9 B exhibits the plot of anodic current versus the "square root of scan rate" that was linearly proportional and the equations was observed as I_{pa} (μA) = 0.256 $v^{1/2}$ – 0.3443 (R^2 = 0.998), which suggested that the electrochemical oxidation of Tyro on ClC_4/TiO_2/GCE is a diffusion-controlled electrochemical process (Erk et al., 2022) . In addition, the "logarithm of the anodic current (log I_{pa})" was observed to be directly proportional to the "logarithm of the scan rate (log v)", according to the "regression equation: log I_{pa} (μA) = 0.454 log v (mV s^{-1}) + 5.177 (R^2 = 0.999)", which resulted a diffusion-controlled process for Tyro oxidation (Fig. 5.9 C). Furthermore, the plot of E_{pa} against ln v was expressed to be linear with the following equation: E_{pa} (V) = 0.014 ln v + 0.823 (R^2 = 0.998) (Fig. 5.9 D). The number of electrons that transfer in the "electro-oxidation reaction" of Tyro

could be estimated using the linear "Laviron's equation's slope". Moreover, it was found that two electrons were transferred during the electro-oxidation of Tyro at "ClC_4/TiO_2/GCE".

The Tafel plot and related voltammograms used to determine the Tyro electro-oxidation at 100 mV/s scan rate are shown in (Fig 5.9 E). Therefore, obtained electron transfer coefficient (α) of Tyro at the ClC_4/TiO_2/GCE surface was based on the slope of Tafel plot (2.3 RT/n (1- α) F), which was found to be approximately 0.97.

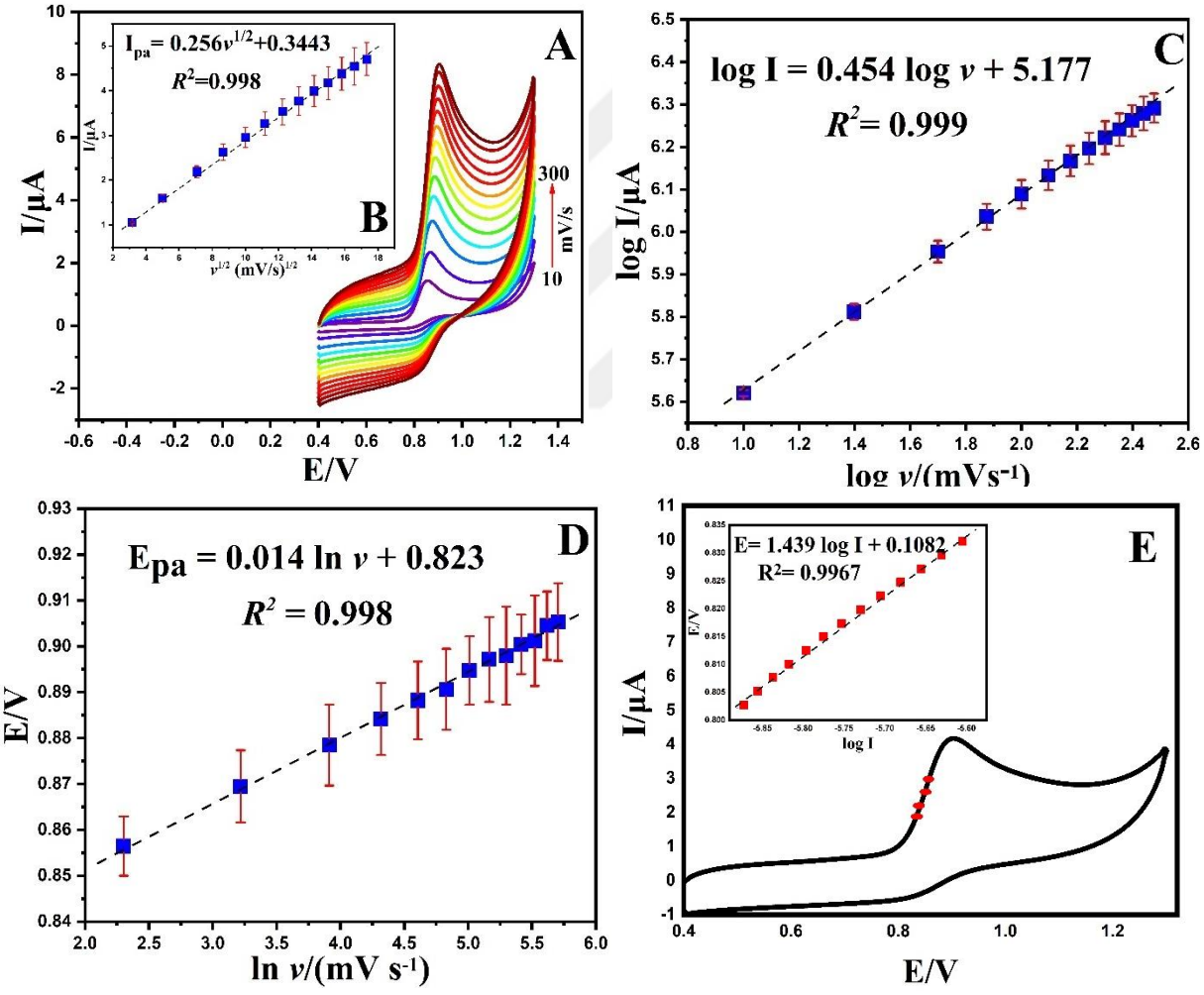

Figure 5.9. (A) cyclic voltammetry (CV) for solution containing 1µM of Tyro in pH= 3 (PBS buffer) at "various scan rates (10-300 mv.s^{-1})", (B) the plot of (I_{pa}) against ($v^{1/2}$) for 1µM of Tyro on the ClC_4/TiO_2/GCE surface, (C) the plot of log I_{pa} versus log *v* that acquired at ClC_4/TiO_2/GCE in pH=3 (PBS buffer) for 1µM of Tyro, (D) the plot of E_{pa} versus ln *v* for Tyro at the ClC_4/TiO_2/GCE surface.

5.6. Evaluation of The Analytical Performance

The DPV technique has been evaluated the analytical performance of ClC_4/TiO_2/GCE by varying the Tyro concentrations under the optimized experimental conditions (Fig. 5.10). Therefore, peak current has been enhanced with increase the Tyro concentration. It was observed that the "anodic current" is linearly proportion with the Tyro concentration in the ranges of 0.8–34.1 μM, with the corresponding regression equation: $I_{pa} = 0.0346\ C_{Tyro} + 0.0665$ ($R^2 = 0.9937$). Moreover, it was overcome the kinetics barrier due to no kinetic limitation and rapid electron transfer. The Tyro limit of detection (LOD)for the developed electrode was observed to be 5.58 nM, according to the equation LOD = 3.3 s/m (Karimi-Maleh et al., 2021). Therefore, it can be indicated that the electrochemically modified electrode has offered analytical performance in term of repeatability, limit of detection and wide linear concentration range.

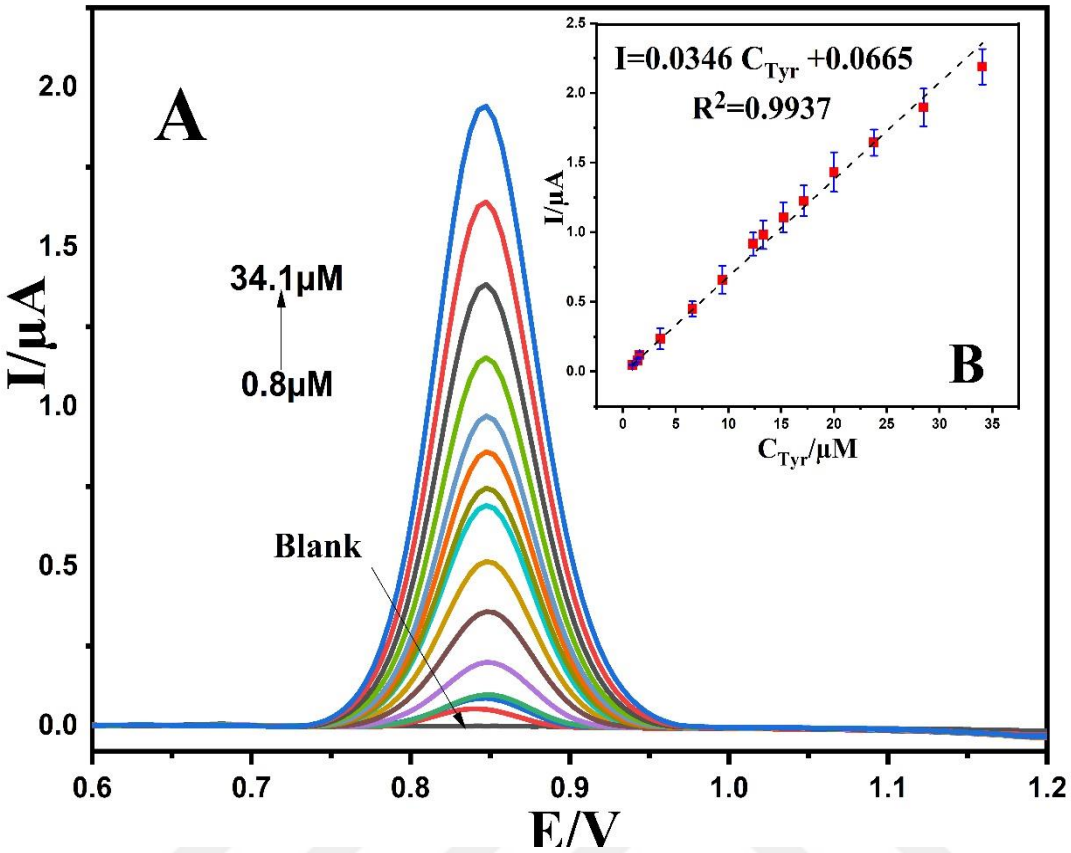

Figure 5.10. (A) Differential pulse voltammetry (DPV) at ClC_4/TiO_2/GCE in pH= 3 (PBS buffer) with diverse concentrations of Tyro, (B) The plot of Tyro concentrations versus I_{pa} at the ClC_4/TiO_2/GCE surface.

The obtained analytical performance of modified electrochemical sensor was compared with the other similar analytical sensor for Tyro investigation has been tabulated in Table 5.1. The result shows that the modified electrode which has low detection limit, selectivity and sensitivity to detect Tyro compared to previously other analytical methods.

Table 5.1. Comparison of the obtained results with similar analytical methods for Tyro detection.

Method	Modifier	Linear range (µM)	LOD (µM)	Ref.
LSV	SWCNHs/GCE	2-30	0.4	(Zhu et al., 2014)
DPV	MIP/RGO/GCE	0.1-400	0.046	(Zheng et al., 2018)
SDLSV	GR/ABPE	0.1-100	0.06	(Deng et al., 2021)
DPV	CNF/CPE	0.2-109	0.1	(Tang et al., 2010)
LSV	Al-CuSe-NPs/SPCE	0.15-10	0.04	(Murtada et al., 2020)
DPV	ClC_4/TiO_2/GCE	0.8-34.1	0.0058	Our work

5.7. Selectivity

The selectivity of the prepared electrode in the presence of other interfering agents was evaluated using the DPV technique under optimal conditions, the interference studies have been interfering agents. For investigation of interferences the presence of 100-fold of the biological compound (glucose, citric acid, urea, and Na_2SO_4) and amino acid (alanine, arginine and cystine) that impact the Tyro voltammetric determination at ClC_4/TiO_2/GCE, in PBS buffer (pH=3) as illustrated in Table 5.2. According to the results, the response anodic current of Tyro had no interfering effect on the analyte signal and the relative standard deviation was obtained to be below 4.0%. Therefore, the results show that the ClC_4/TiO_2/GCE has high selectivity towards the determination of Tyro.

Table 5.2. Selectivity of "ClC_4/TiO_2/GCE" for Tyro in the presence of diverse interfering agents.

Interferent	Tyro:interferent	RSD%
Glucose	1:100	-2.47
Urea	1:100	-2.29
Citric acid	1:100	-2.39
Cystine	1:100	+2.05
Alanine	1:100	-1.74
Na_2SO_4	1:100	-2.12
Arginine	1:100	+2.87

5.8. Reproducibility, Repeatability and Stability of ClC_4/TiO_2/GCE

The proposed electrode's reproducibility was investigated using 1µM Tyro at ClC_4/TiO_2/GCE as illustrated in Fig 5.10 A. Modification of five modified electrodes using the same approaches, and the modified electrodes had used to evaluate the reproducibility. The analyte's relative standard deviation (RSD) value was obtained 1.6%, which demonstrate good reproducibility of the" modified electrode's".

To analyze the repeatability of the response of the ClC_4/TiO_2/GCE the peak currents of DPV investigation of 1µM Tyro for ten times to use the same electrode are measured and then compared as shown in Fig 5.10 B. The results indicate that, the relative standard deviation (RSD) is observed 3%, revealing that the modified electrode has suitable repeatability.

Furthermore, the stability of ClC_4/TiO_2/GCE is an important parameter for investigation. Therefore, stability of the modified electrode was performed at 1.0 µmol L^{-1} Tyro by using differential pulse voltammetry (DPV) at pH= 3 (PBS buffer). The modified electrode that has been kept at "4 °C under optimized conditions" produced the current response is about 98.4% of the original value after 30 days.

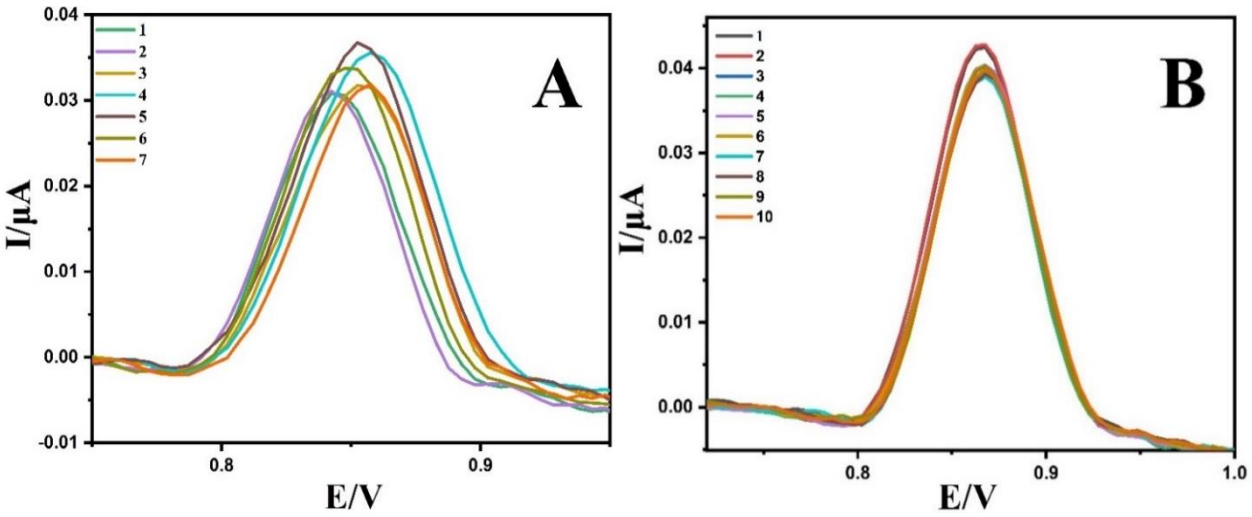

Figure 5.11. Differential pulse voltammetry (DPV) for solution containing 1µM Tyro at the surface of ClC_4/TiO_2/GCE in pH= 3 (PBS buffer) (A) Reproducibility measurements (B) Repeatability measurements.

5.9. Real Samples Analysis

In order to validate the analytical method's applicability of the developed electrode for analyzation of the target analyte in a human plasma sample, milk, and soybean Fig 5.11. The Tyro concentration in real samples can be determined using the standard addition technique by the DPV approach under optimal experimental conditions. Table 5.3 illustrated that the recovery rate is between 99.15–104.60% which observed that confirmed, the high accuracy and sufficient capability of "ClC_4/TiO_2/GCE to determine" Tyrosine in the "real sample".

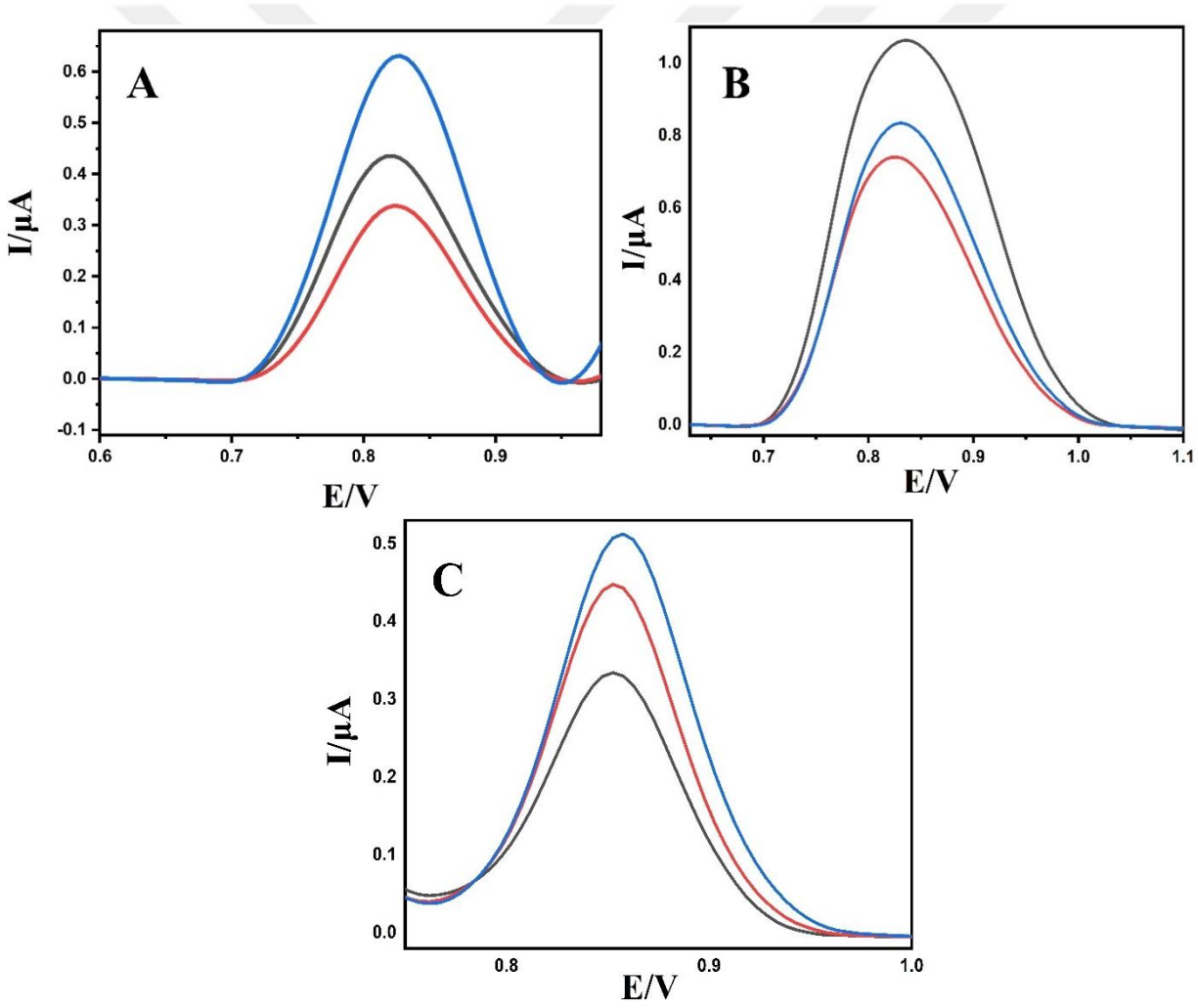

Figure 5.12. DPV of ClC_4/TiO_2/GCE in (A) Human plasma, (B) Milk, (C) Soybean with different Tyro concentrations.

Table 5.3. Determination of Tyro on ClC_4/TiO_2/GCE surface in "real samples".

Sample	Spiked(µM)	Found(µM)	Recovery(%)	RSD
	6	6.27	104	5.69
Human plasma	8	7.93	99	5.94
	10	10.27	102	5.86
	8	8.31	103	3.29
Milk	10	10.18	101	5.78
	30	30.84	102	1.04
	6	6.24	104	1.09
Soyaeabn	8	8.02	100	1.22
	10	10.28	102	1.86

6. CONCLUSION

In summary, the electrochemical technique generally used in the sensor has been applied in the sensing application of Tyro. Therefore, we determined Tyro in selective manner and low limit of detection LOD: 0.0058µM, which enabled the evaluation and detection of Tyro in biological. Furthermore, the developed sensor illustrated outstanding selectivity and reliability and provided the selective identification and detection of Tyro in real samples. To our best knowledge, the modification of glassy carbon surface with ClC_4/TiO_2 has been firstly developed for Tyro detection. The approach offers many attractive advantages such as low limit of detection, portability, low cost, easy-to-use protocol, and wider linear range than previously reported sensors. The analytical performance of electrochemical sensors for Tyro was developed and compared with similar studies conducted so far and it was concluded that sensitive electrochemical sensors have been developed within the scope of the thesis project. In addition, selectivity and percentage recovery studies were performed using a sophisticated electrochemical sensor. In the selectivity studies, Tyro was analyzed in the presence of potential interfering substances by the fabricated electrode. Electrochemical analysis of Tyro sample obtained from plasma, milk and soyabean samples has been successfully applied. The results of the obtained selectivity and percentage recovery showed that the electrochemical sensors we developed are selective towards Tyrosine. In conclusion, we propose that the sensitive and selective electrochemical sensor using ClC_4/TiO_2/GCE that we developed for direct determination of Tyrosine from real samples and used in analysis of Tyro as a point of care diagnostics.

ABSTRACT

Development of electrochemical sensor for determination of Tyrosine

The application of electrochemical sensors as a sensing alternative for the direct detection of Tyrosine (Tyro) in biological samples is demonstrated in this thesis. The crystalline, surface morphology of chloro-tetracarbon titanium dioxide (ClC_4/TiO_2) were investigated using "X-ray diffraction (XRD)", "Fourier transform infrared spectroscopy (FITR)", "Scanning electron microscopy (SEM)" and "Energy dispersive analysis of X-ray (EDAX)". Moreover, the electrochemical properties of the developed sensor were studied using "electrochemical impedance spectroscopy (EIS)", "cyclic voltammetry (CV)", and "differential pulse voltammetry (DPV)". In this study, we: designed an “electrochemical sensor" capable of rapidly detecting Tyro with an extremely "low limit of detection (LOD)" of 0.0058 μM within a broad linear concentration range of 0.8 to 34.1μM. In the existence of interferences, Tyro was detected in human plasma, milk, and soybean as real samples with high sensitivity and satisfactory recovery. The modified electrode also demonstrated high performance for "stability, repeatability, reproducibility, and reusability". Hence, the developed electrochemical sensor with "high stability, repeatability, reproducibility, and reusability" was presented in this study for Tyro determination. Based on the results, ClC_4/TiO_2/GCE can be used as a novel method to detect Tyro.

Keywords: Biological samples, Tyrosine, Voltammetry

ÖZET

Tirozin Belirlenmesi İçin Elektrokimyasal Sensörün Geliştirilmesi

Biyolojik örneklerde doğrudan tirozin (Tyro) analizi için elektrokimyasal sensörlerin uygulanması bu tezde gösterilmektedir. Kloro-tetrakarbon titanyum dioksitin (ClC_4/TiO_2) kristal yüzey morfolojisi "X-ışını kırınımı (XRD)", "Fourier dönüşümü kızılötesi spektroskopisi (FTIR)", "Taramalı elektron mikroskobu (Soltani and Jouyban)" ve "X-ışınının enerji dağılım analizi (EDAX)" kullanılarak araştırıldı. Ayrıca geliştirilen sensörün elektrokimyasal özellikleri "elektrokimyasal impedans spektroskopisi (EIS)", "dönüşümlü voltametri (CV)" ve "diferansiyel puls voltametrisi (DPV)" kullanılarak incelenmiştir. Çalışmamızda, son derece "düşük algılama sınırı (LOD)" 0,0058 μM, 0,8 ila 34,1 μM 'lik geniş bir doğrusal konsantrasyon aralığında Tyro'ı hızlı bir şekilde analiz edebilen "elektrokimyasal sensör" geliştirilmiştir. Girişim yapan maddelerin varlığında Tyro, insan plazmasında, sütünde ve soya fasulyesinde yüksek hassasiyet ve gerikazanım ile gerçek numunelerde tayin edilmiştir. Modifiye elektrot ayrıca "yüksek kararlılık, tekrarlanabilirlik, yeniden üretilebilirlik ve yeniden kullanılabilirlik" için performans göstermiştir. Bu nedenle, Tyro tayini için geliştirilen "yüksek kararlılık, tekrarlanabilirlik, tekrar üretilebilirlik ve yeniden kullanılabilirlik" özelliklerine sahip elektrokimyasal sensör bu çalışmada sunulmuştur. Sonuçlara göre, ClC_4/TiO_2/GCE, Tyro'ı analiz etmek için yeni bir yöntem olarak kullanılabilir.

Anahtar Sözcükler: Biyolojik numune, Tyrozin, Voltametri

REFERENCES

ABO-HAMAD A, ALSAADI MA, HAYYAN M, JUNEIDI I, HASHIM MA, (2016). Ionic liquid-carbon nanomaterial hybrids for electrochemical sensor applications: a review. *Electrochimica Acta,* **193:** 321-343.

AKRAM R, YASEEN M, FAROOQ Z, RAUF A, (2021). Capacitive and Conductometric type Dual-Mode Relative Humidity Sensor based on 5, 10, 15, 20-tetra phenyl porphyrinato nickel (II)(TPPNi). *Polymers,* **13(19):** 3336.

ALLAHVERDIYEVA S, YUNUSOĞLU O, YARDIM Y, ŞENTÜRK Z, (2021). First electrochemical evaluation of favipiravir used as an antiviral option in the treatment of COVID-19: A study of its enhanced voltammetric determination in cationic surfactant media using a boron-doped diamond electrode. *Analytica chimica acta,* **1159:** 338418.

BARD AJ, FAULKNER LR, (2001). Fundamentals and Applications. *Electrochemical Methods*, **2:**580-632.

BARANWAL J, BARSE B, GATTO G, BRONCOVA G, KUMAR A, (2022). Electrochemical Sensors and Their Applications: A Review. *Chemosensors*, **10(9):** 363.

CAMPILLO-BROCAL JC, LUCAS-ELÍO P, SANCHEZ-AMAT A, (2015). Distribution in different organisms of amino acid oxidases with FAD or a quinone as cofactor and their role as antimicrobial proteins in marine bacteria. *Marine drugs*, **13(12):** 7403-7418.

CASETTARI L, VLLASALIU D, LAM JK, SOLIMAN M, ILLUM L, (2012). Biomedical applications of amino acid-modified chitosans: A review. *Biomaterials,* **33(30):** 7565-7583.

CHAITOW L, (1988). Amino acid in Therapy: A guide to the therapeutics application of protein components.First edition. Rochester, New York.

DE SOUZA VIEIRA L, (2022). A review on the use of glassy carbon in advanced technological applications. *Carbon,* **186:** 282-302.

DENG C, DENG Y, WANG B, YANG X, (2002). Gas chromatography–mass spectrometry method for determination of phenylalanine and Tyrosine in neonatal blood spots. *Journal of ChromatograpHy B,* **780(2):** 407-413.

DENG P, XIO J, FENG J, TIAN Y, WU Y, LI Y, HE Q, (2021). Highly sensitive electrochemical sensor for Tyrosine detection using a sub-millimeter electrode. *Microchemical Journal,* **165**: p. 106106.

DEY RS, BERA RK, RAJ C, (2013). Nanomaterial-based functional scaffolds for amperometric sensing of bioanalytes. *Analytical and bioanalytical chemistry,* **405(11):** 3431-3448.

DINU A, APETREI C, (2022). 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 of Molecular Sciences,* **23(3):** 1218.

DJERDJ I, TONEJC A, (2006). Structural investigations of nanocrystalline TiO_2 samples. *Journal of Alloys and Compounds,* **413(1-2):** 159-174.

ELGRISHI N, ROUNTREE K, DEMPSEY J, (2018). A practical beginner's guide to cyclic voltammetry. *Journal of chemical education,* **95(2):** 197-206.

ERK N, BOUALI W, MEHMANDOUST M, SOYLAK M, (2022). An Electrochemical Sensor for Molnupiravir Based on a Metal-Organic Framework Composited with Poly (3, 4-ethylene dioxythiopHene): Poly (sTyroene sulfonate). *ChemistrySelect,* **7(46):** e202203325.

FENG J, DENG P, XIAO J, TIAN Y, WU Y, LIU J, LI G, (2021). New voltammetric method for determination of Tyrosine in foodstuffs using an oxygen-functionalized multi-

walled carbon nanotubes modified acetylene black paste electrode. *Journal of Food Composition and Analysis,* **96:** 103708.

FERNSTROM JD, FERNSTROM MH, (2007). Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *The Journal of nutrition,* **137(6):** 1539S-1547S.

GEORGE JM, ANTONY A, MATHEW B, (2018). Metal oxide nanoparticles in electrochemical sensing and biosensing: a review. *Microchimica Acta*, **185(7):** 1-26.

GU W, WANG M, MAO X, WANG Y, LI L, XIA W, (2015). A facile sensitive l-Tyrosine electrochemical sensor based on a coupled CuO/Cu 2 O nanoparticles and multi-walled carbon nanotubes nanocomposite film. *Analytical Methods*, **7(4):** 1313-1320.

GUO N, YOU X, WU Y, DU D, ZHANG L, SHANG Q, LIU W, (2020). Continuous fluorometric method for determining the monophenolase activity of Tyrosinase on L-Tyrosine, through quenching L-DOPA fluorescence by borate. *Analytical chemistry*, **92(8):** 5780-5786.

GUSEVA MB, NOVIKOV ND, BABAEV VG, ADAMYAN AA, LAVYGIN IA, (2002). Tetracarbon. Google Patents.

HE X, HU C, (2011). Building three-dimensional Pt catalysts on TiO_2 nanorod arrays for effective ethanol electrooxidation. *Journal of Power Sources,* **196(6):** 3119-3123.

ISILDAK Ö, ÖZBEK O, (2021). Application of potentiometric sensors in real samples. *Critical Reviews in Analytical Chemistry,* **51(3):** 218-231.

JAROŠOVÁ R, RUTHERFORD J, SWAIN GM, (2016). Evaluation of a nitrogen-incorporated tetrahedral amorpHous carbon thin film for the detection of tryptophan and Tyrosine using flow injection analysis with amperometric detection. *Analyst,* **141(21):** 6031-6041.

JONGKEES BJ, HOMMEL B, KÜHN S, COLZATO LS, (2015). Effect of Tyrosine supplementation on clinical and healthy populations under stress or cognitive demands—A review. *Journal of psychiatric research*, **70:** 50-57.

KARIMI-MALEH H, LUTFI YOLA M, ATAR N, OROOJI Y, KARIMI F, KUMAR P, (2021). A novel detection method for organophosphorus insecticide fenamiphos: Molecularly imprinted electrochemical sensor based on core-shell Co_3O_4@ MOF-74 nanocomposite. *Journal of colloid and interface science*, **592:** 174-185.

KASAI K, ISHIKAWA T, NAKAMURA T, MIURA T, (2015). Antibacterial properties of L-amino acid oxidase: mechanisms of action and perspectives for therapeutic applications. *Applied microbiology and biotechnology*, **99(19):** 7847-7857.

KASSA A, AMARE M, (2019). Electrochemical determination of paracetamol, rutin and sulfonamide in pHarmaceutical formulations by using glassy carbon electrode–A Review. *Cogent Chemistry*, **5(1):** 1681607.

LI S, XING M, WANG H, ZHANG LI, ZHONG Y, CHEN LEI, (2015). Determination of tryptopHan and Tyrosine by chemiluminescence based on a luminol–N-bromosuccinimide–ZnS quantum dots system. *RSC advances*, **5(73):** 59286-59291.

LIU L, HU M, PAN Y, XIONG M, LIO C, ZHANG Y, LUO K, ZHAO Z, GAO G, YU D, HE J, (2018). Mechanically ductile 3D sp–sp^2 microporous carbon. *Journal of materials science*, **53(6):** 4316-4322.

LÜTKE-EVERSLOH T, SANTOS CNS, STEPHANOPOULOS G, (2007). Perspectives of biotechnological production of L-Tyrosine and its applications. *Applied microbiology and biotechnology*, **77(4):** 751-762.

MACAK J, HILDEBRAND H, MARTEN-JAHNS U, SCHMUKI P, (2008). Mechanistic aspects and growth of large diameter self-organized TiO_2 nanotubes. *Journal of Electroanalytical Chemistry*, **621(2):** 254-266.

MAO A, LI H, CAI Z, HU X, (2015). Determination of mercury using a glassy carbon electrode modified with nano TiO_2 and multi-walled carbon nanotubes composites dispersed in a novel cationic surfactant. *Journal of Electroanalytical Chemistry*, **751:** 23-29.

MAURUTO DE OLIVEIRA GC, P.DE PALMA E, H. KUNITA M, MEDERIOS R, MATOS R, FRANCISCO K, JANEGITIZ B, (2017). Tapioca Biofilm Containing Nitrogen-doped Titanium Dioxide Nanoparticles for Electrochemical Detection of 17-β Estradiol. *Electroanalysis*, **29(11)**: 2638-2645.

MEHMANDOUST M, ÇAKAR S, ÖZACAR M, SALMANPOUR S, ERK N, (2022). Electrochemical sensor for facile and highly selective determination of antineoplastic agent in real samples using glassy carbon electrode modified by 2D-MoS2 NFs/TiO_2 NPs. *Topics in Catalysis*, **65(5):** 564-576.

MEHMANDOUST M, SOYLAK M, ERK N, (2023). Innovative molecularly imprinted electrochemical sensor for the nanomolar detection of Tenofovir as an anti-HIV drug. *Talanta*, **253:** 123991.

MOHAMED MA, ATTY SH, MEREY H, FATTAH T, FOSTER CH, BANKS C, (2017). Titanium nanoparticles (TiO_2)/graphene oxide nanosheets (GO): an electrochemical sensing platform for the sensitive and simultaneous determination of benzocaine in the presence of antipyrine. *Analyst*, **142(19):** 3674-3679.

MOUGHAN PJ, (2003). Amino acid availability: aspects of chemical analysis and bioassay methodology. *Nutrition Research Reviews*, **16(2):** 127-141.

MURTADA K, SALGHI R, RIOS R, ZOUGAGH M, (2020). A sensitive electrochemical sensor based on aluminium dopedcopper selenide nanoparticles-modified screen printed carbon electrode for determination of L-Tyrosine in pharmaceutical samples. *Journal of Electroanalytical Chemistry*, **874**: p. 114466.

NEURAUTER G, BURGI S, HAARA A, GEISLER S, MAYERSBACH P, SCHENNACH H, FUCHS D,(2013). Simultaneous measurement of phenylalanine and Tyrosine by high performance liquid chromatography (HPLC) with fluorescence detection. *Clinical biochemistry*, **46(18):** 1848-1851.

NISHIO T, TOUKAIRIN Y, HOSHI T, ARAI T, NOGAMI M, (2020). Determination of 3-chloro-L-Tyrosine as a novel indicator of chlorine poisoning utilizing gas chromatography-mass spectrometric analysis. *Legal Medicine*, **47:** 101782.

NOURELDEEN DA, BOUSHRA JM, LASHIEN AS, HAKIEM AFA, ATTIA TZ, (2022). Novel environment friendly TLC-densitometric method for the determination of anti-coronavirus drugs "Remdesivir and Favipiravir": Green assessment with application to pharmaceutical formulations and human plasma. *Microchemical Journa**l,*** **174:** 107101.

RANDVIIR EP, BANKS CE, (2013). Electrochemical impedance spectroscopy: an overview of bioanalytical applications. *Analytical methods*, **5(5):** 1098-1115.

REDDY YVM, SRAVANI B, ŁUCZAK T, MALLIKARJUNA K, MADHAVI G, (2021). An ultra-sensitive rifampicin electrochemical sensor based on titanium nanoparticles (TiO_2) anchored reduced graphene oxide modified glassy carbon electrode. *Colloids and Surfaces A: PHysicochemical and Engineering Aspects,* **608:** 125533.

SÁNCHEZ-LÓPEZ E, MARCOS A, AMBROSIO E, MARINA ML, CREGO AL, (2016). Enantioseparation of the constituents involved in the phenylalanine-Tyrosine metabolic pathway by capillary electrophoresis tandem mass spectrometry. *Journal of Chromatography A*, 1467: 372-382.

SANGHAVI BJ, WOLFBEIS OS, HIRSCH T, SWAMI NS, (2015). Nanomaterial-based electrochemical sensing of neurological drugs and neurotransmitters. *Microchimica Acta,* **182(1):** 1-41.

SOHOULI E, GHALKHANI M, ROSTAMI M, RAHIMI-NASRABADI M, AHMADI F, (2020). A noble electrochemical sensor based on TiO_2@ CuO-N-rGO and poly (L-

cysteine) nanocomposite applicable for trace analysis of flunitrazepam. *Materials Science and Engineering: C*, **117:** 111300.

SOLTANI S, JOUYBAN A, (2014). Biological sample preparation: attempts on productivity increasing in bioanalysis. *Bioanalysis*, **6(12):** 1691-1710.

STRADIOTTO NR, YAMANAKA H, ZANONI MVB, (2003). Electrochemical sensors: A powerful tool in analytical chemistry. *SciELO Brasil*.

TAN N-H, FUNG S-Y, (2008). Snake venom L-amino acid oxidases and their potential biomedical applications. *Malaysian Journal of Biochemistry and Molecular Biology*, **16(1):** 1-10.

TASHKHOURIAN J, DANESHI M, NAMI-ANA S, (2016). Simultaneous determination of Tyrosine and tryptophan by mesoporous silica nanoparticles modified carbon paste electrode using H-point standard addition method. *Analytica Chimica Acta*, **902:** 89-96.

YIN T, QIN W, (2013). Applications of nanomaterials in potentiometric sensors. *TrAC Trends in Analytical Chemistry*, **51:** 79-86.

WANG J, (2006). Analytical Electrochemistry. Third edition. New Jersey :John Wiley&Sons,Inc., Hoboken Publishers.

ZHENG M, GAO F, WANG Q, CAI X, JIANG SH, GAO F,(2013). Electrocatalytical oxidation and sensitive determination of acetaminopHen on glassy carbon electrode modified with graphene–chitosan composite. *Materials Scienceand Engineering:* **33(3):** p. 1514-1520.

ZHU S, ZHANG J, ZHAO X, WANG H, XO G, YOU J, ACTA M, (2014). Electrochemical behavior and voltammetric determination of L-tryptophan and L-Tyrosine using a glassy carbon electrode modified with single-walled carbon nanohorns. *Microchimica Acta*, **181(3):** p. 445.451-.

ZHENG W, ZHAO M, LIU W, YU SH, NIU L, LI G, LI H, LIU W, (2018).Electrochemical sensor based on molecularly imprinted polymer/reduced graphene oxide composite for simultaneous determination of uric acid and Tyrosine. *Journal of Electroanalytical Chemistry*, **813**: p. 75-82.