ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

ELECTROCHEMICAL DETERMINATION OF GALLIC ACID IN RED WINE, BLACK TEA AND GREEN TEA BASED ON CHOLINE CHLORIDE MODIFIED GLASSY CARBON ELECTRODE

By

Getachew Amsalu

Advisor: Dr. NEGUSSIE NEGASH

August, 2024 G.C
Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF CHEMISTRY

Electrochemical determination of Gallic acid in red wine, black tea and green tea based on choline chloride modified glassy carbon electrode

A thesis submitted to the department of chemistry in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry, Addis Ababa University

By

Getachew Amsalu

Advisors: Dr. Negussie Negash

August, 2024 G.C
Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

DEPARTMENT OF CHEMISTRY

This is to certify that this thesis prepared by Getachew Amsalu entitled “electrochemical determination of Gallic acid in red wine, black tea and green tea based on choline chloride modified glassy carbon electrode” and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Chemistry compiles with regulation of the university and meets the accepted standards with respect to originality and quality.

Approved by board of examiners

Advisors	**Signature**	**Date**
1. Dr. Negussie Negash	____________	_____________
Examiners		
1. Prof. B. S. Chandravanshi	_____________	_____________
2. Dr. Solomon Mihretie	_____________	_____________

DECLARATION

I hereby declare that this MSc thesis is my original work and has not been presented for a Degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Getachew Amsalu

Signature: ___________________________

This thesis has been submitted for examination with my approval as university advisor.

Name: Dr. Negussie Negash

Signature: ___________________________

ACKNOWLEDGEMENTS

I want to start by expressing my gratitude to the Almighty God for giving me the strength and capacity to carry out this research. Without his blessings, it would not have been accomplished.

I would like to express my sincere and deepest gratitude to my advisors Dr. Negussie Negash for his excellent guidance, valuable suggestions, insightful criticism, genuine support and kindness throughout the work. I also would like to extend my sincere thanks to Dr. Endale Tesfaye for his continuous encouragement, assistance and technical support throughout the laboratory work. I would also like to acknowledge the Ministry of Education, Addis Ababa University and Department of Chemistry for giving me the chance to study my master’s degree and conduct this research work. Last but not least, my heartfelt gratitude shall go to my family members and friends who assisted me in any way in my studies.

Table of Contents page

List of figures

List of tables

List of Abbreviation

I_{pa}	Anodic peak current
ABS	Acetated buffer solution
E_{pa}	Anodic peak potential
BRB	Britton-Robinson buffer
BGCE	Bare glassy carbon electrode
I_{pc}	Cathodic peak current
E_{pc}	Cathodic peak potential
CBS	citrate buffer solution
CME	Chemically modified electrode
ChCl	Choline chloride
R^2	Correlation coefficient
CV	Cyclic voltammetry
$\triangle E_p$	Difference in peak potentials
EIS	Electrochemical impedance spectroscopy
G.A	Gallic acid
GCE	Glassy carbon electrode
HPLC	high-performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of detection
LOQ	Limit of quantification

PBS	Phosphate buffer solution
RSD	Relative standard deviation
Sec	Seconds
SWV	Square wave voltammetry

Abstract

A sensitive and selective square wave voltammetric approach for determination of Gallic acid (G.A) was developed based on choline chloride modified glassy carbon electrode (ChCl/GCE). The modification was simple and cost effective, which was performed through electrochemical deposition of choline chloride onto the glassy carbon electrode surface using cyclic voltammetry technique. Electrochemical methods including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used for characterization of the modified electrode. It was also effectively employed for the detection of G.A using cyclic voltammetry and square wave voltammetry (SWV) techniques. From the electrochemical investigation, choline chloride exhibited excellent electro catalytic behavior for the detection of G.A and increased electron transfer rate. The effects of several electro analytical experimental conditions were also optimized for sensitive determination of G.A. The irreversible electrochemical behavior of G.A at the ChCl/GCE is an adsorption controlled process involving transfer of electrons. Under the optimized conditions, the SWV result indicated that the oxidation peak current of G.A at the ChCl/GCE was directly proportional to its concentration over linear range of 1.6 μM to 240 μM with limit of detection 0.126 μM, limit of quantification 0.419 μM. The ChCl/GCE has also showed excellent selectivity, reproducibility and long-time stability towards the electrochemical oxidation of G.A. Moreover, the practical applicability of the prepared ChCl/GCE was evaluated in order to determine G.A in red wine, black tea and green tea samples with recovery ranging from 92.20 % – 106.8 %.

Key words: Gallic acid, Choline chloride, Square wave voltammetry, Modified glassy carbon electrode.

1. Introduction

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is a naturally occurring and the most abundant phenolic acid in plant and the building block of polyphenols (hydrolysable tannins) [1, 2]. Gallic acid is organic acid which contain multiple hydroxyl group. It inhibits lipid peroxidase and break free radical chains by providing multiple protons [3].The fast and simple Gallic acid determination was important for quality control of foods. The phenolic content or antioxidant capacity of foods was typically expressed by Gallic acid amount present there [3]. Gallic acid was widely distributed across all part of the plant such as leaves, roots and bark [1, 2]. A large number of plants were rich sources of Gallic acid either in free form or as a part of tannin molecule. It was present in a wide variety of fruits such as bananas, citrus fruits and mangoes, as well as in tea leaves (green tea and black tea), beverages (red wine), and various medicinal plants [4, 5]. Gallic acid is known to have hepatoprotactives, cosmetics, pharmaceuticals, and anti-HIV, anti-inflammatory, anticancer and antioxidant scavenging ability against free radical activity. It also seems to have antifungal, antiviral and antibacterial properties. Gallic acid was found to show cytotoxicity against cancer cells without harming healthy cells. Gallic acid was used as a remote astringent in cases of internal hemorrhage (6).But it has no any significant side effect on health or organ damages [7]. Up to now there are several analytical methods were used for determination of Gallic acid like spectrophotometry [8], high-performance liquid chromatography (HPLC) [9], gas-capillary electrophoresis [10] thin layer chromatograph [11], mass spectrometry (MS), flow injection–chemiluminescence (FCL) and electro chemical method [6]. However the listed methods required sophisticated laboratories, well trained instrumental operators, an expensive instrument, complicated analysis procedure and time consuming [12]. But among these mentioned methods recently electro chemical techniques was mostly used due to its simplicity, portability, low cost, time saving, convenient operation, speedy response, high efficiency, sensitivity and selectivity, provide information about the reaction mechanisms [13]. However chemically modified electrodes have a great importance to determine the interest analyte with high selectivity and sensitivity. Therefore a simple, rapid, cost effective, sensitive and selective method would be developed for the determination of G.A. Glassy carbon electrode was widely used as a working electrode for the determination of interest analyte. Because of easy modification, low back ground current, wide potential window, low charge transfer resistance and low cost [14]. But electro chemical determination of analyte at bare glassy carbon electrode causes to decrease the rate of electron transfer due to inactivation of the surface of the electrode by the adsorption of the reaction product [15]. So far a modification of working electrode surface was very important to avoid the electrode fouling and to maximize the selectivity, sensitivity and stability of the electrode surface. Modifiers of the electrodes increase the selectivity and sensitivity towards the analyte due to increasing the electro active surface area of electrode by its oxygen containing functional groups [16]. In this study choline chloride modified glassy carbon electrodes (ChCl/GCE) was prepared by CV technique for the determination of Gallic acid. The electrochemical behavior of Gallic acid at the surface of ChCl/GCE was investigated and the analytical performance of ChCl/GCE for Gallic acid determination in Red wine, black tea, and green tea samples was evaluated.

1.1. Rational and motivations

Phenolic compounds are one of the largest categories of plant secondary metabolites found in many different plant species and used for medical purposes. Gallic acid was one of the natural phenolic compounds and most commonly exist in banana, citrus fruits and mangoes, green tea, black tea, red wine, beverages and various medicinal plants. Gallic acid was commonly used as an anti-inflammatory, antibacterial, antiviral, hepatoprotactives, and production of cosmetics, pharmaceuticals, anti HIV, anticancer and antioxidant drug. Gallic acid has no any side effect in excess amount. Moreover; Gallic acid was used in analytical chemistry as food additive and preservations. Due to the therapeutic activity of Gallic acid, it was found important to develop a very sensitive method for its analysis. The conventional analytical methods developed for analysis of Gallic acid are quite expensive; require complicated and tedious sample preparation procedures, need skilled personnel and not suited for onsite measurements. Therefore, to overcome these limitations many researchers at different time have developed electro analytical methods to determine Gallic acid by modifying electrodes using organic polymers, inorganic complexes and different single and composite nanomaterial’s. However, these methods showed low sensitivity and selectivity in some cases and it involved complicated and longtime preparation procedure. Considering these aspects, in this study simpler, faster, cheaper and environmentally friendly square wave and cyclic voltammetric method was developed for sensitive and selective determination of Gallic acid based on choline chloride modified glassy carbon electrode.

1.2. Objectives of the Study

1.2.1. General Objective

The general objective of this research work is to develop simple and low cost electro chemical sensor for sensitive, selective, and rapid determination of Gallic Acid.

1.2.2. Specific objective

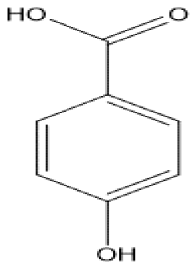
The specific objective of this study was

- ✓ Modify conventional glassy carbon electrode with choline chloride
- ✓ Characterize choline chloride modified and Bare GCE electrodes using cyclic voltammetry(CV) and electro chemical impedance spectroscopy (EIS)
- ✓ Study the catalytic effect of the modified electrode towards the electro chemical oxidation of Gallic acid.
- ✓ Set the optimum working conditions for the determination of Gallic acid.
- ✓ Establish the analytical performance characteristics such as linear response range, detection limit, repeatability, reproducibility, and stability of the modified electrodes.
- ✓ Investigate the selectivity of the method towards potentially interfering substances
- ✓ Investigate the analytical application of modified electrodes for quantitative determination of Gallic acid in red wine, black tea and green tea sample.

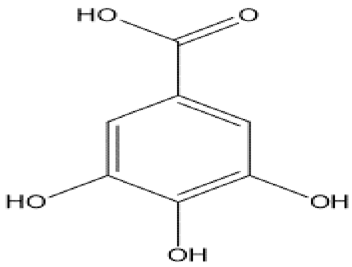
2. Literature review

2.1. Gallic Acid

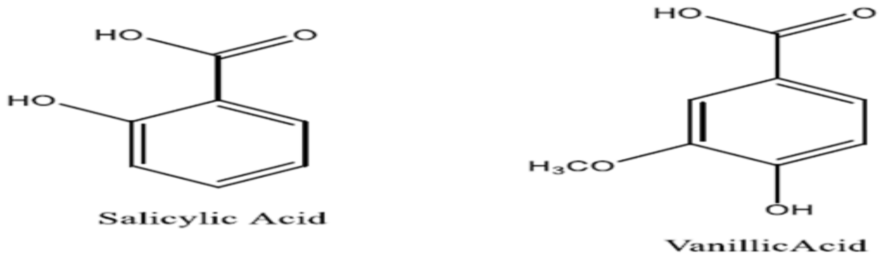
There is different hydroxyl benzene or also known as phenolic compounds were found in our world. Phenolic compounds include a large class of compounds that are commonly occurred in nature. They were widely found in fruits and vegetables with a wide range of phenolic contents [10]. In fact the benefits related to consumption of phenolic rich foods are associated with their antioxidant activities which are determined by their reactivity as hydrogen or electron donating agent [17]. Among naturally occurring phenolic compounds phenolic acids and flavonoids have particular interest due to their biological properties. Phenolic compounds are widely found in plant-derived foods and due to their metabolites they have health benefits on human. It is important for anticancer drugs and over 50% of medicinal used in antitumor [18]. Some of the phenolic compounds, such as Gallic acid (3,4,5-trihydroxybenzoic acid, GA) and its derivatives have different biological activities against various cell lines [19], like antibacterial, anti-inflammatory, and antitumor activities, scavenging of free radicals and protection against cardiovascular diseases, anticancer and antidiabetic [20]. G.A has also beneficial effects of widespread use as antioxidants in the pharmaceutical and cosmetic industries [21]. G.A is one of the main natural phenolic acids existing in tea leaves (both green and black tea), grapes (grape seeds), fruits such as strawberries, berries and other fruits, as well as in many other processed herbs and drinks [22]. Phenolic compounds were the most antioxidant in diet. Their chemical structures were made from effective antioxidant molecules due to the presence of hydroxyl group bonded to aromatic ring. The arrangement of the hydroxyl group on the aromatic ring has a very large influence on the antioxidant activity of phenolic acids [23]. The antioxidant or anti-radical activities of phenolic acids could be affected by the position of hydroxyl group and other functional groups [24]. Some of the phenolic acids were



p-hydroxybenzoic Acid



Gallic Acid


Salicylic Acid

VanillicAcid

Figure 1: Chemical structure of phenolic acid

Gallic acid was a phenolic acid which has three hydroxyl groups attached to the aromatic ring with in ortho- position with respect to each other. Due to the presence of these groups Gallic acid has higher antioxidant capacities than other phenolic acids [25]. Gallic acid contains four acidic protons with Varity of pKa values =4(COOH), 8.7, 11.4 and 13(OH) [26].The three hydroxyl group attached to the aromatic ring leads to the formation of hydrogen peroxide, semiqunone and Quinone [27].Antioxidants were organic molecules or compounds found naturally in plants and animals. It was used as inhibitors to the oxidations of substrate and they also scavenge free radicals and form other new stable radicals. Antioxidants are components of phenolic compounds [28].

2.1.1. **Physicochemical properties of Gallic acid**

In pure state Gallic acid was colorless, odorless, white crystals, has relative molecular mass of 170.11 g/mol, melting-point 260 ^{0}C, pKa 4(COOH) and 10 for (OH) and it was more soluble in water, alcohol, ether, glycerol, acetone but insoluble in chloroform and benzene. It could be decomposed at temperatures between 235 °C and 240 °C producing CO_2 and CO [29].

2.1.2. Occurrence of Gallic acid

The name Gallic acid derived from oak galls, which were used to prepare tannic acid. Gallic acid was found in the number of land and aquatic plants [30]. Food staff containing various amount of Gallic acid especially fruits and vegetables such as strawberry, grapes, banana, blueberry, apple, red wine, green tea, black tea, avocado, blackcurrant, guava, mango, mulberry, and pomegranate [31, 32].

2.1.3. Application of Gallic acid

Antioxidants were compounds that can bind to and remove free radicals formed in the human body. Free radicals are unstable molecules that may cause cellular damage when their levels become too high [18]. Amount of free radicals in the body leads to cause various chronic conditions, such as autoimmune disorders, aging, cardiovascular disease, neurodegenerative diseases, cancer, heart disease and diabetes [19].

2.2. Choline chloride

Choline chloride (2-hydroxyethyl trimethylammonium chloride, $C_5H_{14}ClNO$, ChCl) is a water-soluble organic molecules and a quaternary ammonium salt which can be used as a catalyst in reactions. It appears as an odorless, white crystalline substance. It contains choline cation with chloride anion as shown in the Figure-2. Choline is a bifunctional molecules containing both quaternary ammonium cation ($–N^+(CH_3)_3$) and a hydroxyl (–OH) functional groups [33]. It is an essential nutrient which is considered as the synthetic form of vitamin B4 and used as animal food supplement mostly for chickens where it accelerates growth. It is also a precursor to biologically active compounds such as acetylcholine (a neurotransmitter) and phosphatidylcholine (membrane component) [34]. ChCl is widely used as an organic salt to produce solvent with urea, ethylene glycol, glycerol, and many other compounds [35]. It is also used as a clay control additive in fluids used for hydraulic fracturing [36]. ChCl is considered very cheap since it is a waste product of soybean oil production also nontoxic to both humans and the environment. It is approved by the Food and Drug Administration (FDA) as a human nutrient [35].

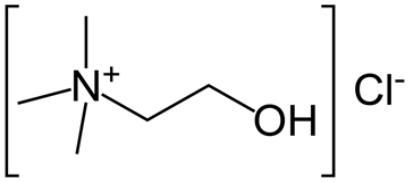


Figure 2: Chemical structure of choline chloride

Currently, there has been considerable interest in developing ChCl modified electrodes for electro analysis due to its low cost, non- toxicity, biodegradability and biocompatibility. For instance, choline chloride modified glassy carbon electrode has been used for simultaneous determination of dopamine, serotonin and ascorbic acid [37]. But mostly choline chloride has been used as a supporting material to construct nanoparticles-modified electrodes, such as gold nanoparticles on choline chloride modified glassy carbon electrode for sensitive detection of nitrite [38], zirconia-choline chloride-gold nanoparticles modified carbon paste electrode for simultaneous detection of Gallic acid and uric acid [39], gold nanoparticles modified choline chloride functionalized graphene oxide for detection of meloxicam [40], silver nanoparticles modified choline chloride functionalized graphene oxide modified carbon paste electrode for detection of celecoxibe[41] and others. The chemistry of choline chloride modification could be related to the covalent bonding of choline to the oxidized carbon surface. The hydroxyl (–OH) group of choline chloride enables it to be immobilized or assembled onto the edge plane sites of the carbon electrodes through covalent bonding to form ether linkage. Furthermore, the quaternary ammonium cation polar head group ($-N^+ (CH_3)_3$) of choline chloride can provide a positively charged stable surface which can facilitate electrostatic interaction and decrease the resistance of the electron transfer at the electrode surface [37, 38].

2.3. Electrochemical sensors

Chemical sensors are solid state device that gives data and general information about their immediate environment. The sensors were used for the transformation of signals that generated by a chemical reaction of interested analyte in to usable quantitative or qualitative information then changed to analytical signals. The sensor signals were typically electronic in nature like current, voltage and conductance usually formed by analytes. Chemical sensors were made of physical transducers and chemically sensitive layers. The transducers could transform the response from chemically sensitive layer in to detectable signals and observed on the modern instruments/software. Electro analytical methods were widely used in scientific studies and monitoring of industrial materials, pharmaceutical compounds, biological samples, and the environment [42]. An electrochemical sensor has broad aspects in physical and analytical chemistry, material science, biochemistry, solid-state physics, device fabrication, electrical engineering, and even statistical analysis [43]. Electrochemical sensors represent the most rapidly growing class of chemical sensors. Chemical sensors can be classified according to the property to be determined as: electrical, optical, mass or thermal sensors and they are designed to detect and respond to an analyte in the gaseous, liquid or solid state. Compared to optical, mass and thermal sensors, electrochemical sensors are especially attractive because of their remarkable detection ability, experimental simplicity and low cost. Due to these properties electrochemical sensors has a wide range of applications in the fields of clinical, industrial, environmental and agricultural analyses [44]. Electrochemical sensors are classified into three main types: potentiometric, voltammetric/amperometric and conductometric. Potentiometric sensors were based on measuring the potential of an electrochemical cell without producing appreciable current. Voltammetric sensors were based on the measurement of the current flowing through the working electrode immersed in a solution containing electro-active compounds, while a potential scanning was imposed on it. It measures the current-potential behavior at an electrode surface. In voltammetric sensors, the potential was varied in some systematic manner to cause oxidation or reduction of the electro active chemical species at the electrode. The resulting current was proportional to the concentration of the oxidized/reduced electro active species [45, 46]. Voltammetric sensors are applicable in the use of applied potential between a reference and a working electrode to cause the oxidation or reduction of an electro active species and measure the resulting current. They were based on the measurement of current at a fixed operating potential. If this potential was conveniently chosen, then the magnitude of the current was directly proportional to concentration. This current was the result of electrochemical oxidation or reduction of the electro active compound. Conductometric sensors involve the measurement of conductivity at a series of frequencies [44].

2.3.1. Chemically modified electrodes

Electrodes encounter specific phenomena that reduce their applicability; among these are fouling of the electrode by unwanted precipitation or adsorption processes and the slow electrochemical reaction rates of some species that require application of over potential to cause the desired reaction to occur. These phenomena often can be controlled by modification of the electrode surface which is done by deliberately attaching or incorporating chemical species onto the electrode surface to alter its electrochemical features. According to IUPAC definition a Chemically Modified Electrode (CME) was an electrode made of conducting or semiconducting material that was coated with a selected monomolecular, multimolecular, and ionic or polymer film chemical modifiers by means of charge transfer (faradic) or no net charge transfer(non-faradic) reactions that shows the chemical, electrochemical and optical properties of the films. Chemically modified electrodes were electrical conductors that has surface modified for different applications [47]. Currently the chemical modification of carbon surfaces with different functional groups becomes an active area of research in electrochemistry. The stronger attachment to the electrode surface can be accomplished by covalent linking of the desired component to surface of the electrode. The electrode surface is usually pretreated by an oxidative reaction to form active surface groups. Then the surface is treated with the linking agent [45]. There were different electrode modifiers, including: nano materials, polymers, perm selective membranes, organic ligands, lipids, surfactants, organic and inorganic micro particles, organometallic or inorganic catalysts, ion exchangers, biological materials, clays, zeolites, silicates and so on. The reactions of the bare electrode with modifiers are shown in figure 3.

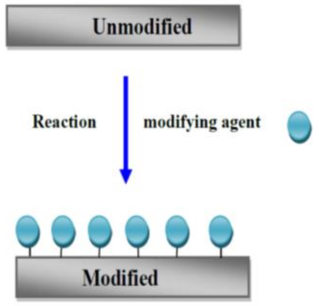


Figure 3: Immobilization of a modifying agent on the electrode surface

The use of coating electrode surface with adsorbed layers or film can change electron transfer rate on an electrode surface. Electro catalysis at modified electrode was usually an electron transfer reaction between the electrodes and the nearby solution decided by an immobilized modifier, which continues at lower over potential; otherwise occur at bare electrode [46].

There are four methods of modifying electrodes these are:-

(1) Chemisorption (Adsorption): Here the forces involved are the valence forces of the same as those operating in the formation of chemical compounds. The chemical film is strongly, ideally and irreversibly adsorbed (chemisorbed) onto the electrode surface. This methods usually produces monolayer (or less) coverage. This type of modification are the substrate-coupled self-assembled monolayers (SAMs) in which uncorrelated molecules spontaneously chemisorbs at specific sites on the surface of the electrode [48].

(2) Covalent Bonding: Linking agents, such as, organosilanes or cyanuric chloride are used to covalently attach from one to several monomolecular layers of the chemical modifier to the electrode surface [46].

(3) Composite: The chemical modifier is simply mixed with an electrode matrix material, as in the case of an electron-transfer mediator (electro-catalyst) combined with the carbon particles (plus binder) of a carbon paste electrode. Alternatively, intercalation matrices such as certain, zeolite, clays and molecular sieves can be used to contain the modifier [49].

(4) Polymer Film Coating: Electron conductive and nonconductive polymer films are held on the electrode surface by combination of chemisorption and low solubility in the contacting solution or by physical anchoring in a porous electrode. The polymer film can be organic, organometallic or inorganic [50].

2.3.2. Purpose of developing chemically modified electrodes

For the advancement in the field of electrochemical sensors chemists commonly used modified electrodes rather than bare surface electrodes to obtain an interested result in their investigation. The reasons behind that bare surface of electrodes are required certain chemical and physical properties that did not naturally exist in it which used as electrical conductors. Then to solve these problem bare electrodes required chemical modification for best used. Atoms, molecules, and nano particles are attached to the bare surface electrodes to modify their electronic and structural properties, which leading to change their function [51].

2.3.3. Application of chemically modified electrode

Chemically modified electrodes have various advantages compared to unmodified electrodes. CME provide a powerful route to change the performance of electrodes. The modification of sensors facilitated the processes involved in electro analytical chemistry were catalyzing the redox reaction of species that shows high over potential at unmodified electrodes, They also increase sensitivity, selectivity and stability of the electrode in the electrochemical detection, resist fouling concentrating species, improving electro catalytic properties, and limiting of interference in samples. Electro catalysis at a modified electrode is usually an electron transfer reaction which is catalyzed by a suitable electron transfer mediator attached to the electrode surface. The function of the mediator is to facilitate the charge transfer between the analyte and the electrode in order to lower over potential that would be applied at the bare electrode [52].

2.4. Conducting polymers (CPs)

The terms polymer and monomer were derived from the Greek words poly (many), mono (one) and meres (part) which is a large molecule, or macromolecule, composed of many repeated subunits, known as monomers [53]. Polymers have long been known as insulating materials and are often used to insulate cables and electrical devices. However, there are also a number of polymers that are electrically conductive [54]. In general, conducting polymers include electronically conducting polymers and ionically conducting polymers. Ionically conducting polymers are usually called polymer electrolytes. Electronically conducting polymers can also include conjugated conducting polymers and the insulating polymers blending with conducting materials. Conjugated Conducting polymers possess alternating single and double bonds and the optical, electro chemical, and electrical properties of the conducting polymers due to their pi conjugated systems. The most known parameters that affect physical properties of conducting polymers are their conjugation length, degree of crystallinity, and intra and inter chain interactions [55]. Conducting polymers have unique chemical structures and are easy to assemble or deposited into many electronic devices. CPs provides the advantage of chemical diversity, low density, flexibility, corrosion resistance, easy-to-control shape and morphology, and tunable conductivity over their existing inorganic counter parts. CPs has been modified with other heterogeneous material components to overcome their inherent limitations in terms of solubility, conductivity, and long-term stability. Conducting polymers such as polythiophene, polyfuran, polypyrrole, poly (phenylene), poly fluorene and Polyaniline, Polyvinylenes, polyarylenes and polyheterocycles are the major classes. Furthermore, electro polymerization has been successfully used for the immobilization of polymers to control their thickness and uniformity. The electrochemical polymer modified electrodes are highly selective and sensitive to a wide range of electro active substances [56].

2.5. Electro analytical techniques

Electro analytical techniques were concerned with the relation between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential, or charge and their relationship to chemical parameters. Such use of electrical measurements for analytical purposes has found a wide range of applications, including environmental monitoring, industrial quality control, and biomedical. These techniques are capable of producing exceptionally low detection limits and wealth of characterization information describing electrochemical addressable systems. Electrochemical techniques were a group of quantitative analytical methods that depend on the electrical properties of a solution of the analyte. They use relatively inexpensive equipment produce unique characterization information for molecules and chemical systems, qualitative and quantitative analytical data, thermodynamic and kinetic data. Electrochemical techniques are powerful versatile analytical techniques that offer high sensitivity, low detection limits, accuracy and precision as well as a large linear dynamic range [57].

There are many different electrochemical techniques. Among these, potentiometry, voltammetry, and electrochemical impedance spectroscopy (EIS) represent the most used electrochemical techniques for sensor and biosensor fabrication and detection of analyte.

2.5.1. Advantage of electrochemical techniques

Electro analytical methods are well established and use relatively inexpensive equipment to produce unique characterization information for molecules and chemical systems qualitative and quantitative analytical data, thermodynamic data, and kinetic data. Electro analytical methods are sensitive; they are able to detect sub micro molar concentrations and sub Pico mole amounts of electro active material. Therefore, the methods are selective; they are able to control the potential of an electrode, which makes it possible to determine the electrochemical "spectrum" of electro active species in solution [57].

2.5.2. Voltammetry

Voltammetry refers to a class of electro analytical techniques that is used to designate the current voltage measurement obtained at a given electrode. Voltammetric techniques can be used for qualitative detection of electro active species, quantitative determination of amount of dissolved inorganic and organic compounds, to study in detail the electron transfer process of species and for understanding the redox behavior of species including both kinetic and thermodynamic aspects of the electron transfer processes. It is the most power full and versatile technique for research purposes based on the measurement of current flowing through an electrode immersed in solution containing electro active compounds while a potential is imposed upon it. Voltammetry is typically performed using a three electrode system connected to a potentiostat, which accurately controls the applied potential. The redox reaction takes places at the working electrode, because the working electrode the reaction of interest is taking place. This electrode could be made of several materials. Usually, it has a very little surface in order to assume quickly and accurately the potential imposed by the electrical circuit. The second electrode is a reference electrode, which maintains a constant potential throughout the experiments, and the third electrode is the counter electrode, which complete the electrical circuit. The counter electrode also known as the auxiliary electrode, is often much larger than working electrode to minimize current density at the electrode surface [47]. Generally the potential is applied between working electrode and the reference electrode whereas the current is measured between the working electrode and counter electrode. There are different types of voltametric techniques; these are cyclic voltammetry, square Wave Voltammetry and differential pulse voltammetry. The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell [58]. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of E, i and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces change in the concentration of an electro active species at the electrode surface by electrochemically reducing or oxidizing it. The plot of the resulting current as a function of the applied potential obtained from a voltammetry experiment is known as voltammogram. Voltammetric techniques provide various benefits, including high levels of sensitivity and selectivity, a broad range of temperatures, wide linear concentration range, rapid response, the capacity to assess kinetic and mechanistic parameters and the simultaneous analysis of many analytes [58].

The general schematic representation of voltametric techniques are shown in Figure 4.

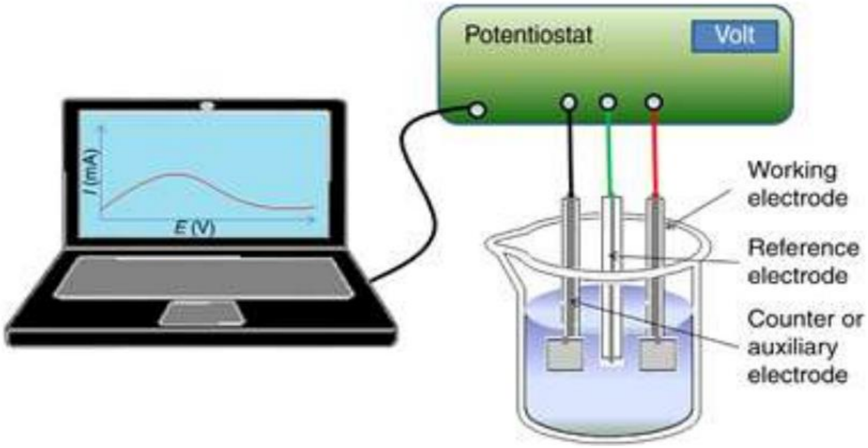


Figure 4: Electro analytical set up

2.5.3. General use of voltammetric method

- Quantitative determination of organic and inorganic compounds in aqueous and non-aqueous solutions
- Measurement of kinetic rates and constants
- Determination of electron transfer and reaction mechanisms
- Determination of thermodynamic properties of solvated species
- Fundamental studies of oxidation and reduction processes in various media
- Determination of complexion and coordination values
- Quantitative determination of pharmaceutical compounds
- Determination of metal ion concentrations in water to subparts-per-billion levels
- Determination of redox potentials
- Detection of eluted analytes in high-performance liquid chromatography (HPLC) and flow injection analysis [47].

2.5.4. Cyclic voltammetry

Cyclic voltammetry is the most widely used voltametric technique for acquiring qualitative information about electrochemical reactions. The function of cyclic voltammetry is ability to rapidly provide considerable information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron transfer reactions, and on coupled chemical reactions or adsorption processes. Cyclic voltammetry is often the first experimental approach performed in an electrochemical study of a compound, a biological material, or an electrode surface. The effectiveness of CV results from its capability for rapidly observing the redox behavior over a wide potential range. The resulting voltammogram is an analogous to a conventional spectrum in that it conveys information as a function of an energy scan. CV consists of cycling potential of an electrode, which is immersed in an unstirred solution, and measuring the resulting current. The potential of this working electrode is controlled by reference electrode such as a silver/silver chloride (Ag/AgCl). The controlling potential which is applied across these two electrodes can be considered an excitation signal. The excitation signal for CV is a linear potential scan with a triangular wave form as shown in Figure 5. This triangular potential excitation signal sweeps the potential of the electrode between two values, sometimes called the switching potentials [60]. In Cyclic voltammetry, the direction of the potential is reversed at the end of the first scan. Thus, the waveform is usually the form of an isosceles triangle with the advantage that the product of the electron transfer reaction that occurred in the forward scan can be probed again in the reverse scan. It is powerful tool for the determination of formal redox potentials, detection of chemical reactions that precede or follow the electrochemical reaction and evaluation of electron transfer kinetics. Different voltammetric techniques are notable mainly by the potential function that is applied to the working electrode and by the material used as the working electrode.

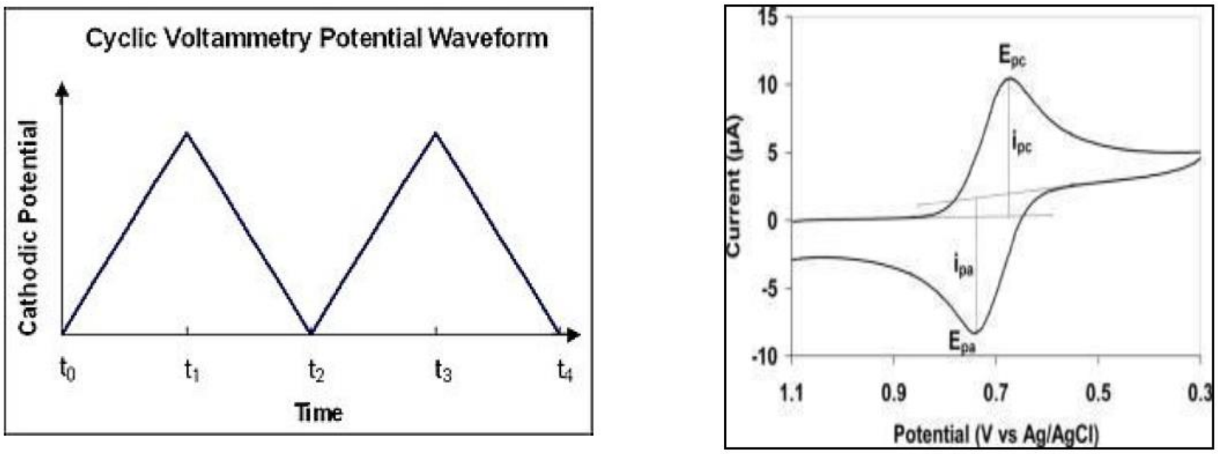


Figure 5: The triangular potential-excitation signal of cyclic voltammogram

A cyclic voltammogram is found by measuring the current at the working electrode throughout the potential scan. The current measured can be deliberated as the response signal to the potential excitation signal. The voltammogram shows of current (vertical axis) verses potential (horizontal axis). Because the potential varies linearly with time, the horizontal axis can also be thought as time axis [59]. The important parameter of a cyclic voltammogram are the magnitude of the anodic peak current (Ipa), cathodic peak current (Ipc), anodic peak potential (Epa) and cathodic peak potential (Epc). The formal reduction potential E^o, for reversible couple is centered between Epa and Epc.

$$E^o = \frac{Epa + Epc}{2} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (1)$$

The number of electrons transferred in the electrode reaction (n) for reversible couple can be determined from the separation between peak potentials (for reverse couple) is given by

$$\Delta E = E_{pa} - E_{pc} = 0.059 / n \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2)$$

Thus, a one electron process such as the reduction of $[Fe (CN)_6]^{3-}$ to $[Fe (CN)_6]^{4-}$ exhibits a

$\Delta E = 0.059$ V. The peak current for a reversible system is described by the Randles-Sevcik equation for the forward sweep of the first cycle

$$I_p = 2.69\times10^{5} n^{3/2} A\, D^{1/2}\, C\, v^{1/2} \text{---} [3]$$

Where n is the number of electrons, A is electrode area (cm^2), D is the diffusion coefficient (cm^2/s, C is the concentration (mol/cm^3) and ν is the scan rate (in V/s). Thus, a great deal of information learned about the system by varying known parameters, such as concentration and scan rate. The peak current is linearly proportional to the bulk concentration, C, of the electro active species, and the square root of the scan rate, $\upsilon^{1/2}$. Thus, an important diagnostic is a plot of the ip vs $\upsilon^{1/2}$. If the plot is linear, it is reasonably safe to say that the electrode reaction is controlled by diffusion, which is the mass transport rate of the electro active species to the surface of the electrode across a concentration gradient. If the anodic peak current ipa is equal to the cathodic peak current ipc, so that the relationship,

$$\frac{ipc}{ipa}=1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (4)\ [52].$$

2.5.5. Square Wave Voltammetry

Square wave voltammetry is the most sophisticated technique in the family of pulse voltammetric techniques. Square wave voltammetry is a large-amplitude differential technique in which the wave form composed of a symmetric square wave, superimposed on a base staircase potential applied to the working electrode. The current is tasted twice during each square wave cycle, once at the end of the forward pulse and once at the end of the opposite pulse. Since the square wave modulation amplitude is very large, the reverse pulses cause the reverse reaction of the product [57].

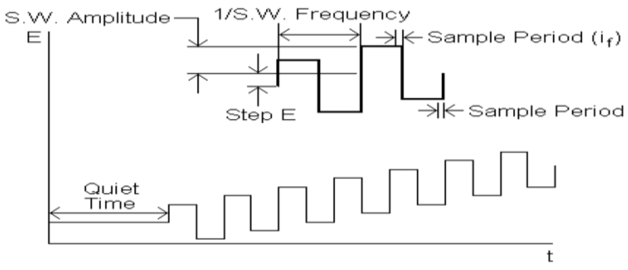
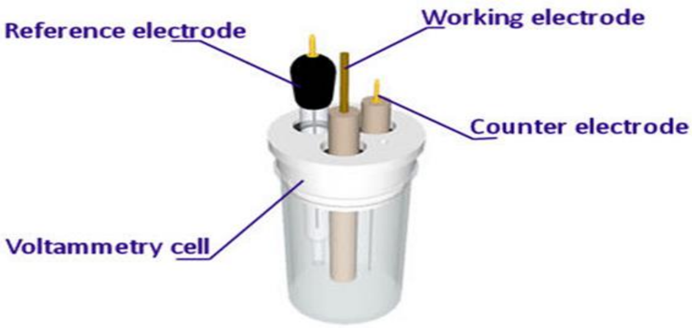


Figure 6: Applied potential wave form for square wave voltammetry

In general, square wave voltammetry possesses excellent sensitivity. The sensitivity is higher than from differential polar graphic techniques coupled to the effective discrimination against the charging background current, very low detection limit can be attained. The major beneficial of square wave voltammetry is its speediness. The analysis time is very short, complete voltammogram can be recorded within a few seconds as compared to 2-3 min in differential pulse voltammetry. An additional advantage of square wave voltammetry is that its fast scans capability to determine changes in voltammetric responses with time nondestructively [52].

2.6. The Electrochemical Cell

Electrochemical cell is an instrument where voltametric measurements were carried out. An electrochemical cell consists of solution of analyte (interest), supporting electrolyte and the electrodes which used to voltametric measurement. The electrochemical cell in voltamertric measurement is consisting of three electrodes. These are working electrode, reference electrode and counter electrode those connected with potensistat [47].



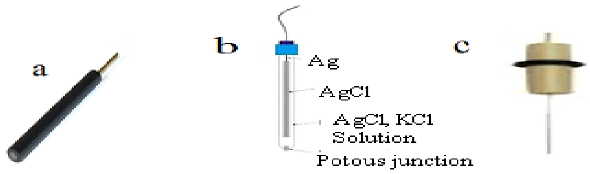


Figure 7: Types of electrodes (a) working electrode, (b) reference electrode, and (c) counter electrode.

2.6.1. **Working Electrodes**

Working electrode is an electrode the reaction of the interest is takes place. The reduction – oxidation of the substance occur at the appropriate applied potentials. During the reaction there is mass transport of the new materials to the electrode surface and generate current. The potential of the working electrode was thus calculated from the difference between the applied potential and the potential of the counter electrode. The surface of the working electrode can have a significant effect on the current response in voltammetry experiments. The working electrode is often used in conjunction with an auxiliary electrode and a reference electrode in a three electrode system. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode can be referred to as either cathodic or anodic [60]. Working electrode is renewable which is easily pretreated and polished. The most common method for surface cleaning of glassy carbon electrode is mechanical polishing. Some examples of polishing materials are diamond, alumina, and silicon carbide. There are different kind of working electrode; such as Platinum electrode (Pt), Gold electrode (Au), Glassy carbon electrode (GCE), Silver electrode (Ag) and Carbon paste electrode. Generally the potential is applied between working electrode and reference electrode whereas the current is measured between working electrode and counter electrode [61].

2.6.2.Reference Electrodes

Reference electrodes are electrodes which have a stable and well known electrode potential and used to give a value of potential to which other potentials can be referred in terms of a potential difference, as potentials can only be registered as differences with respect to a chosen reference value. When an external potential is applied to the working electrode the potential of the reference electrode shouldn't change and it also needs to be insensitive to the composition of the analyte solution. The three most commonly used reference electrodes are standard hydrogen electrode (SHE), silver/silver chloride electrode (Ag/AgCl) and calomel electrode ($Hg_2Cl_2(s)/Hg$) [62].

2.6.3.Counter Electrodes (Auxiliary Electrodes)

The counter electrode must be inert in the conditions to which it is exposed in order to avoid contamination of the solution [59]. Good examples of counter electrodes are platinum, thin pieces of gold and graphite. The fundamental requirement is that to pass sufficient current into the solution without needing an excessive cell voltage or creating a non-uniform current distribution on the working electrode. Counter electrode (Auxiliary electrode) is a non-reactive and stable electrode with high surface area to prevent the limitation of current passing through an electrode which is used to close the current circuit in the electrochemical cell [63].

a) Serve to supply the current required by the W.E. without limiting the measured response.

b) Current should flow readily without the need for a large over potential.

c) The reaction products of C.E. should not interfere with the reaction being studied.

d) Should have a large area compared to the W.E. and should ensure equipotentiality of the W.E.

e) Usually long Pt wire (straight or coiled) or Pt meshes (large surface area).

2.6.4. Supporting Electrolyte

Supporting electrolyte: is a solution containing ionizable salts or compounds that are not redox active in a given potential window and whose ionic strength is much greater than the dissolved electro active substance. An electrochemical experiment required supporting electrolytes to increase the conductivity of the solution, eliminate electro migration effects, maintain a constant ionic strength, pH and complete the electrical circuit. They are also necessary to limit analyte migration through the use of electrolyte in high concentration [46]. High electrolyte concentration relative to the analyte concentration ensures the charge balances migrate to the electrode surface (electro migration), resist quickly oxidized or reduced. The supporting electrolyte should be highly soluble in the selected solvent, chemically and electrochemically inert in the experiment conditions, and they should be prepared from highly purified reagents. Supporting electrolyte may be an inorganic salt, a mineral acid, a buffer solution, a chelating reagent, strong acids or bases [63].

2.7. Potentiostat

Potentiostat is a device that applies an exact potential and monitors the changes in the current in the electrochemical system. The modern way potentiostat consists of various components like electrometer circuits, various converters and amplifiers, as well as microprocessors with internal memory. The system worked by scanning or controlling the potential of working electrode with respect to the reference electrode at which no current flows, the current only passes through the working and the counter electrodes. The potential of the counter electrode needs to be adjusted to run an electrochemical reaction opposite of the working electrode reaction [64].

2.8. Glassy Carbon Electrode

Glassy carbon electrodes have been widely used in electro analytical chemistry because of their excellent mechanical and electrical properties such as broad potential window, low back ground current, high temperature resistance, hardness, low density, low electrical resistance, low friction, extreme resistance to chemical attack and impermeability to gases and liquids. To enhance their analytical performance and to create active and reproducible glassy carbon electrodes surface pretreatment is usually employed. The electron transfer rate or adsorptive behavior of the GCE is also increase with modification [46].

Figure 8 : Common glassy carbon working electrode

3. EXPERIMENTAL

3.1. Chemicals and reagents

Gallic acid from Carloerba reagent, choline chloride (≥99%), acetic acid (99.8%) and ethanol (≥99.5%) were obtained from Sigma-Aldrich, USA. potassium hexacyanoferrate (III) (≥99%), potassium chloride (99.5%), ortho-phosphoric acid (85%), from Riedel-de Haën, France. sodium hydroxide and hydrochloric acid were purchased from Sigma-Aldrich. Ascorbic acid (99%), sodium acetate (99-101%) was obtained from BDH, England. Citric acid (99%), disodium hydrogen phosphate (≥ 98%), sodium dihydrogen phosphate and sodium citrate were obtained from Research Lab Fine Chem Industries, India. urea (≥99.5%) were obtained from Hopkins and Williams LTD, England. Sucrose (≥95%) from Pharmacos, LTD, England and boric acid (99.5%) was obtained from Carloerba reagent. All of the chemicals and reagents used in this study were of analytical grade, and they were used as received without any further purification.

3.2. Apparatus and instrumentation

All electrochemical experiments for the determination of Gallic acid was involved by model CHI630A electrochemical workstation (CH Instruments, USA) which is controlled by a desktop computer used for the electrochemical measurements of CV and SWV. Electrochemical impedance spectroscopic experiments were performed on a CHI760D electrochemical workstation (CH Instruments, Inc., Austin, Texas, USA) and ZAHNER EIS machine (Germany). A standard three electrode system was used for all electrochemical experiments where bare glassy carbon electrode (BGCE) or choline chloride modified glassy carbon electrode (ChCl/GCE) were used as working electrodes, Ag/AgCl (3 M KCl solution) as reference electrode and platinum wire as counter electrode. During solution preparation, a digital balance (Model: Scientech: ZSA 120) was used to weigh solid chemicals. A pH meter (senses ion TM + MM150, China) was used for pH measurements. After polishing of the glassy carbon electrodes ultrasonic cleaner power (YJ5120-B, shanghai, china) was utilized for cleaning the electrode. Centrifugator (Model: 800-1) was used for centrifugation of wine and tea sample. The measurements were all carried out at room temperature.

3.3. Preparation of standard and choline chloride solutions

A standard stock solution of Gallic acid (4 mM) was prepared using distilled water and stored at 4 ^{o}C until use. The working solutions of lower concentrations were prepared daily by appropriate dilution of the stock solution with 0.1 M BRB pH6. 0.1 M BRB solutions were prepared from the mixing of equimolar (0.1 M) of phosphoric acid, boric acid and acetic acid. 2.0 mM choline chloride solution containing 0.01 M KCl was prepared in 0.1 M phosphate buffer solution (pH 7). Phosphate buffer solution (PBS) was prepared by mixing 0.1 M sodium dihydrogen phosphate and 0.1 M disodium hydrogen phosphate and the required pH of the solution was adjusted by NaOH and HCl.

3.4. Preparation of ChCl/GCE

Before the deposition process, the BGCE was polished carefully with 0.05 µm alumina slurry on a polishing pad and rinsed repeatedly with distilled water. Then, the GCE was sonicated for 3 min in a 50:50 solution of ethanol and distilled water to remove any remaining polishing material from the electrode surface. Then after, the cleaned GCE was electrochemically activated in PBS (pH 7) using CV technique in the scanning potential between -0.2 to 1.5 V for five cycles at a scan rate 100 mV/s. Next for the electrochemical modification of the GCE, the cleaned GCE was inserted into 2.0 mM ChCl solution containing 0.01 M KCl. CV technique was used in the potential scanning range between -1.7 to 1.8 V at scan rate of 25 mV/s for 9 cycles**.** At the end, the modified electrode was rinsed with distilled water to eliminate any physically adsorbed or unreacted components from the electrode surface which is represented as ChCl/GCE.

3.5. Collection and preparation of real samples

The liquid red wine and the solid state tea samples were purchased from a supermarket in Addis Ababa, Ethiopia   and kept in a refrigerator until analysis. For taking analysis purpose the sample of wine and 2 g of black and green was dissolved in 50 ml of 0.1 M BRB pH6. Then wine and tea solutions would be centrifuging for 15 min and filtered each sample. 5.0 mL clear solution of wine was diluted to 50.0 mL with 0.1 M BRB pH 6 and 5mL clear solution of tea was diluted to

50.0 mL with 0.1 M BRB pH 6. For the recovery test, the samples were spiked with stock solution of GA in BRB pH 6.

4. RESULTS AND DISCUSSION

4.1. Fabrication of ChCl/GCE

The electrochemical deposition of ChCl at the surface of activated GCE was performed by CV at 2.0 mM ChCl solution containing 0.01 M KCl in the potential window of -1.7 to 1.8 V and scan rate of 25 mV/s for 9 cycles. Figure: 10 shows the CV curves for the electrochemical deposition of ChCl at the activated surface GCE. In the formation of ChCl/GCE, ChCl was strongly attached on the GCE surface by forming a covalent bonding rather than simply attaching to the electrode surface in physical adsorption. During this process the BGCE was oxidized to form free radical cations on its surface which allow to actively attaching to the hydroxyl group of ChCl molecules to form the ether linkage. There is also another group of ChCl, quaternary ammonium cation ($–N^+(CH_3)_3$) polar head group, forming a positively charged stable surface and is suitable for electrostatstic interaction and decreases electron transfer resistance at the electrode surface [22, 23].

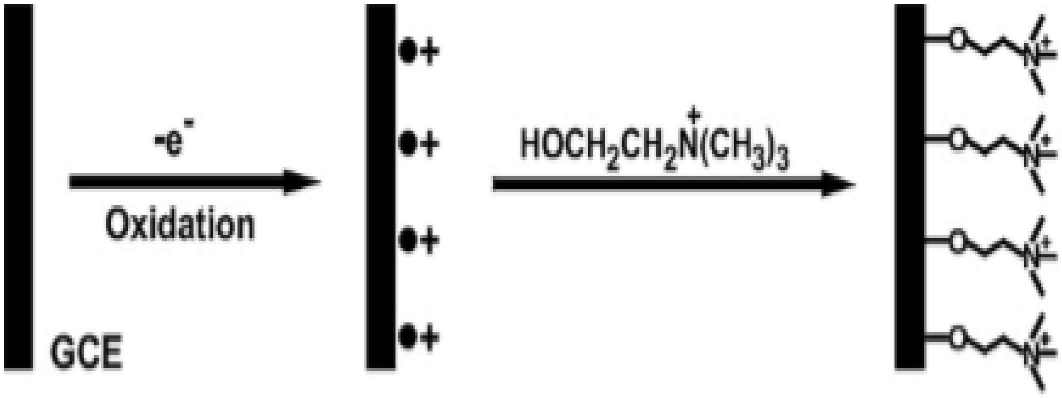


Figure 9: representation of the attachment of ChCl onto the GCE

Due to such properties of deposition ChCl on the surface of the activated GCE which affect the rate of electron transfer. The deposition process was optimized by changing the number of scanning cycles from 5 to 12 cycles. As shown in Figure: 11, the peak current response of 100 μM G.A in 0.1 M BRB increased up to 9 cycles due to increase in active sites at the electrode surface. The peak current would be decreased when an excess amount of ChCl was deposited on the surface of the activated GCE, which blocks electron transfer between the electrode and analyte (G.A). Thus, 9 cycles of deposition scanning was selected for further determination.

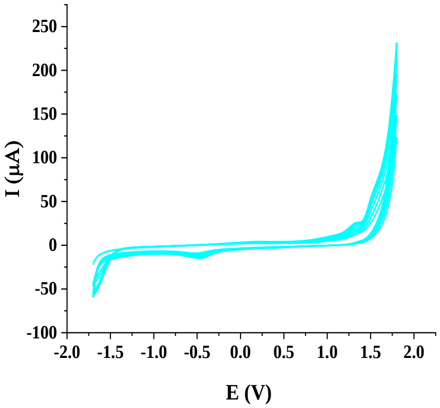


Figure 10 : Cyclic voltammogram for deposition of 2.0 mM ChCl solution containing 0.01 M KCl in 0.1 M BRB pH 6 at GCE for 9 cycles and scan rate of 25 mV/s in the potential window −1.7 and 1.8 V

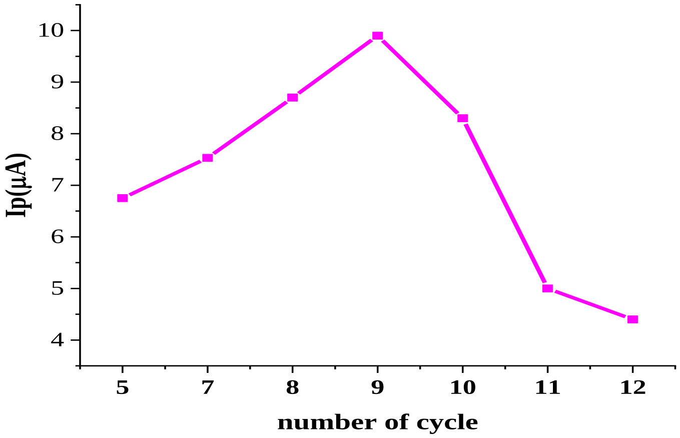


Figure 11 : Plot of scan cycles versus peak current response of 100 μM G.A in 0.1 M BRB

4.2. Electrochemical characterization of the BGCE and ChCl/GCE

Before starting the analysis of G.A at the developed electrode, the charge-transfer kinetic properties and the nature of electron transfer between the electrodes (BGCE, ChCl/GCE) and electrolyte solution were characterized by CV and EIS, considering $[Fe(CN)_6]^{3-/4-}$ system as a probe. To investigate the interface characteristics of electrodes with surface modifications electrochemical impedance spectroscopy is known to be a great tool. Figure: 12 displays the Nyquist plots of EIS obtained for 5.0 mM $[Fe\ (CN)\ _6]^{3-/4-}$ in 0.1 M KCl to estimate the charge transfer resistance at the BGCE (a) and ChCl/GCE (b). The charge transfer reaction at the interface of the electrode leads to formation of a semicircle and the diameter of the semicircle reflects the electrode's charge transfer resistance (Rct)]. The BGCE has exhibited a semicircle with Rct of around 939.5 Ω. After modification, the Rct value has decreased substantially to 620 Ω. The Rct value has decreased due to electrostatic interaction between the positively charged group of ChCl attracts more negatively charged $[Fe(CN)_6]^{3-/4-}$ to the modified electrode surface. This result indicates that the presence of ChCl on the surface of GCE enhances the reduction of Rct value and accelerates the rate of electron transfer at the electrode surface. Electron transfer rate at ChCl/GCE is higher compared to the BGCE. These results were in agreement with those obtained from EIS, demonstrating that ChCl was successfully modified at the surface of GCE [65].

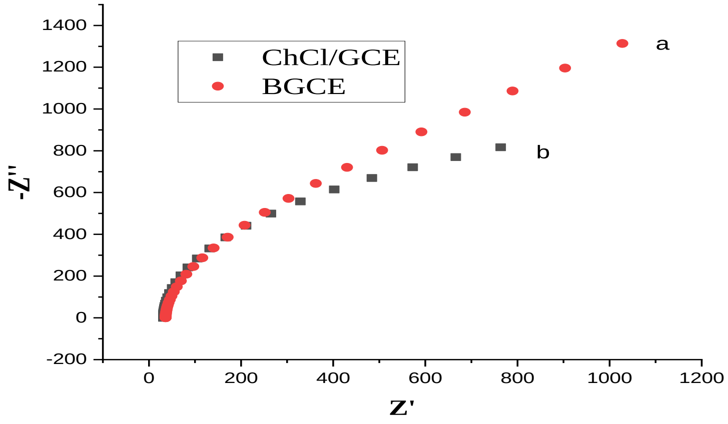


Figure 12 : Nyquist plots obtained for 5.0 mM $[Fe\ (CN)\ _6]^{3-/4-}$ in 0.1 M KCl at BGCE (a) and ChCl/GCE (b) at frequency range of 0.1 Hz to 100 kHz.

As shown in Figure: 13 [Fe (CN) $_6$]$^{3-/4-}$ shows a well-defined anodic and cathodic peak that corresponded to the expected electrochemical behavior of redox couples at both BGCE and ChCl/GCE in acyclic voltammetry. However, as compared to BGCE, the ChCl/GCE produced a larger redox peak current response due to the formation of positively charged ChCl layer which led to stronger attraction of [Fe(CN)6]$^{3-/4-}$ on the surface of the electrode and the increased electro active surface area of the electrode. In order to investigate whether the ChCl layer could increase the surface area, the electro active surface areas of both the BGCE and ChCl/GCE were estimated from cyclic voltammogram recorded for [Fe (CN) $_6$]$^{4-/3-}$ in 0.1 M KCl at different scan rates using Randles-Sevcik equation [23].

$$\mathrm{Ip} = (2.63 * 10^5)\mathrm{n}^{\frac{3}{2}}\mathrm{ACD}^{\frac{1}{2}}\mathrm{v}^{\frac{1}{2}} \ldots\ldots\ldots \quad (3)$$

where, I_p is the peak current (A), n is number of electron transferred in the redox reaction (n=1), A is the electro active surface area of electrode (cm^2), C is the concentration of [Fe(CN)$_6$]$^{4-/3-}$ (5×10^{-6} mol/cm^3), ν is the scan rate in (V/s) and D is the diffusion coefficient of 5.0 mM [Fe(CN)$_6$]$^{4-/3-}$ (7.26×10^{-6} cm^2/s). From the plot of peak currents of [Fe (CN) $_6$]$^{4-/3-}$ versus square root of scan rates a linear relationship was found. Based on the slope of the plot the electro active surface area was calculated using equation 3. The electro active surface area of the BGCE and ChCl/GCE are calculated to be 0.338 cm^2 and 0.618 cm^2, respectively. The electro active surface area of the ChCl/GCE is found to be two fold wider than that of the BGCE, indicating that the electrode's electrochemical active sites are also increased.

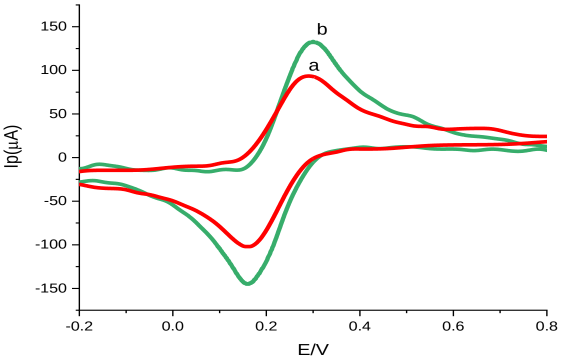


Figure 13 : Cyclic voltammograms of 5.0 mM [Fe (CN) $_6$]$^{4-/3-}$ in 0.1 M KCl at BGCE (a) and ChCl/GCE (b) at scan rate 100 mV/s

4.2. Electro chemical behavior of Gallic acid

The electro chemical behavior of G.A was studied using both CV and SWV technique as shown in figure 14 and 15. The CV and SWV response of 100 µM G.A in 0.1 M BRB (pH6) at a scan rate100 mvs^{-1}at BGCE and ChCl/GCE in both figures, low peak current response was observed at BGCE. This indicates that there is low rate of electron transfer at BGCE but the peak current response of G.A was increased significantly at ChCl/GCE this suggesting that increasing the sensitivity of G.A at ChCl/GCE can be contributed to large the surface area and increase oxygen containing group like hydroxyl group and quaternary ammonium cations at the electrode surface which leads adsorption easier and provide more reaction site. The oxidation peak current obtained for G.A at the ChCl/GCE is much higher compared to the BGCE .The increase in peak current response was due to the strong electro catalytic activity of ChCl for the oxidation reaction of G.A which facilitates the electron transfer process and the increase in electro active surface area that increased adsorption sites. The strong attraction of G.A on electrode surface is mainly due to strong dipole-dipole interaction between ChCl and G.A.

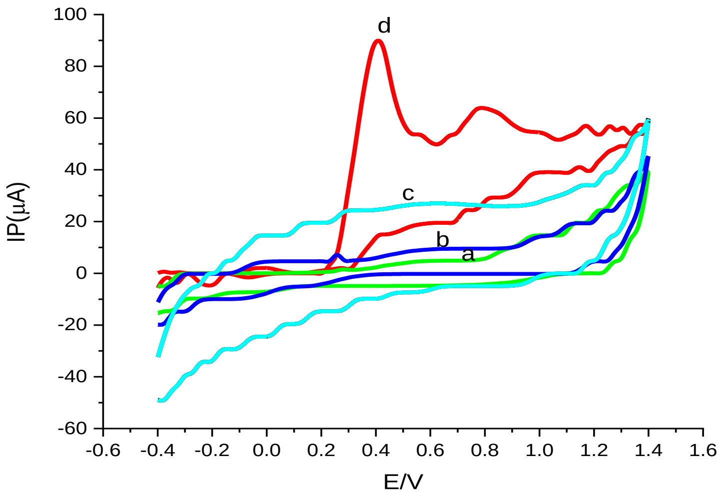


Figure 14 : cyclic voltammogram of BGCE in blank solution (a), ChCl/GCE in the blank solution (b), BGCE in the presence of 100 µM G.A (c) and ChCl/GCE in the presence of 100 µM G.A (d)

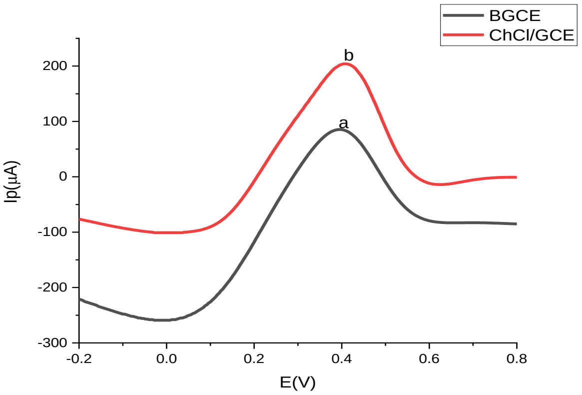


Figure: 15 Square wave voltammogram of 100 μM G.A in 0.1 M BRB at BGCE (a) and ChCl/GCE (b)

The oxidation reaction mechanism of Gallic acid is presented in figure 16: the first step abstraction of proton from the Para (hydroxyl) group to produce free radical when the free radicals rearrange leads to cause second proton abstraction and the formation of semiqunone. Hence Gallic acid is oxidized by the process involving loss of $2e^-$ and $2H^+$ to form Quinone.

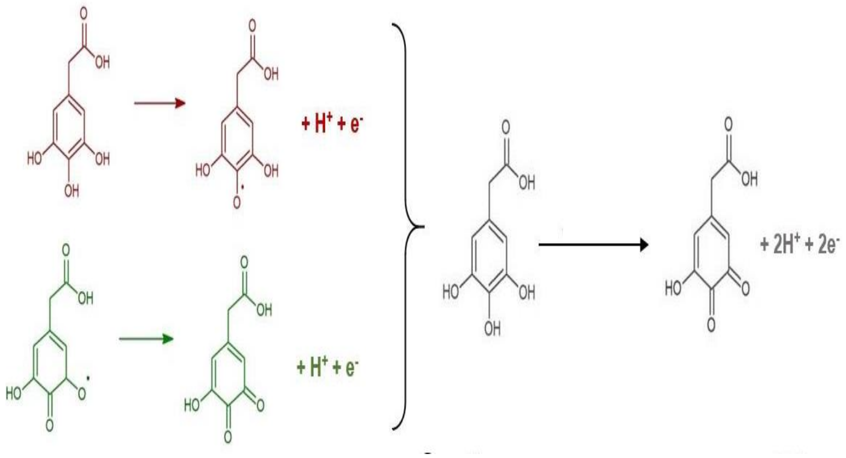


Figure 16 **:** the proposed mechanism for the oxidation of G.A

4.4. Optimization of experimental conditions

4.4.1. Effect of supporting electrolytes

In order to obtain high sensitivity in the electrochemical behavior of 100 µM Gallic acid at the modified electrode the influence of supporting electrolyte solutions was studied in the experiment. 0.1 M different electrolyte solutions such as acetate buffer solution (ABS), citrate buffer solution (CBS), phosphate buffer solution (PBS) and Britton Robison buffer solution (BRB) were studied at ChCl/GCE for the electrochemical oxidation of G.A using SWV. In all listed buffer solutions G.A acid gave electrochemical response but the peak current response obtained using BRB was higher than the other supporting electrolyte solutions. Then BRB was selected as preferable supporting electrolyte at ChCl/GCE for further experimental work.

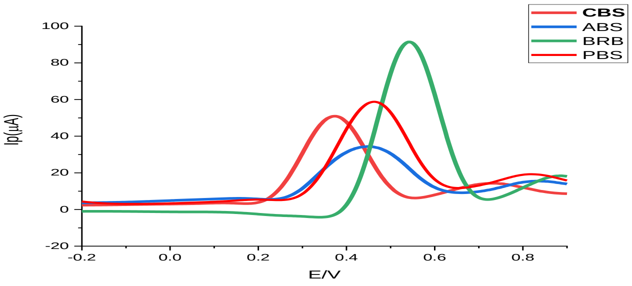


Figure 17 : square wave voltammogram of 100 µM G.A at different supporting electrolyte

4.4.2. **Effect of pH**

The pH effect of the supporting electrolyte solution on the electrochemical oxidation of G.A was studied in 0.1 M BRB in the pH range of 3-9 using CV technique. The current response and the peak potential of G.A were changed with increasing the pH from 3 to 9 which indicates the oxidation of G.A is pH dependent. As shown in the figure 18: oxidation peak current response G.A increase with increasing pH from 3 to 6 and then decrease with further increasing the pH this indicates the detection of G.A at ChCl/GCE is feasible only in the acidic conditions. So that pH 6 was selected for further investigation.

The dependence of the oxidation peak potential (Ep) of G.A on the pH of the buffer solution was also studied. The peak potential of G.A shifted linearly towards the negative position with increasing the pH due to the concentration of H^+. As shown figure 19: A plot of peak potentials versus pH values was found to be linear in the range of 3 to 9 with regression equation of

Epa (V) = -0.0564 pH + 0.736 with (R^2 = 0.998) and the slope of 56.4 mV/ pH. The slope is nearly equal to the Nernstian value of 59 mV/pH at 25 °C indicating that in the electrochemical oxidation of G.A, equal number of electrons and protons are involved [3].

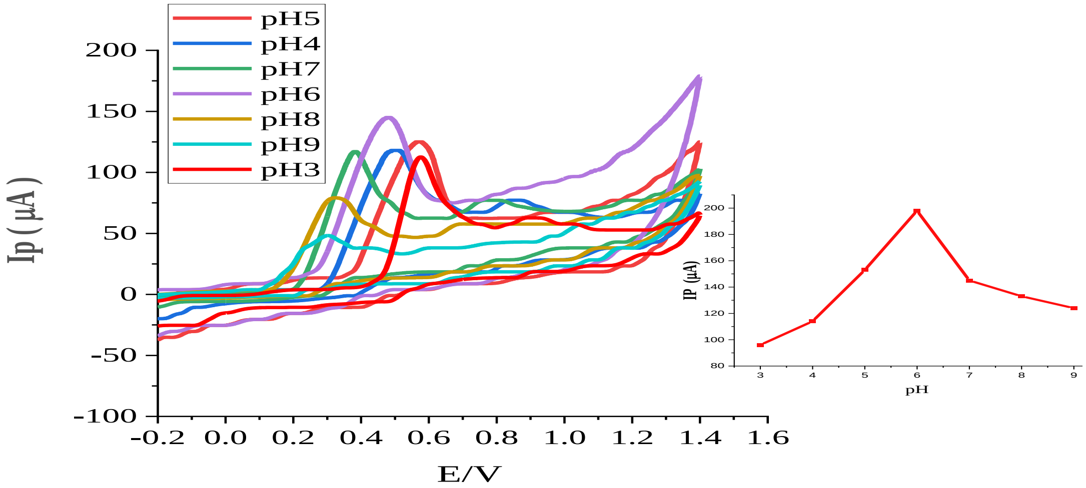


Figure 18 : Plot of peak currents versus pH

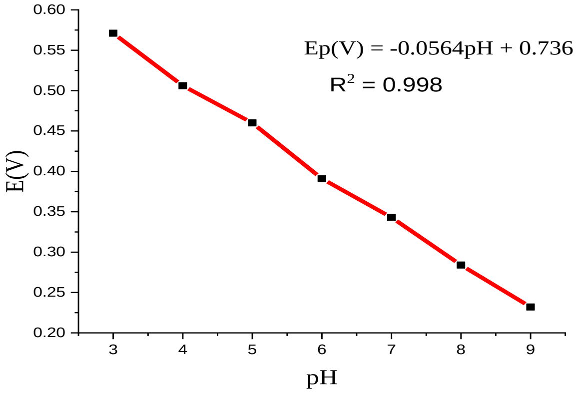


Figure 19 : Plots of peak potential of GA versus pH of buffer solution

4.4.3. Effect of scan rate

The study of scan rate used for deciding the process involved in the electrode surface is adsorption or diffusion controlled .The effect of scan rate on the peak current of 100 µM Gallic acid in 0.1 M BRB pH 6 at ChCl/GCE was studied using CV technique in the range of 25-300 mV/s. As shown in figure 19: the plot of peak current versus scan rate for the oxidation of G.A shows a linear relationship that is oxidation peak current increased as increasing the scan rate. The relationship between peak current and scan rate could be expressed by a linear regression equation Ip (µA) =0.4224 (mv/s) +21.303 (R^2 = 0.995) .This results indicate that the electrochemical reaction of G.A at ChCl/GCE was adsorption controlled process.

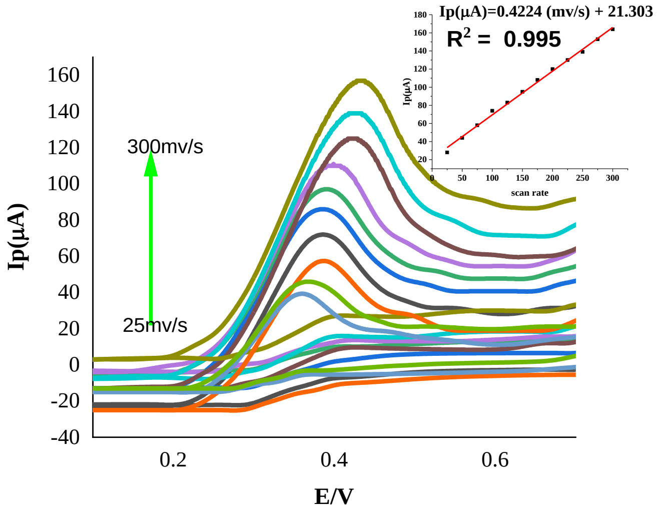


Figure 20 : Cyclic voltammogram of the oxidation of 100 µM of G.A in 0.1 M of BRB at ChCl/GCE on different scan rates 25, 50, 75, 100, 125, 150, 175, 200, 225, 150, 275, 300 mv/s

4.4.4. Effect of accumulation time and potentials

Accumulation time and potential can be optimized for obtained maximum peak current response during electro chemical oxidation of G.A. The effect of both accumulation time and potential on the oxidation peak current of G.A was studied by SWV. The effect of accumulation time was studied in the range of (30-300 sec) as shown in figure 20: The peak current response increase up to 180 sec and decreased slowly with further increasing accumulation time. This indicates the adsorptions of G.A on the modified electrode surface was saturated or stabilize then 180 sec accumulation time was selected for further analysis. Since the electrochemical oxidation of G.A at the surface of ChCl/GCE is an adsorption controlled process the effect of accumulation potential was also studied using SWV technique. Effect of accumulation potential on the oxidation of G.A at the ChCl/GCE was investigated in the range of -500 to 400 mV but the peak current response of 100 µM G.A in 0.1 M BRB remained nearly the same, indicating that accumulation potential had no significant effect on the oxidation peak current response of G.A at the ChCl/GCE. As a result, an open circuit accumulation was used to conduct further analysis.

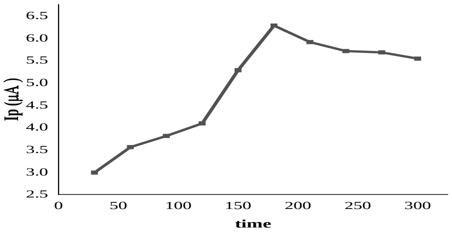


Figure 21: Effect of open circuit accumulation time on the peak current of 100 µM G.A in 0.1 M BRB at ChCl/GCE

4.5. Effect of SWV parameter

A square wave voltammetric technique was used for the determination of G.A at ChCl/GCE due to its higher sensitivity, removal of background current and higher speed. Square wave parameters such as the pulse amplitude, frequency, and increment (step potential) were also optimized before any analytical measurement. The effect of square wave parameters on the oxidation of 100 µM G.A was performed for the parameter range of frequencies(15-90 Hz), amplitude (0.02-0.28 V) and step potential (0.002-0.012 V) to the peak current response of 100 µM in 0.1 M BRB (pH 6) at ChCl/GCE by measuring the peak current enhancement [52]. The optimized square wave parameters were obtained pulse amplitude 0.22 V, frequency 30 Hz, and increment 0.004 V used for further experimental work.

4.6. Analytical Performance of the Method

Under the optimized experimental conditions, the relationship between the oxidation peak current of G.A and concentration was investigated by using SWV technique [53]. Figure: 21 shows the SWV responses for various concentrations of G.A at the ChCl/GCE. The peak current increased up on increasing G.A concentration and a linear response was obtained in the concentration range 1.6 µM to 240 µM with the linear regression equation:

Ip (µA) =1.34 (µM) - 1.49547, (R^2 = 0.998) .The limit of detection (LOD) and limit of quantification (LOQ) values calculated using the equation LOD = 3s/m and LOQ = 10s/m where s, represents the standard deviation of the peak current of the lower most concentration in the calibration curve and m, is the slope of the calibration curve. The values of LOD and LOQ were 0.126 µM and 0.419 µM, respectively.

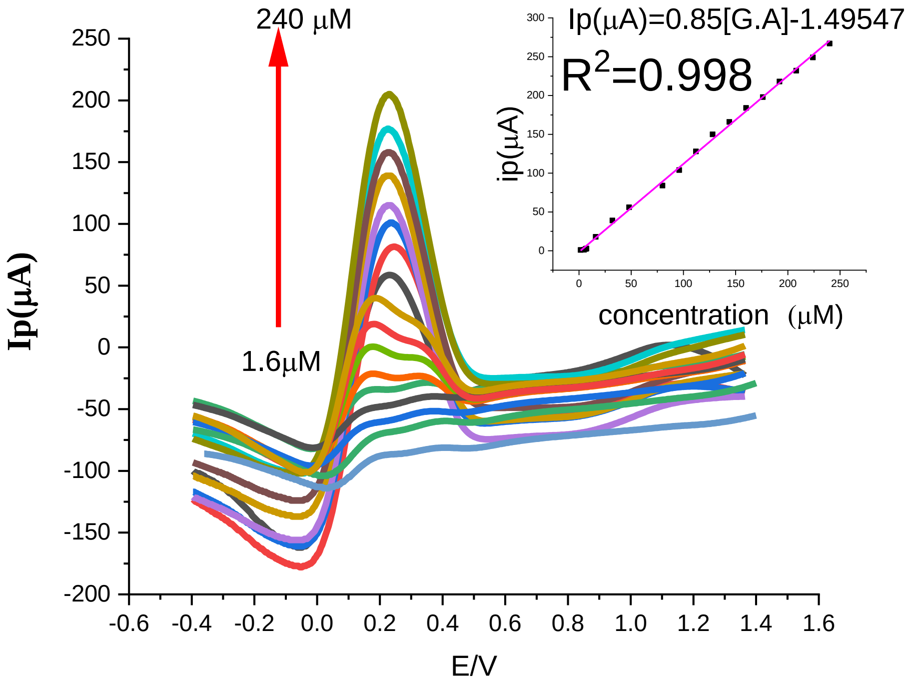


Figure 22: square wave voltammograms of different concentration of G.A at ChCl/G*CE in 0.1 M* BRB pH 6, pulse amplitude 0.22 V, frequency 30 Hz, and increment 0.004 V. Inset: Plot of SWV anodic peaks current versus G.A concentration from 1.6 µM to 240 µM.

The analytical performance of the newly developed method was also compared with other reported data in terms of linear range and LOD. When compared with the reported literatures for the determination of G.A at different modified electrodes, ChCl/GCE can be improves the detection performance of G.A with low cost, time saving and simple operation.

Table: 1 shows comparison of the developed method for the determination of G.A at ChCl/GCE with other reported method.

Modified electrode	Technique	Linear range (μM)	LOD (μM)	Reference
rGO/GCE	SWV	8–400	0.42	[4]
Polyepinephrine/GCE	SWV	1.0 – 20.0	0.66	[11]
rGO/GCE	SWV	20–144	30.8	[3]
PDDA-GR-Pt/GCE	SWV	0.03–1	0.007	[27]
ZrO_2 nanoparticles/CPE	DPV	1-1000	0.124	[39]
Graphene/GCE	DPV	0.08-20	0.0012	[38]
ChCl/GCE	SWV	1.6-240	0.126	THIS WORK

Where, RGO/GCE: reduced graphene Oxide, PDDA-GR-Pt: Poly (diallyldimethylammoniumchloride) (PDDA)-functionalized graphene supported platinum nanoparticles Nano composite, CPE: Carbon paste electrode, zirconia-nanoparticles, SWV: Square-wave voltammetry, DPV: Differential pulse voltammetry

4.7. Repeatability, Reproducibility and Stability of modified electrode

The important properties of the modified electrode including reproducibility, repeatability and stability were also studied in the experiments under optimized conditions. To evaluate reproducibility of the modified electrode for determination of G.A four different ChCl/GCE were prepared under the same condition. The peak current response of 100 µM G.A was recorded. The relative standard deviation (RSD) thus electrodes were 1.64 %. Similarly to evaluate the repeatability of prepared electrode four successive measurement of the oxidation peak current of

100µM G.A was performed by using a single electrode. The result gives RSD of 3.063 %. The stability of the electrode was studied by measuring the peak current of 100 µM G.A after storage of ChCl/GCE for one day, one week and three week the peak current response decreased by 7.47 %. The above results indicated that the prepared modified electrodes have good reproducibility, repeatability and stability of the sensor.

4.8. Interference Study

Under the optimum experimental conditions, the selectivity of the ChCl/GCE for the determination of G.A was evaluated by a fixed amount of G.A (40 μM) in 0.1 M BRB in the presence of 1:1, 1:5, 1:10 and 1:50 concentration ratio of the interfering substance including caffeine, ascorbic acid, sucrose, urea, and citric acid using SWV. The tolerance limit was considered as the maximum the concentration of interfering species that cause an error less than 5 % in the determination of G.A. The results show that 50 fold additions of common interfering species had no effect on voltammetric responses of G.A. The change of the peak currents of G.A was less than 5 %, suggesting that the ChCl/GCE has good selectivity for the determination of G.A. Figure: 22 and table: 2 shows influence of potential interferents on the determination of 40 μM GA at ChCl/GCE (n= 4) in BRB (pH 6) after accumulation at a potential for 180 sec. SWV parameters: frequency, 30 Hz; step potential, 0.004 mV and pulse amplitude, 0.22 mV.

Table: 2 Effect of interferents on the determination of 40 μM Gallic acid in 0.1 M BRB at ChCl/GCE

Interferents	Concentration (µM)	Changes in (%)
Caffeine	2000	2.60
Sucrose	2000	0.38
Citric acid	2000	-2.00
Urea	2000	-2.43
Ascorbic acid	2000	0.67

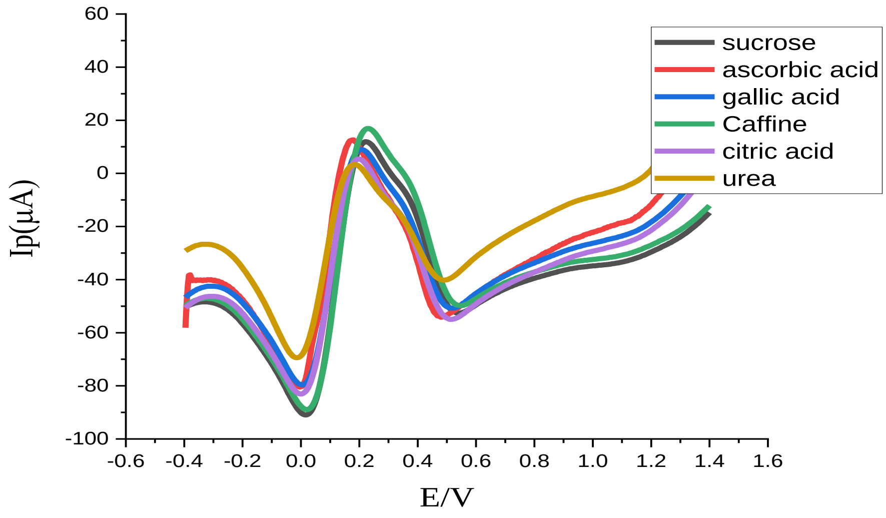


Figure 23 : SWV voltammograms of 40 µM Gallic acid with different concentrations of some known interfering compounds in 0.1 M BRB at pH 6 and a scan rate 100 mv/s

4.9. Application of the Method in Real Samples

The newly developed electrochemical sensor based on the ChCl/ GCE was used to analyze G.A in a green tea, black tea and red wine by SWV under the optimized experimental conditions. The real samples were centrifuged for 15 minute and filtered to remove the solid residue and analyzed without further treatment. Peak currents were observed for each unspiked real samples and its peak current increased upon successive addition of G.A standard solutions for each real samples, this suggesting the real samples contain G.A (the concentration of G.A in the real sample is higher than the detection limit). The application of the developed method was evaluated by the recoveries studies. Different known concentrations of G.A standard solution were added to each real sample with spiking methods to do the recovery test. The square wave voltammetric peak current for each sample was recorded. The calculated recovery values for the each real sample solutions (5 ml real sample and 50 ml BRB) are summarized in the following tables. The recovery percentage at the ChCl/GCE was obtained in the range of 92.20% – 106.8% which is highly satisfactory. Therefore, this voltammetric method can be applied for G.A determination in black tea, green tea and red wine with high accuracy.

Table: 3 Results obtained for detection of Gallic acid in real samples (n = 4) at ChCl/GCE

sample	Spiked G.A (μM)	Found G.A (μM)	Recovery (%)
Red wine	0	2.80	-
	6.40	5.90	92.20 %
	25.6	24.99	97.60 %
	51.2	51.95	101.5 %
Black tea	0	3.58	-
	6.40	6.67	104.2 %
	25.6	27.34	106.8 %
	51.2	49.43	96.50 %
Green tea	0	4.51	-
	6.40	6.54	102.2 %
	25.6	27.14	106.0 %
	51.2	49.90	97.50 %

5. CONCLUSION

Gallic acid is a nontoxic phenolic acid which have diverse biological activities including anti-

inflammatory, anticancer and antioxidant properties. In this work, a simple, fast, and low cost SWV methods based on choline chloride modified glassy carbon electrode can be used for detecting Gallic acid. The ChCl/GCE increased the electrochemical current response of Gallic acid compared to the BGCE which is due to the remarkable catalytic behavior of choline chloride and the increased electro active surface area. Under the optimized experimental conditions, the peak current is directly proportional to the concentration of Gallic acid in the range of 1.6 μM to 240 μM with a detection limit of 0.126 μM. Moreover, the ChCl/GCE was also successfully applied for determination of Gallic acid in real samples (red wine, green tea and black tea) in which the recoveries obtained are satisfactory. The fabricated ChCl/GCE has also provided the features of excellent repeatability, reproducibility, long-time stability with high selectivity and sensitivity that would make it an excellent candidate for Gallic acid detection in the black tea, green tea and red wine.

6. Reference

1. Mohapatra, P. D., Mondal, K. C., Pati, B. R. (2006). Production of tannase through submerged fermentation of tannin-containing plant extracts by Bacillus licheniformis KBR6. *J. Microbiol*, *300*, 297-301.
2. Daglia, M., Di Lorenzo, A., F Nabavi, S., S Talas, Z., M Nabavi, S. (2014). Polyphenols: well beyond the antioxidant capacity: Gallic acid and related compounds as neuroprotective agents: you are what you eat. Current pharmaceutical biotechnology, 15(4), 362-372.
3. Ma, J., Luo, X. D., Protiva, P., Yang, H., Ma, C., Basile, M. J., Kennelly, E. J. (2003). Bioactive novel polyphenols from the fruit of Manilkara zapota (Sapodilla). *Journal of Natural Products*, *66*(7), 983-986.
4. Álvarez, R., Araya, H., Navarro-Lisboa, R., Lopez de Dicastillo, C. (2016). Evaluation of polyphenol content and antioxidant capacity of fruits and vegetables using a modified enzymatic extraction. *Food technology and biotechnology*, *54*(4), 462-467.
5. Ghaani, M., Nasirizadeh, N., Ardakani, S. A. Y., Mehrjardi, F. Z., Scampicchio, M., Farris, S. (2016). Development of an electrochemical nanosensor for the determination of Gallic acid in food. *Analytical Methods*, *8*(5), 1103-1110.
6. Kumar, S., Singh, A., Kumar, B. (2017). Identification and characterization of phenolics and terpenoids from ethanolic extracts of Phyllanthus species by HPLC-ESI-QTOF-MS/MS. *Journal of pharmaceutical analysis*, *7*(4), 214-222.
7. Rajalakshmi, K., Devaraj, H., Devaraj, S. N. (2001). Assessment of the no-observed-adverse-effect level (NOAEL) of Gallic acid in mice. *Food and chemical toxicology*, *39*(9), 919-922.
8. Dhama, P. K., Ain, S., Kumar, B., Ain, Q. (2022). Development and evaluation of topical ointment formulation containing Gallic acid as an active pharmaceutical ingredient against bacterial infection and oxidative damage. *Annals of Phytomedicine: International Journal*, *11*(1), 439-449.
9. Shen Hua, S. H., Wang Zheng, W. Z., Li Lei, L. L., Li HongYan, L. H. (2014). Simultaneous determination of gallic acid, methyl gallate and ethyl gallate from gallnut extracts by HPLC.

10. Tsering, J., Gogoi, B., Veer, V., Kalita, P., Tag, H. (2014). HPTLC determination of Gallic acid in methanol extract of Quercus griffithii Acorn. *Int J PharmTech Res*, *6*(4), 1341-1347.
11. Asfaw, T. B., Tadesse, M. G., Tessema, F. B., Woldemariam, H. W., Legesse, B. A., Esho, T. B., Kumar, P. (2023). Optimization, identification, and quantification of selected phenolics in three underutilized exotic edible fruits using HPTLC. *Sustainability*, *15*(12), 9568.
12. Kuceki, M., de Oliveira, F. M., Segatelli, M. G., Coelho, M. K. L., Pereira, A. C., da Rocha, L. R., Tarley, C. R. T. (2018). Selective and sensitive voltammetric determination of folic acid using graphite/restricted access molecularly imprinted poly (methacrylic acid)/SiO2 composite. *Journal of Electroanalytical Chemistry*, *818*, 223-230.
13. Gao, Y., Wang, L., Zhang, Y., Zou, L., Li, G., Ye, B. (2016). Highly sensitive determination of Gallic acid based on a Pt nanoparticle decorated polyelectrolyte-functionalized graphene modified electrode. *Analytical methods*, *8*(48), 8474-8482.
14. Beitollahi, H., Ivari, S. G., Torkzadeh-Mahani, M. (2016). Voltammetric determination of 6-thioguanine and folic acid using a carbon paste electrode modified with ZnO-CuO nanoplates and modifier. *Materials Science and Engineering: C*, *69*, 128-133.
15. Barek, J. (2021). How to Improve the Performance of Electrochemical Sensors via Minimization of Electrode Passivation. *Chemosensors*, 9, 12.
16. Koyun, O., Gorduk, S., Arvas, M. B., Sahin, Y. (2018). Electrochemically treated pencil graphite electrodes prepared in one step for the electrochemical determination of paracetamol. *Russian Journal of Electrochemistry*, *54*, 796-808.
17. Naczk, M., Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of pharmaceutical and biomedical analysis*, *41*(5), 1523-1542.
18. Abdel-Hamid, R., Newair, E. F. (2013). Adsorptive stripping voltammetric determination of Gallic acid using an electrochemical sensor based on polyepinephrine/glassy carbon electrode and its determination in black tea sample. *Journal of Electroanalytical Chemistry*, *704*, 32-37.

19. Petrea, N., Ginghina, R., Pretorian, A., Petre, R., Barsan, G., Otrisal, P., Mosteanu, D. (2018). Experimental survey regarding the dangerous chemical compounds from military polygons that affect the military health and the environment. *Rev. Chim*, *69*(7), 1640-1644.
20. Kong, F., Su, Z., Guo, X., Zeng, F., Bi, Y. (2018). Antidiabetic and lipid-lowering effects of the polyphenol extracts from the leaves of Clausena lansium (Lour.) Skeels on streptozotocin-induced type 2 diabetic rats. *Journal of food science*, *83*(1), 212-220.
21. Nayeem, N., Asdaq, S. M. B., Salem, H., AHEl-Alfqy, S. (2016). Gallic acid: a promising lead molecule for drug development. *Journal of Applied Pharmacy*, *8*(2), 1-4.
22. López-Cobo, A., Gómez-Caravaca, A. M., Švarc-Gajić, J., Segura-Carretero, A., Fernández-Gutiérrez, A. (2015). Determination of phenolic compounds and antioxidant activity of a Mediterranean plant: The case of Satureja montana subsp. kitaibelii. *Journal of Functional Foods*, *18*, 1167-1178.
23. Badhani, B., Sharma, N., Kakkar, R. (2015). Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. *Rsc Advances*, *5*(35), 27540-27557.
24. Palafox-Carlos, H., Gil-Chávez, J., Sotelo-Mundo, R. R., Namiesnik, J., Gorinstein, S., González-Aguilar, G. A. (2012). Antioxidant interactions between major phenolic compounds found in 'Ataulfo'mango pulp: chlorogenic, Gallic, protocatechuic and vanillic acids. *Molecules*, *17*(11), 12657-12664.
25. Ji, H. F., Zhang, H. Y., Shen, L. (2006). Proton dissociation is important to understanding structure–activity relationships of Gallic acid antioxidants. *Bioorganic & medicinal chemistry letters*, *16*(15), 4095-4098.
26. Eslami, A. C., Pasanphan, W., Wagner, B. A., Buettner, G. R. (2010). Free radicals produced by the oxidation of Gallic acid: An electron paramagnetic resonance study. *Chemistry Central Journal*, *4*, 1-4.
27. Gülcin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of toxicology*, *86*, 345-391.
28. Pham-Huy, L. A., He, H., Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS*, *4*(2), 89.

29. Fernandes, F. H. A., Salgado, H. R. N. (2016). Gallic acid: review of the methods of determination and quantification. *Critical reviews in analytical chemistry*, *46*(3), 257-265.
30. Santo, A., Zhu, H., Li, Y. R. (2016). Free radicals: From health to disease. *React. Oxyg. Species*, *2*, 245-263.
31. Perego, C., Carati, A., Ingallina, P., Mantegazza, M. A., Bellussi, G. (2001). Production of titanium containing molecular sieves and their application in catalysis. *Applied Catalysis A: General*, *221*(1-2), 63-72.
32. Sheldon, R. A. (2000). Atom efficiency and catalysis in organic synthesis. *Pure and applied chemistry*, *72*(7), 1233-1246.
33. Rahman, M. A., Park, D. S., Shim, Y. B. (2004). A performance comparison of choline biosensors: anodic or cathodic detections of H2O2 generated by enzyme immobilized on a conducting polymer. *Biosensors and Bioelectronics*, *19*(12), 1565-1571.
34. Zeisel, S. H. (1992). Choline: an important nutrient in brain development, liver function and carcinogenesis. *Journal of the American College of Nutrition*, *11*(5), 473-481.
35. de Almeida Pontes, P. V., Shiwaku, I. A., Maximo, G. J., Batista, E. A. C. (2021). Choline chloride-based deep eutectic solvents as potential solvent for extraction of phenolic compounds from olive leaves: Extraction optimization and solvent characterization. *Food Chemistry, 352*, 129346.
36. Tran, V. T., Catenza, K. F., Donkor, K. K., Schmidt, K. J., Crabtree, H. J., Warrender, N. A. (2022). Analytical characterization of choline chloride in oilfield process waters and commercial samples by capillary electrophoresis. *Canadian Journal of Chemistry*, *100*(7), 552-559.
37. Jin, G. P., Lin, X. Q., Gong, J. M. (2004). Novel choline and acetylcholine modified glassy carbon electrodes for simultaneous determination of dopamine, serotonin and ascorbic acid. *Journal of Electroanalytical Chemistry*, *569*(1), 135-142.
38. Wang, P., Mai, Z., Dai, Z., Li, Y., Zou, X. (2009). Construction of Au nanoparticles on choline chloride modified glassy carbon electrode for sensitive detection of nitrite. *Biosensors and Bioelectronics*, *24*(11), 3242-3247.
39. Shahamirifard, S. A., Ghaedi, M., Razmi, Z., Hajati, S. (2018). A simple ultrasensitive electrochemical sensor for simultaneous determination of Gallic acid and uric acid in

human urine and fruit juices based on zirconia-choline chloride-gold nanoparticles-modified carbon paste electrode. *Biosensors and Bioelectronics*, *114*, 30-36.

40. Bahrani, S., Razmi, Z., Ghaedi, M., Asfaram, A., Javadian, H. (2018). Ultrasound-accelerated synthesis of gold nanoparticles modified choline chloride functionalized graphene oxide as a novel sensitive bioelectrochemical sensor: Optimized meloxicam detection using CCD-RSM design and application for human plasma sample. *Ultrasonics Sonochemistry*, *42*, 776-786.
41. Parsaee, Z., Karachi, N., Abrishamifar, S. M., Kahkha, M. R. R., Razavi, R. (2018). Silver-choline chloride modified graphene oxide: Novel nano-bioelectrochemical sensor for celecoxib detection and CCD-RSM model. *Ultrasonics Sonochemistry*, *45*, 106-115.
42. Panov, G. I. (2000). Advances in oxidation catalysis; oxidation of benzene to phenol by nutrous oxide. *Cattech*, *4*, 18-31.
43. Uslu, B., Ozkan, S. A. (2007). Solid electrodes in Electroanalytical chemistry: present applications and prospects for high throughput screening of drug compounds. *Combinatorial chemistry & high throughput screening*, *10*(7), 495-513.
44. Kimmel, D. W., LeBlanc, G., Meschievitz, M. E., Cliffel, D. E. (2012). Electrochemical sensors and biosensors. *Analytical chemistry*, *84*(2), 685-707.
45. Rahman, M. A., Kumar, P., Park, D. S., Shim, Y. B. (2008). Electrochemical sensors based on organic conjugated polymers. *Sensors*, *8*(1), 118-141.
46. Alam, M. T., Gooding, J. J. (2015). Modification of carbon electrode surfaces. *Electrochemistry of Carbon Electrodes*, 211-240
47. Bard, A. J., Faulkner, L. R., White, H. S. (2022). *Electrochemical methods: fundamentals and applications*. John Wiley & Sons.
48. Durst, R. A. (1997). Chemically modified electrodes: recommended terminology and definitions (IUPAC Recommendations 1997). *Pure and applied chemistry*, *69*(6), 1317-1324.
49. Chillawar, R. R., Tadi, K. K., Motghare, R. V. (2015). Voltammetric techniques at chemically modified electrodes. *Journal of analytical chemistry*, *70*, 399-418.
50. Jang, J. (2006). Conducting polymer nanomaterials and their applications. *Emissive materials nanomaterials*, 189-260.

51. Tembe, S., Inamdar, S., Haram, S., Karve, M., D'Souza, S. F. (2007). Electrochemical biosensor for catechol using agarose–guar gum entrapped tyrosinase. *Journal of biotechnology*, *128*(1), 80-85.
52. Spitalsky, Z., Tasis, D., Papagelis, K., Galiotis, C. (2010). Carbon nanotube–polymer composites: chemistry, processing, mechanical and electrical properties. *Progress in polymer science*, *35*(3), 357-401.
53. Heeger, A. J. (2001). Semiconducting and metallic polymers: the fourth generation of polymeric materials. *The Journal of physical chemistry B*, *105*(36), 8475-8491.
54. Le, T. H., Kim, Y., Yoon, H. (2017). Electrical and electrochemical properties of conducting polymers. *Polymers*, *9*(4), 150.
55. Farghaly, O. A., Hameed, R. A., Abu-Nawwas, A. A. H. (2014). Analytical application using modern electrochemical techniques. *International Journal of Electrochemical Science*, *9*(6), 3287-3318.
56. Arribas, A. S., Bermejo, E., Chicharro, M., Zapardiel, A., Luque, G. L., Ferreyra, N. F., Rivas, G. A. (2007). Analytical applications of glassy carbon electrodes modified with multi-wall carbon nanotubes dispersed in polyethylenimine as detectors in flow systems. *Analytica Chimica Acta*, *596*(2), 183-194.
57. Stojek, Z. (2010). The electrical double layer and its structure. *Electroanalytical methods: Guide to experiments and applications*, 3-9.
58. Ventura, K., Smith, M. B., Prat, J. R., Echegoyen, L. E.,Villagrán, D. (2017). Introducing students to inner sphere electron transfer concepts through electrochemistry studies in diferrocene mixed-valence systems. *Journal of Chemical Education*, *94*(4), 526-529.
59. Christian, G. D., Dasgupta, P. K., Schug, K. A. (2013). *Analytical chemistry*. John Wiley & Sons.
60. Allen, B. J., Larry, R., Faulkner. (2000).Electrochemical methods Fundamentals and Application 471(2) 04372-04379.
61. Simões, F. R., Xavier, M. G. (2017). Electrochemical sensors. *Nanoscience and its Applications*, *1*, 155-178.
62. Yi, Y., Zhu, G., Wu, X., Wang, K. (2016). Highly sensitive and simultaneous electrochemical determination of 2-aminophenol and 4-aminophenol based on poly (l-

arginine)-β-cyclodextrin/carbon nanotubes@ graphene nanoribbons modified electrode. *Biosensors and Bioelectronics*, *77*, 353-358.

63. Elgrishi, N., Rountree, K. J., McCarthy, B. D., Rountree, E. S., Eisenhart, T. T.,Dempsey, J. L. (2018). A practical beginner’s guide to cyclic voltammetry. *Journal of chemical education*, *95*(2), 197-206.
64. Harsini, M., Fitriany, E., Farida, A. N., Suryaningrum, D., Asy’ari, D. N., Widyaningrum, B. A., Kurniawan, F. (2021). Polymelamine/gold nanoparticle-modified carbon paste electrode as voltammetric sensor of uric acid. *Malaysian Journal of Analytical Sciences*, *25*(2), 286-295.
65. Raj, M. A., John, S. A. (2013). Fabrication of electrochemically reduced graphene oxide films on glassy carbon electrode by self-assembly method and their electro catalytic application. *The Journal of Physical Chemistry C*, *117*(8), 4326-4335.