# DETERMINATION OF ASCORBIC ACID IN SOME CITRUS FRUITS BY USING PENCIL GRAPHITE ELECTRODE

**A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY**

**By**
**ASHEL MUSARA**

**In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering**

**NICOSIA, 2019**

# DETERMINATION OF ASCORBIC ACID IN SOME CITRUS FRUITS BY USING PENCIL GRAPHITE ELECTRODE

**A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY**

**By**
**ASHEL MUSARA**

**In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering**

**NICOSIA, 2019**

**Ashel MUSARA: DETERMINATION OF ASCORBIC ACID IN SOME CITRUS FRUITS BY USING PENCIL GRAPHITE ELECTRODE**

**Approval of Director of Graduate School of**
**Applied Sciences**

**Prof. Dr. Nadire ÇAVUŞ**

**We certify this thesis is satisfactory for the award of the degree of Master of Science in Food Engineering**

**Examining Committee in Charge:**

| | |
|---|---|
| Assist. Prof. Dr. Suleyman Aşır | Head of the Jury, Department of Material Science and Nanotechnology Engineering, NEU |
| Assist. Prof. Dr. Perıhan Adun | Department of Food engineering NEU |
| Dr. Hazal Özyurt | Supervisor, Department of Food Engineering, NEU |

I hereby declare that, all the information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

Name, Last Name:
Signature:
Date:

## ACKNOWLEDGEMENTS

This work would not have been possible without the financial support of the Food engineering department. I would first like to thank my Thesis advisor Dr. HazalÖzyurt, of the faculty of Engineering, in the Food engineering department for the support, encouragement throughout the entire research study. The door to Dr. HazalÖzyurt's office was always open whenever I needed assistance or had a question about my research or writing. She consistently allowed this paper to be my own work, but steered me in the right direction whenever she thought I needed it.

I am also indebted to Ass. Prof. Dr. PerihanAdun, Chairman of the Department of Food Engineering, she has been supportive of my career goals and who worked so hard to provide me with the protected academic time to pursue these goals. More so, I am extremely grateful to FevziyeDindar, it has been a pleasure to work with her during this entire research study. Each of the members of my Dissertation Committee has provided me extensive personal and professional guidance and taught me a great deal about both scientific research and life in general. I would especially like to thank Mr. BugraDemircioglu, the course coordinator of the department of Food engineering.

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents, whose love and guidance are with me in whatever I pursue. They are the ultimate role models. Most importantly, I wish to thank my loving and supportive girlfriend, Nomsa Tendai, for providing me with an unending inspiration.

**To my parents...**

## ABSTRACT

In this study, we focused on the use of a pencil graphite electrode (PGE) as a sensor for determination of ascorbic acid (AA) mostly known as vitamin C. The performance of the pencil graphite electrode was studied using a differential pulse voltammetric (DPV) method. The amount of ascorbic acid (mg/100g) in 6 citrus fruit samples were determined by electrochemical method using a pencil graphite electrode as working electrode, Ag/AgCl as reference and platinum as the auxiliary electrode in an aqueous solution of pH 4.8 (acetate buffer solution and 0.02 M of NACl solutions). Ascorbic acid, a water-soluble vitamin, is the most common electroactive biological compound found in some fruit species. For the qualitative purposes the peak position was obtained at 0.342 - 0.40V by DPV technique in all samples. Linear calibration was achieved with a correlation coefficient of 0,999. Limit of Detection was 0,557 mg/100g. Vitamin C amount in various citrus fruits were changed in a range of 60.32- 214.06mg/100g. Differential Pulse Voltammetry by using bare PGE was found promising approach which is quick, easy, cost-effective and environmentally friendly in determination of vitamin C in fruits.



***Keywords:***PGE; Ascorbic acid; DPV; citrus fruits;voltammetric peak

# ÖZET

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| **ABS:** | Acetate Buffer Solution |
| **Ag/AgCl :** | Silver/silver Chloride |
| **AA:** | Ascorbic acid |
| **AgNPs:** | Silver nanoparticles |
| **Ag/AgCl:** | Silver Silverchoride |
| **CE:** | Counter electrode |
| **CV:** | Cyclic voltammetry |
| **DHAA** | Dehydroascorbic acid |
| **DNA:** | Deoxyribonucleic acid |
| **DPV:** | Differential pulse voltammetry |
| **DNA** | deoxyribonucleic acid |
| **EIS:** | Electrochemical impedance spectroscopy |
| **eC:** | Counter electrode potential |
| **eW:** | Working electrode potential |
| **EA:** | Applied potential |
| **HPLC:** | High-performance liquid chromatography |
| **$H_2O_2$:** | Hydrogen peroxide |
| **KB:** | Ketjenblack |
| **L-AA:** | L- ascorbic acid |
| **I:** | Current |
| **(t):** | Circuit the current |
| **KCl:** | Potassium chloride |
| **LOD:** | Detection limit |
| **miR:** | microRNA |
| **NADH:** | Nicotinamide adenine dinucleotide |

| | |
|---|---|
| **NC:** | Nucleic Acid |
| **PBS:** | Phosphate Buffer Solution |
| **PGE:** | Pencil graphite electrode |
| **Res:** | Reference Electrodes |
| **RE:** | Reference electrode |
| **RISC:** | RNA-induced silencing complex |
| **RNA:** | Ribonucleic acid |
| **Rs:** | Solution resistance |
| **ss-DNA:** | single stranded DNA |
| **TBS:** | Tris Hydrochloride Buffer Solution |
| **SCE:** | Saturated calomel electrode |
| **TAA:** | Total ascorbic acid |
| **TCEP:** | Tris (2-carboxyethyl) phosphine, |
| **TEM:** | Transmission electron microscopy |
| **Thi:** | Thionine |
| **UV-Vis:** | Ultraviolet-visible spectroscopy |
| **UV:** | Ultraviolet |
| **V:** | Potential |
| **WE:** | Working electrode |
| **XRD:** | X-ray powder diffraction |
| **Z:** | Impedance |
| **Z':** | Real resistance |
| **Z":** | Imaginary resistance |
| **ZnO:** | Zinc oxide |

# CHAPTER 1

# INTRODUCTION

Food analysis is a critical subject in the field of food industry. This is proven by an exponential increase in new food supplement and nutraceuticals, which makes the availability of fully validated methods of analysis an important aspect. According to Spinola (2014) there has been much concern with regards to nutritional quality of foods in the field of food chemistry which in this particular subject attracted attention from both consumers and manufactures particularly in ascorbic acid determination. Previous work has shown that ascorbic acid commonly known as vitamin C is highly sensitive to temperature and pH alterations during processing and storage and this has attracted attention of manufactures and concerned consumers. It has been reported in previous researches that ascorbic acid is an essential nutrient; which plays a noble rolein the growth and development of the body and ismuch associated with the enzyme functionality within the body (Wonsawat, 2014).

By definition, vitamins are organic molecules which a body needs to sustain life, these compounds cannot be synthesized hence should be supplied by diet (Tarrago-Trani& Phillips, 2012; Ganjal et al., 2017). Vitamins are sub divided into two significant categories which are fat -soluble and water-soluble vitamins respectively. Vitamins A, D, E and K are fat-soluble organic molecules and the water-soluble ones include the entire vitamin B complex altogether with vitamin C. Of critical importance in this particular thesis is ascorbic acid. Literature reviews that, vitamin C a water-soluble compound is easily leached out through urine (Badea et al., 2018) because it cannot be stored in the body for the prolonged period. Ascorbic acid is a water-soluble vitamin synthesized by most animals from glucose.

Like any other nutrient, vitamin C has different components and molecular structure respectively. Vitamins C, is an organic compound with formula $C_6H_8O_6$, (Majed et al.,

2013) originally called hexuronic acid with a white or slightly yellow crystal or powder.It is water-soluble, slightly alcohol-soluble and insoluble in chloroform, ether, and benzene.

Over the past decade, considerable attention has been given on the improvement of analytical techniques which are available for vitamin C determination in different matrices. Some of these techniques include direct titration, fluorometric methods, chromatographic methods, and spectrometric methods (Norfun et al., 2016). However, major drawbacks of some of these methods such as titration, fluorometric methods, and chromatographic methods are time-consuming, some are costly, some need special training operators, or they suffer from the insufficiency of sensitivity and or selectivity respectively (Norfun et al., 2016).

The most commonly used methods are spectrophotometric and electrochemical detectors. Previous researches, on validation of chromatographic methods for vitamin C determination mostly regards a narrow range of analyzed food matrices such as citrus fruits and vegetables (Olana et al., 2015). Literature suggests that amongst these methods, the electrochemical method presents some advantages such as high sensitivity (Norfun et al., 2016) and the ability to assay the electro active species even colored samples.

However, the above batch-wise methods are time-consuming due to sample pre-treatments. They also consume large amounts of samples, expensive and/or toxic reagents and solvents.

This study was carried out with the aim of examiningthe qualitative and quantitative measurement of ascorbic acid(vitamin C) contentin some citrus fruits by using voltammetry. The results were compared to those obtained by others from previous researches.

# CHAPTER 2

# THEORETICAL FRAMEWORK

## 2.1 Vitamins C (ascorbic acid)

Vitamins are organic molecules that a body needs to sustain its life. Instead, a human body cannot synthesize the vitamins hence they should be supplied through diet (Tarrago-Trani&Phillips, 2012; Ganjal et al., 2017). Vitamins are divided into two categories which are fat -soluble and water-soluble vitamins. Vitamins A, D, E and K are classified as fat-soluble organic molecules and the water-soluble ones are vitamins C and all the vitamin B complex, but of significant in this particular paper is ascorbic acid.

According to Badea et al., (2018) water-soluble vitamins cannot be stored in the body for the prolonged period because they easily get excreted through urine. Vitamin C which is also known as ascorbic acid is a water-soluble vitamin synthesized by most animals from glucose. Ascorbic acid is an organic compound with a formula $C_6H_8O_6$ (Majed et al., 2013), originally called hexuronic acid, and it's a white solid compound, but impure samples can appear yellowish.


**Figure 2.1:**Chemical structure of Ascorbic acid

### 2.1.1Sources of vitamin C

Historically Vitamin C was first isolated in 1928 by the Hungarian biochemist and Nobel Prize winner Szent-GyorGyi. A water-soluble vitamin needs to be supplied more than the fat-soluble ones, (Pisoschiet al 2014). Apart from other mammals, humans, rats and other primates cannot synthetize ascorbic acid, hence the need for exogenous input, particularly through diet. Citrus fruits, green vegetables such as broccoli, Brussels and leafy vegetables (Tarrago-Trani&Phillips., 2012) are the chief sources of ascorbic acid. A report of fish and milk containing vitamin C was published. Vitamin C is also found in milk, and in some types of meat such as kidney, liver fish (Burzle., 2012), but it is widely spread in fruits and vegetables.

### 2.1.2 Vitamin C characteristics

Vitamin C refers to all compounds exhibiting equivalent biological activity to L-ascorbic acid (L-AA), including its oxidation products (dehydroascorbic acid, DHAA), isomers (isoascorbic acid, IAA), esters (ascorbyl palmitate), and synthetic forms (6-deoxy-L-AA, 2-phosphate-L-AA). It is also applied by the food industry as an additive, preventing the oxidation of food products. L-ascorbic acid is susceptible to oxidation by oxygen and is oxidized to dehydroascorbic acid, and its oxidation can be induced by exposure to high temperatures and pH, light, presence of oxygen or metals and enzymatic action.

Dehydroascorbic acid exhibits equivalent biological activity to L-ascorbic acid, so it is critical to measure both molecules to know the total ascorbic acid (TAA) or total vitamin C content in foodstuffs. The equilibrium between L- ascorbic acid and dehydroascorbic acid is dependent on the sample pre-harvest and post-harvest conditions. Most crops contain up to 10% DHAA of total vitamin C and it tends to increase during storage (Lee & Kader, 2000).

Irreversible hydrolysis of dehydroascorbic acid produces the biologically inactive 2,3-diketo-l-gulonic acid(DKGA), followed by its degradation to other by-products, including oxalic acid, l-threonic acid, CO2, l-xylonic acid, and l-xylose. According to Nimse& Pal, (2015) some reducing agents can convert dehydroascorbic acid back to L-AA in

vivo(glutathione dehydrogenase) and in vitro systems (homocystein;dl-1,4-dithiothreitol, DTT; dimercaptopropanol, BAL; and tris (2-carboxyethyl) phosphine, TCEP. Taking into consideration that the potential degradation of L-ascorbic acid depends on the storage, sample preparation, and extraction conditions, is very critical to obtain reliable results.

### 2.1.3 Significance of vitamin C

Vitamins are a class of nutrients that are essentially required by the body for its various biochemical and physiological processes. Popular literature suggests vitamins as a class of nutrients that are essentially required by the body for its various biochemical and physiological processes. Ascorbic acid helps in metabolism of tyrosine, folic acid and tryptophan (Tadese et al., 2014). It regulates blood cholesterol level and helps in the synthesis of the ascorbic acid carnitine and catecholamine that regulate nervous system (Elgailan et al., 2017; Majed et al., 2013). Ascorbic acid is an antioxidant that protects the body from the harmful effects of free radicals and pollutants.

According to Badea et al., (2015), vitamin C is of paramount importance in the formation and maintenance of the collagen and as a powerful antioxidant (Elgailan et al., 2017; Majed et al., 2013), protecting the body against oxidative stress, and present in the human diet as a vital vitamin. Previous studies have shown that vitamin C is essential for the natural synthesis of dopamine in the human body. On the other hand, large doses of ascorbic acid proved to reduce the risk of kidney stone formation in women. The deficiency of vitamin C compound in the body result in causing scurvy, anaemia and various infection and mental disorders (Burzle., 2012). However, Pisoschi et al., (2014) argued that vitamin C if excessively consumed can cause gastric irritation, and some of its metabolites like oxalic acid, causes renal problems. Furthermore, it may result in the inhibition of natural processes occurring in food and can contribute to taste/aroma deterioration in food and beverages if in excess.

### 2.1.4 Industrial functions of ascorbic acid

According to Majed et al., (2013), vitamin C can be used as nutrititive food additive, antioxidant (Pisoschi et al., 2014), reducing agent, stabilizer and color stabilizer. Thus, determination of this compound is very essential for pharmaceutical and biological studies

(Burzle., 2012, Pisoschi et al 2014). Vitamin C is well known of its reductive properties that make it functional as an antioxidant agent in foods and drinks that protect the body from the harmful effects of free radicals and pollutants (Olana et al., 2015, Tadese et al., 2014). That is why ascorbic acid is often sold through the counter by the pharmaceuticals as a supplementary source to human diet to serve as a free-radical scavenger. Vitamin C plays a very significant role as a quality indicator in food stuffs and beverages. Thus, products should be monitored carefully during storagesincevitamin Cis a labile substance which is prone to degradation by atmospheric oxygen and biochemical enzymes is a good quality indicator. This oxidative process is catalysed by extreme heat, light and heavy metal cations, (Majed et al., 2013), this gives ascorbic acid a critical role as quality dictator.

### 2.1.5 Oxidation of ascorbic acid

Ascorbic acid is an electro-active biological compound, hence it prone to oxidation, and this aids in its electrochemical determination. Ascorbic acid forms with dehydroascorbic acid an irreversible redox couple. Its electrocatalytical oxidation showed only the anodic oxidation peak, it has been concluded that there is a direct dependence between the current intensity corresponding to ascorbic acid electro-oxidation and the square root of the potential sweep rate, (Pisoschietal., 2014).

Szultkaetal (2014) describes ascorbic acid oxidation-reduction properties have a connected with its ability to donate two hydrogen atoms to the neutralizing molecule of an oxidant. The process can be reversible and be converted to a molecule of vitamin C into dehydroascorbate. Ascorbic acid together with its oxidized form is crucial in oxidation stress; therefore, the amount of vitamin C is measured as collective contents of ascorbic acid and DHA (Szultkaetal, 2014). However, DHA has a biological activity which is similar to that of ascorbic acid, hence it is important to effectively measure the quantities of both molecules to know total vitamin C content in foodstuffs (Szultkaetal, 2014).

In a solution, vitamin C is stable; and its stability is dependent on the solvent and factors such as pH, temperature, or exposure to light. Under certain conditions, vitamin C can show a pro-oxidant effect, which scientist suggested that ascorbic acid can as well reacts with hydrogen peroxide ($H_2O_2$) (Szultkaetal, 2014). In biological models, AA reduces or

prevents the $H_2O_2$-induced lipid peroxidation. Furthermore, ascorbic acid inhibits OH-deoxyguanisine and protects thymocytes against oxidation-induced apoptosis, as well as protects aged dermal fibroblasts from the $H_2O_2$-induced cytotoxicity, (Szultkaetal, 2014). Figure 2.2 shows the summary of oxidation-reduction of ascorbic acid.

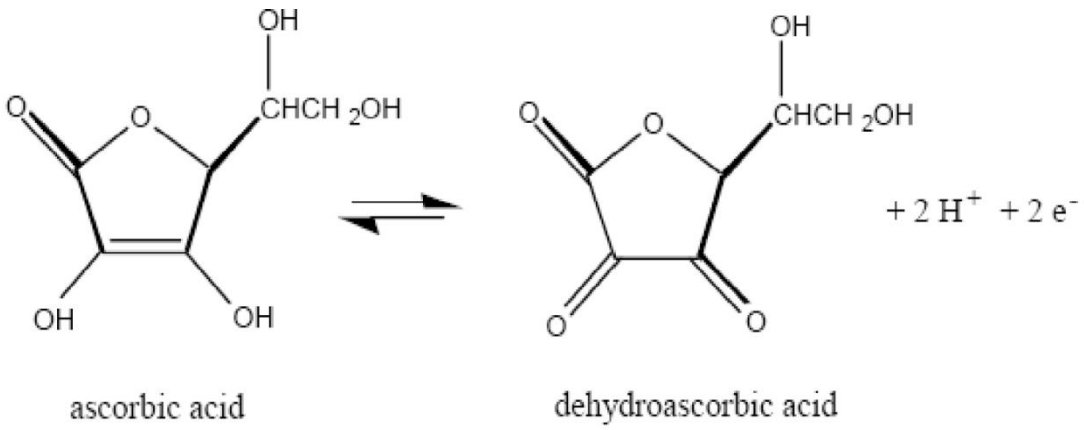



**Figure2.2:** Oxidation of Ascorbic acid

### 2.2 Analysis of vitamin C

Vitamin C quantitative analytical methods have been reported these include high performance liquid chromatography, titrimetric, spectrophotometry methods, flurometric methods, solid phase analysis and chemiluminescence methods (Pardakhty et al., 2016). Of the abovementioned methods, they have major demerits which are high cost, cumbersome in sample preparation and time consuming, which requires big infrastructure back up and expert knowledge, background interference for fluorometry and destroying of sample for chromatography.

Methods for vitamin C determination in foods which have been reported, are categorically designed for a specific food type for example fruits and vegetables and or fortified products, they validated for a particular food or tested on a limited number of products (Tarrago-Trani et al., 2012), and it is important to note that the wide range of food matrices validated methods for the analysis of vitamin C are lacking. According (Tarrago-Trani et al., 2012), the most broadly used certified standard methods includes fluorimetric and

titrimetric techniques, such as the Association of Official Analytical Chemists (AOAC) methods 967.21, 967.22, 984.26 that were developed for specific matrices. The titrimetric method (AOAC 967.21) applies to juices and vitamin preparations while the fluorimetric method (AOAC 967.22) applies to vitamin fruit juices, infant formula and fortified breakfast cereal (Tarrago-Trani et al., 2012).

Several methods of ascorbic acid determination have been reported using spectrometry and amperometry (Elgailan et al 2017). The development of fast, simplistic and inexpensive analytic methods is one of the growing areas of interest. Since quick decisions are to be made in the industries for instance in the field of food processing, a need for fast and simple methods is a necessity. Similarly, liquid chromatography, capillary electrophoresis and gas chromatography have been reportedly used in ascorbic acid determination from different species of citrus fruits. Spectrophotometry is one of the most frequently used simple methods because vitamin C is able to absorb UV rays (Tarrago-Trani&Phillips, 2012). Since Ascorbic acid is able to absorb UV rays (Elgailani., 2017), the method is suitable for use with Vitamin C tablets, fresh packaged fruit juices and solid fruits and vegetables.

### 2.2.1 Chromatographic methods

Among chromatography techniques, HPLC happens to be the most used than gas chromatography. Many chromatographic methods have been reported for the determination of ascorbic acid. Some of them are dedicated to the determination of ascorbic acid and total ascorbic acid content as the sum of ascorbic acid and DHAA after reduction of DHAA. In the past 10 years, methods used HPLC in conjunction with a variety of detection techniques (Szultkaetal., 2014), including UV-Vis, DAD, electrochemical detection, and MS. Fluorescence detection is not very popular because it requires derivatization. LC-MS is especially promising because of short analysis time, as well as high sensitivity and selectivity (Szultkaetal., 2014). The HPLC methods proved to be of more use in a wide range of food samples due to their high complexity, which demands high selectivity and sensitivity for the analysis (Eschetal., 2010). Of importance to note is that only L-ascorbic acid can be determined by the traditional iodometrictitration, whereas, HPLC methods can be used to quantify appropriately L-AA and DHAA, (Spinolaa et al., 2014).HPLC is more

sensitive than spectrophotometric, titration and or enzymatic methods and the sensitivity depends to a large degree on selection of an adequate detector (Novakova et al., 2008). In the fields of food safety, UPLC is used for determination of pesticides residues and their metabolites (Leandro, Hancock, Fussell, & Keely, 2006; Li et al., 2013), and heterocyclic aromatic amines (Barceló-Barrachina et al., 2006). Major applications of UPLC in pharmaceutical analyses include quality control and stability monitoring of products, drug discovery and development. The most commonly used arespectrophotometric and electrochemical detectors (Novakova et al., 2008). Papers describing validation of chromatographic methods for the determination of vitamin C mostly regard a narrow range of analyzed food matrices such as broccoli, green peas, strawberries, fruit beverages, fruits, vegetables, fruits and vegetables.

Reversed-phase (RP)-HPLC is one of the most common approach used (Spínolaa et al., 2014, due to the non-volatile and hydrophilic nature of vitamin C, also Ultra-high performance liquid chromatography (UHPLC) has recently been used to analyse vitamin C in foods, observing as key advantages the shorter time of analysis and the much lower solvent consumption when compared to other analytical approaches (Spínolaa et al., 2014).

However, liquid chromatography, capillary electrophoresis, and gas chromatography have been reportedly used in ascorbic acid determination (Elgailani., 2017) from different species of citrus fruits. Of the abovementioned methods, they have major demerits which are high cost, cumbersome in sample preparation and time consuming, which requires big infrastructure back up and expert knowledge, background interference for fluorometry and destroying of sample for chromatography (Tarrago-Trani&Phillips, 2012).

### 2.3 Biosensor

A biosensor is a measurement device constituting of a biological element that functions as a target recognition entity, in conjunction with a transducer that converts a biological recognition episode to a measurable signal. Looking at the literature related to biosensors over the last few decades, it undoubtedly reveals that biosensors are attractive not only in academia field but also in industry due to their specificity in reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical

compounds. Biosensorsconstitute a biological sensing element either intimately connected to or integrated within a transducer. Recent advances and new trends in biosensors involve the use of optical, electrochemical and piezoelectric biosensors based on the detection method. The ultimate purpose of this device is to yield a digital electronic signal, which entails the direct proportional to the concentration of a certain (bio) chemical of particular interest in the presence of interfering species (Gerard et al., 2002). Biosensors can provide an invaluable method for agro–food diagnostics since they are convenient, portable and do not need intense particular skills to operate.

The technology of biosensors is as a result of combined interdisciplinary efforts of engineers, chemists, physicists and biologists. Biosensor technology exploits the unique properties of a biological recognition event on a transducing device, (Malhotra and Chaubey., 2003). In such an event, the interaction of the analyte with the bioreceptor is converted into a suitable output that is easily readable by the user. This approach not only exploits the molecular binding event, but also brings researchers from different areas of science and engineering to bridge their skills. Biosensors have been widely researched and developed as a tool for chemical, biochemical, medical, agricultural and environmental monitoring.

Biosensors in the food industry can be used to analyze the nutrients, to detect natural toxins and antinutrients, for monitoring of food processing, and for detection of genetically modified organism. Through the immunogenic reactions, and enzymatic reactions, biosensors can be employed to determine the concentration of antibiotics, proteins or vitamins and or pesticides found in foods. This is accomplished because of their compact size, specificity, sensitivity, response linearity, real time analysis, nearly reagent less operation, reproducibility, simple pretreatment protocols, low cost of construction, and simplicity of use. A key technology in developing biosensors is combining the biological components to the surface of transducers. A biosensor set up is comprised of three parts, which are

- a biological receptor for biochemical recognition of sample analyte
- a transducer to translate recognition event into a readable signal
- a detection technique for signal analysis and processes,

The ultimate performance of the biosensors is made possible by the technique combination of these two system components through the technique called immobilization (MugurumaandKarube., 1999).

However most commonly used techniques of immobilization are adsorption of inert carriers, physical entrapment in gel lattices, cross-linking by bifunctional reagents into the macroscopic particles, covalent binding of water insoluble matrices, microencapsulation within the wall spheres, electrostatic interaction and electrochemical entrapment, etc.

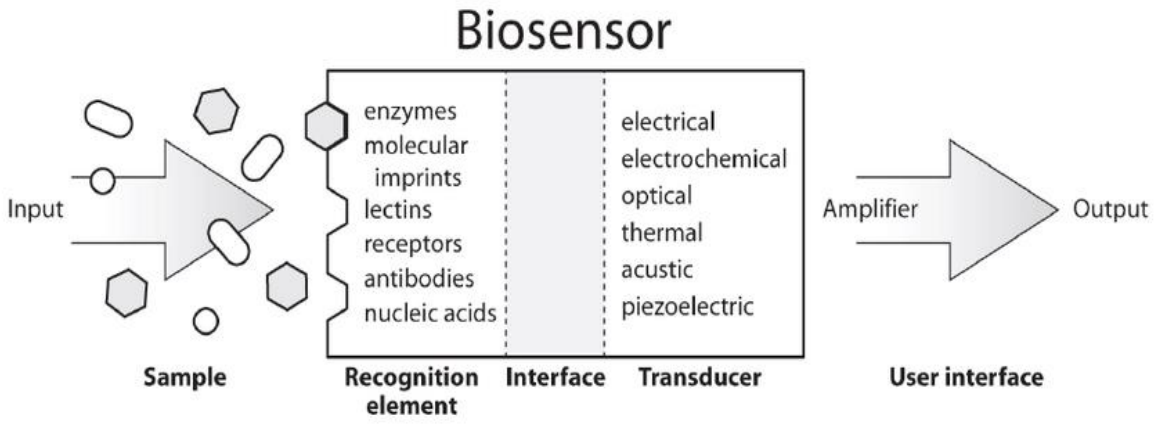



**Figure 2.3:** Shows a general configuration of a biosensor

**Classification of Biological Recognizers**

Biosensors can be classified in agreement to the type involved active biological component in the mechanism or the mode of signal transduction or combination of these two aspects. Figure 3.2 shows some analytes (substrate) possible to be analyzed immobilizing the biological components, separately, in several transducers. The choice of the biological material and the adjusted transducer depends on the properties of each sample of interest and the type of the physical magnitude to be measure. The type of the biocomponent determines the degree of selectivity or specificity of the biosensor. Thus, the biological recognizers are divided in three groups: biocatalytic, bioaffinity and hybrid receptors (Mello and Kubota., 2002).

**Biocatalytic receptors**

Biocatalysis refers to the use of biological organisms also called enzymes so as to speed up chemical reactions. Biocatalyticprocessesmake use of natural catalysts, like whole cells

such as bacteria, fungi eukaryotic cells, yeast, cells organelles and plant or animal tissues to perform chemical transformations. Biotechnologically produced enzymes and or modified enzymes are called chemoenzymatic synthesis and their reactions are called chemoenzymatic reactions. Drawbacks such as poor selectivity and slow response cases of microbial sensors can be augmented through the use of specific enzymes because of their selectivity (Davis et al., 1995).

**Bioaffinity receptors**

The affinity-based biosensors can be nucleic acid, antibodies and or a chemoreceptor. The interaction of most ligands with their binding sites can be characterized in terms of a binding affinity and it is made possible through selectivity interactions to form a thermodynamically stable complex. The potential use of antibodies is due to their general applicability and their specificity and selectivity of the antigen-antibody reaction as well as their high sensitivity. The use of monoclonal antibodies is more common in immunosensor studies because they are quite homogenous with respect to their molecular structure, which possesses the same binding technique and can be cloned in large quantities. The physicochemical change induced by antigen-antibody binding does not generate an electrochemically detectable signal (Davis et al., 1995). However, the use of antibodies as bioreceptors has its demerits which are high cost, a limited life span and high susceptibility to high temperatures.

**Hybrid receptors**

The hybrid receptors such as DNA and RNA probes have shown promising applications. These nucleotides are employed as bioreceptors through the immobilization of a single-stranded oligonucleotide onto a transducer surface to detect its complementary target sequence the DNA hybridization event is then translated into a signal. The principle of selective detection is based on the detection of a unique sequence of nucleic acids through hybridization (Mello and Kubota, 2002). The nucleic acid structure is a double helix conformation of two polynucleotide strands. Each strand of a nucleic acid is constituted of a polymeric chain that contain bases: A, T, C, G. These bases are complementary by two through three hydrogen bonds in the C-G base pair and two in the T-A base pair. This base-pairing property gives ability of one strand to recognize its complementary strand to

form a duplex. DNA sensors consist to immobilize, onto a solid support, well-defined sequences of single strands as a biological receptor. A DNA probe is added to DNA or RNA from an unknown sample or the reverse process is possible. If the probe hybridizes with the unknown nucleic acid because of pairing of complementary base recognition, detection and identification are possible (Mello and Kubota, 2002). Nucleotides consist of 3 main parts: 5 carbon sugar, phosphate groups andbases.

**Transducers**

The transducer is an integral part of a biosensor that converts one form of energy into another. The transducer converts the bio-recognition event into a measurable signal through a process called signalization. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte–bioreceptor interactions. Classification of biosensors is done in several types according to the transducer: calorimetric, optical, piezoelectric and electrochemical transducers. Optical biosensors are based on the measurement of light absorbed or emitted as a consequence of a biochemical reaction. In such a biosensor, the light waves are guided by means of optical fibers to suitable detectors (Peterson and Vurek, 1984). Calorimetric biosensors also detect an analyte on the basis of the heat evolved due to the biochemical reaction of the analyte with a suitable enzyme. Different substrates, enzymes, vitamins and antigens have been determined using thermometric biosensors. Piezoelectric biosensors operate on the principle of generation of electric dipoles on the subjecting an anisotropic natural crystal to mechanical stress.

They are used for the measurement of organophosphorus compounds, ammonia, and nitrous oxide substances. All these biosensors suffer from certain drawbacks for example, optical biosensors, though very sensitive, cannot be employed in turbid media. Thermal biosensors cannot be utilized with systems with very little heat change. Moreover, they are not easy to handle. Electrochemical biosensors have emerged as the most commonly used biosensors and presents more merits than other types of biosensors. These biosensors are rapid, easy to handle and are of low cost (Malhotra and Chaubey, 2003).

**Electrochemical transducers**

Among the chemical sensors electrochemical biosensors emerged as the most commonly used biosensors (Wang, 1994; Skoog et al., 2006). In electrochemical DNA biosensor, it is important to observe the differences of peak currents between single stranded DNA and double stranded DNA. There are quite amount of electrochemical methods for the biosensors such as differential pulse voltammetry, cyclic voltammetry, chronoamperometry, square wave voltammetry, chronopotentiometery (Thevenot et al., 2001). Biosensors based on the electrochemical transducer have the advantage of being economic and present fast response. They can be operated in turbid media, have comparable instrumental sensitivity and are more amenable to miniaturization. Also, possibility of automation allows application in a wide number of samples (Malhotra and Chaubey, 2003). During a biointeraction process, electrochemical species such as electrons are consumed or generated producing an electrochemical signal, which can in turn be measured by an electrochemical detector. Electrochemical biosensors have more benefits compared to the other sensors due to their simplicity user-friendliness, low cost, suitability for mass production, portability, sensitivity and selectivity (Malhotra and Chaubey, 2003).

## 2.4: Potentiostat

A potentiostat is an electronic instrument that controls the voltage between two electrodes.

### 2.4.1 Two Electrode Configurations

This configuration consists of a Working Electrode where the chemistry of interest occurs and a Counter Electrode which acts as the other half of the cell. Both electrodes are contained in an electrochemical cell. The potentiostat implements this control by injecting current into the cell through an Auxiliary, or Counter, electrode. In almost all applications, the potentiostat measures the current flow between the Working and Counter electrodes. The controlled variable in a potentiostat is the cell potential and the measured variable is the cell current.

The applied potential (EA) is measured between the working and counter electrode and the resulting current is measured in the working or counter electrode lead. The counter electrode in the two electrode set-ups serves two functions. It completes the circuit

allowing charge to flow through the cell, and it also maintains a constant interfacial potential, regardless of current. In twoelectrode systems, it's very difficult to maintain a constant counter electrode potential (eC) while current is flowing. This fact, along with a lack of compensation for the voltage drop across the solution (iRS) leads to poor control of the working electrode potential (eW) with two electrode systems. The roles of passing current and maintaining a reference voltage are better served by two separate electrodes.

### 2.4.2 Three Electrode Configuration

The three electrode systems consist of a working electrode, counter electrode, and reference electrode. The reference electrode's role is to act as a reference in measuring and controlling the working electrode potential, without passing any current. The reference electrode should have a constant electrochemical potential at low current density. Additionally, since the reference electrode passes negligible current, the iR drop between the reference and working electrode (iRU) is often very small. Thus, with the three electrode systems, the reference potential is much more stable, and there is compensation for iR drop across the solution. This translates into superior control over working electrode potential. The most common lab reference electrodes are the Saturated Calomel Electrode and the Ag/AgCl electrode. In the three electrode configurations, the only role of the counter electrode is to pass all the current needed to balance the current observed at the working electrode. The counter electrode will often swing to extreme potentials in order to accomplish this task.

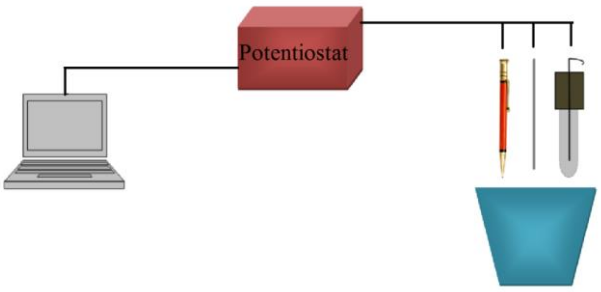



**Figure 2.4:** Schematics of conventional three-electrode-system connected to potentiostat and personal computer

### 2.4.3. Electrode types

**Reference Electrode**

The Reference Electrode is used to measure the Working Electrode potential. A Reference Electrode should have a constant electrochemical potential as long as no current flows through it(Skoog et al., 2006). The most common lab Reference Electrodes are the silver/silver chloride Ag/AgCl and the saturated calomel electrode (SCE). The Reference Electrode is used to measure the Working Electrode potential. A Reference Electrode should have a constant electrochemical potential as long as no current flows through it.

There are many different types of REs. For instance, a standard hydrogen electrode meets factors above, but in practice it is hard to use hydrogen electrode as a reference electrode. A reference electrode is critical to acquiring good electrochemical data. A drift in the reference electrode potential can cause quantitative and qualitative errors in data collection and analysis beyond simple inaccuracies in the measured potential. If the reference electrode impedance is too high it can cause instability in high performance potentiostat.

There are some factors that need to be considered when a RE is used:

- Concentrations of ions must remain same.
- Must not be affected by the experiment solution, ions, potential, change of the
- Current must be polarized
- The reaction in the reference electrode must be reversible

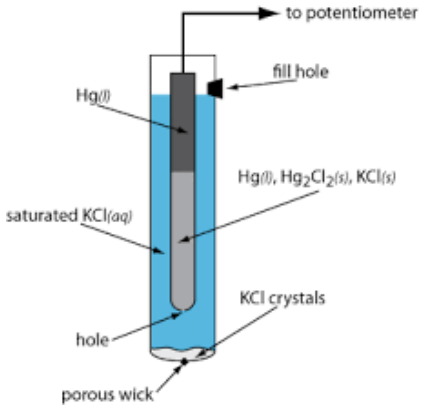



**Figure 2.5:** Schematic of reference electrode

**Working electrodes**

The Working Electrode is the electrode where the potential is controlled and where the current is measured. The working electrode (WE) is the electrode where the analyte is studied. The Working Electrode serves as a surface on which the electrochemical reaction occurs therefore needs to have some stability properties and easy preparation. For many physical electrochemistry experiments, the Working Electrode is an "inert" material such as gold, platinum, or glassy carbon, for example Pencil Graphite Electrode (PGE)is a solid working electrode with easy preparation and low cost. The Working Electrode can be bare metal or coated. For batteries, the potentiostat is connected directly to the anode or cathode of the battery. Various studies have been done with different working electrodes of Graphite, carbon paste, glassy carbon, gold, microarrays.

Literature records reflect that, there are numerous different types of working electrodes. Some of these working electrodes are shown in the Table 2.1.

**Table 2.1:** Classification of working electrodes

| **Carbon based electrodes** | **Solid Metallıc electrodes** | **Mercury based electrodes** | **The other electrodes** |
|---|---|---|---|
| Pencil Graphite | Gold | Hanging mercury drop | composites |
| Glassy carbon | Platinum | Amalgam electrodes | ITO (Indium tin oxide) |
| Carbon paste<br><br>Carbon fiber | Bismuth | Dropping mercury | Rotary spinning electrode |

**Counter (auxiliary) electrodes**

The Counter Electrode in lab cells is generally an inert conductor like platinum or graphite. In electrochemical measurements, potential is controlled and the main objective is to observe the current, and usually working electrode signals form in a wrong way. Instead, the counter, or auxiliary electrode is a conductor that completes the cell circuit (Esch et al.,2010). Current flows into the solution (electrolyte) through the Working Electrode leaves the solution via the Counter Electrode. The Counter Electrode in lab cells is generally an inert conductor like platinum or graphite. Working Electrode potential is measured under nearly zero potential. Counter Electrode does not affect the reaction in the electrochemical cell also need to be larger than Working Electrodes and kept near the Working Electrode.

As a Counter Electrode platinum, tantalum, tungsten, carbon wires have been used. The collection of the electrodes, the solution, and the container holding the solution are referred to as an electrochemical cell.

**2.5 The Electrometer**

The electrometer circuit measures the voltage difference between the working and the reference electrode. Its output serves two purposes: it acts as a feedback signal within the potentiostat, and (*I)* current is the voltage signal that is measured and displayed to the user. An ideal electrometer has infinite impendence and zero current. In reality the reference electrode does pass a very small amount of current. Current that passes through the reference electrode can change its potential, but this current is usually so close to zero that the change is negligible. The capacitance of the electrometer and the resistance of the reference electrode form an RC circuit. If the RC time constant is too large it can limit the effective bandwidth of the electrometer. The electrometer bandwidth must be higher than the bandwidth of all other components in the potentiostat.

**The I/E Converter**

The current to voltage converter measures the cell current. The cell current is forced through a current measurement resistor, Rm. The resulting voltage across this resistor is a measure of cell current. During the course of an experiment, cell current can change by

several orders of magnitude. Such a wide range of current cannot be accurately measured by a single resistor. Modern potentiostat have a number of Rm resistors and an "I/E auto ranging" algorithm that selects the appropriate resistor and switches it into the I/E circuit under computer control. The bandwidth of the I/E converter depends strongly on its sensitivity. Unwanted capacitance in the I/E converter along with Rm forms an RC circuit. In order to measure small currents, Rm must be sufficiently large.

**The Control Amplifier**

The control amplifier compares the measured cell voltage to the desired cell voltage and drives current into the cell to force these voltages to be the same. The control amplifier works on the principle of negative feedback. The measured voltage enters the amplifier in the negative or inverting input. Therefore, a positive perturbation in the measured voltage creates a decrease in the control amplifier output, which counteracts the initial change. The control amplifier has a limited output capability, for the PARSTAT is 10 V and 200 mA.

**The Signal**

In modern potentiostat, the signal circuit is a computer-controlled voltage source. Proper choice of number sequences allows the computer to generate constant voltages, voltage ramps and sine waves at the signal current output.

**Computer Controlled Instrumentation**

Most potentiostat now utilize a microprocessor for signal generation and data acquisition. Computers are very useful for generating complex voltage waveforms. These waveforms are first created as numerical arrays in memory, which are sent to a Digital to analog converter. The DAC produces an analog voltage proportional to the digital numerical arrays. The analog voltage is then sent to the control amplifier of the potentiostat. Conversely, in data acquisition, the voltage responses from the electrometer and I/E converter are digitized into numerical arrays and recorded at fixed time intervals. The accuracy of the analog to digital conversion depends on the number of bits used for a given voltage signal.

## 2.6Pencil graphite electrode

The pencil graphite leads are composite structure which constitutes graphite (~65%), clay (~30%), and a binder wax, resins, or high polymer (David et al., 2017). The usefulness of Pencil graphite electrodes (PGEs) has risen in the past decade and this has been prompted by its applicability in the analysis of various types of inorganic and organic compounds in the food industry. This type of working electrodes is constituted by graphite pencil leads.

In terms of cost, (David et al., 2017) PGEs are cheap and readily available, easy to use and are disposable electrodes and lessens the time-consumed needed for electrodes surface cleaning. Comparing them to other working electrodes, PGEs constitute lower background currents (Ardjmand& Rad, 2009), good reproducibility, higher sensitivity, and an adjustable electro active surface area (Purushothama& Nayaka, 2017), which makes it possible for the analysis of low concentrations and small sample volumes without any deposition/ pre-concentration step.

Pencil graphite leads used as working electrodes are currently known as pencil graphite electrodes (PGEs). According to Purushothama& Nayaka, (2017), using various types of voltammetric techniques in order to quantify a variety of analytes from a wide range of samples, the PGEs used showed reproducible signals, yielding well-defined voltammetric peaks, (David et al., 2017). Pencil graphite electrodes yielded high sensitivity and reproducibility, and thereby being a viable, renewable, and economical tool (Ardjmand& Rad., 2009).

It has been reported that, independent of the producer and hardness, PGEs have electrical resistance (David et al., (2017), lower than 5ohm, being thus suitable as electrode material. Pretreated PGE have a good electrochemical reactivity, sensitivity and selectivity in comparison with other modified carbon-based electrodes because the PGE are hydrophilic nature (water loving) and their surface increases at positive potentials on pretreatment and provides a good adsorption surface (Purushothama& Nayaka., 2017).

From previous papers, it is evident that the pencil graphite electrodes are found to be more versatile in contrast with other carbon-based electrodes like glassy carbon electrodes and carbon paste electrodes (Ardjmand& Rad., 2009). This is because of the fact that the pencil

graphite electrodes constitute high reactivity, more sensitivity, low-cost, low background current, ease of surface modification, and easy preparation technique compared to other electrodes (Purushothama& Nayaka, 2017). Even with sp2 hybridized carbon, the pencil graphite electrode (PGE) shows good conductivity and adsorption properties. Various literatures have been reported on PGE for electro-analytical applications including in ascorbic acid determination (Purushothama& Nayaka., 2017).

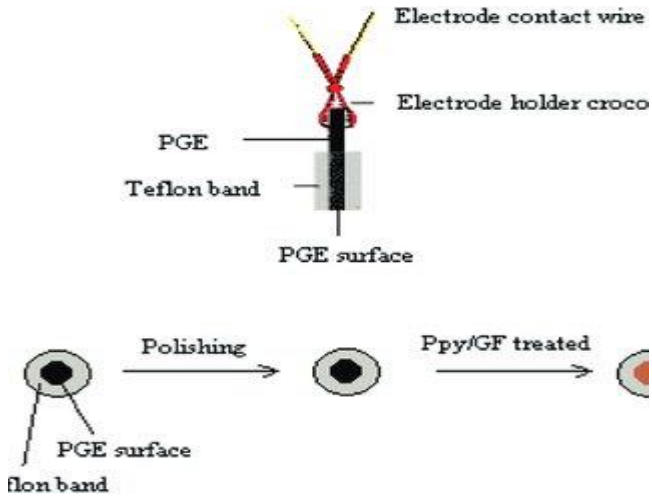



**Figure2.6:**Shows a pencil graphite lead

Using pencil graphite as disposable electrodes (PGEs) in electrochemical ascorbic acid sensing make the method easier and more rapid compared with the conventional DNA biosensors. The use of pencil graphite electrodes has several advantages, such as avoidance of contamination among samples, ease of use due to without any need to pretreatment, constant sensitivity, selectivity and reproducibility. Testing for the presence and level of nutrients in drinks and food samples is one of the applications of (PGEs). Characteristics such as low-cost, easy-to-use, portability, and miniaturizable, as well as maintaining the level of accuracy and sensitivity of laboratory diagnostics, made pencil graphite electrode gain more relevance in the field of food analysis.

## 2.7 Immobilization

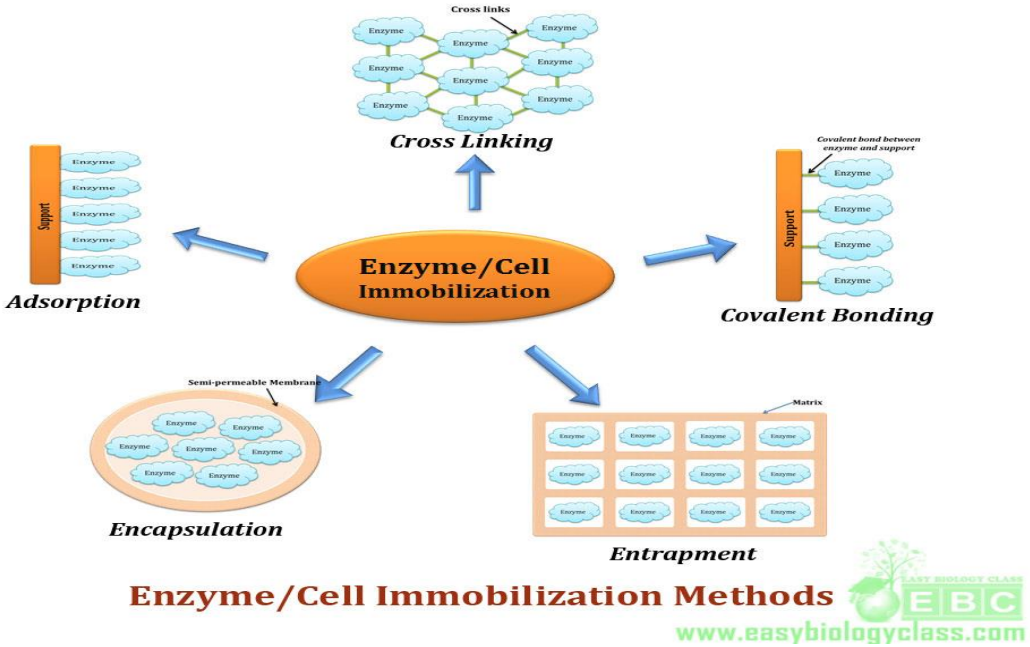



**Figure 2.7:** Immobilization methods

### 2.7.1 Adsorption

It is a type of physical binding where fluid phase of certain components in dissolved form holds on to solid adsorbents due to surface adhesion. Adsorption is only achieved through three different ways observed and these are seen together or sequentially in many adsorption cases.

### 2.7.2 Physical adsorption

- It is formed by Van der Waals forces between solid surface and adsorbent. It is significant to note that physical adsorption is reversible and there might be heat as result of condensing energy.

### 2.7.3Chemical adsorption

- Chemical Adsorption is comprised of attraction between functional groups of solid surface and adsorbent. It is irreversible and Adsorption released heat is bigger than reaction heat: Electrode is coated with monomolecular layer generally

### 2.7.4 Ionic adsorption

- With the electrostatic forces, ions are held on to the charged areas on the surface. In ionic Adsorption it is important to note that between adsorbent and adsorberions, smaller charged ions are absorbed.

### 2.7.5 Covalent attachment

- It depends on engaging, functional groups to the bare electrode. There is a covalent bond with the functional group and the substrate. At first Murray et. al. in 1978 used this technique and they interacted hydroxyl groups on the electrode surface and organocylane. Carbon based electrodes are available to create functional groups on their surfaces. Especially with Glassy carbon electrode there are lots of studies about covalent attachment.

### 2.7.6Entrapment

- The enzymes have a three-dimensional conformation which has a critical effect on its catalytic action. Consequently, when immobilizing an enzyme, it is necessary to use such methods and chemicals that the functional tertiary structure will not be affected. The principle behind the entrapping technique is to form a cross-linked polymeric network around the material to be trapped. This is usually performed by mixing the monomers, a cross-linking agent, and the material to be entrapped in a buffered solution and then adding a catalyst system, which initiates the polymerization process. Most enzymes have been entrapped in acrylic polymers, probably because of the great possibilities of varying the monomers used and thereby the chemical and physical properties of the polymer that is formed. Other materials used include starch, silicone rubber, silica gel, fibrin, and collagen.

### 2.7.7 Covalent binding

- Minehan et. al. stated that DNA Adsorption onto electrode coated PPy is diffusion controlled. If a covalently immobilized DNA were to be claimed successful, there should not be any non-specific bondings. That's the reason immobilization step is crucial. These non-specific bondings affects the results thereby descreases the sensitivity and reliability.

## 2.8 Electroanalytical techniques

Electroanalytical techniques depend on measuring the potential and/or the current of solution of the analyte in an electrochemical cell and provide low detection limits. The detection principles of electroanalytical techniques can be divided into amperometry, potentiometry, impedimetry and voltammetry. The electroanalytical techniques has numerous advantages of low cost, ease of miniaturization, portability, ease of assembly and the ability to work with turbid samples (Lim & Ahmed., 2016; Skoog et al., 2017).

### 2.8.1Voltammetrictechniques

Voltammetric techniques depend on the measurement of current (i) in an electrochemical cell as a function of applied potential (E). Voltammetry is commonly used to analyse biological, physical, and inorganic studies of oxidation and reduction process, (Skoog et al., 2017) adsorption process on surfaces and electron transfer mechanisms at surfaces of modified electrodes. More so, voltammetric techniques have many advantages such as excellent sensitivity, large useful linear concentration range, rapid analysis time, a wide range of temperatures, and determination of several analytes simultaneously. Voltammetry gives ample time to analytical chemists for quantitative determination of several dissolved organic and inorganic substances (Nolan et al., 1997). The voltammetric experiment is carried out in an electrochemical cell which is made up of sample dissolved in a solvent, supporting electrolyte, a working electrode, a counter electrode and a reference electrode (Nolan et al., 1997). The oxidation/reduction process of the analyte occurs at the surface of working electrode. The current in the cell passes between the working electrode and the counter electrode (Skoog et al, 2003). The schematic diagram of three-electrode system of an electrochemical cell is shown in Figure 1.6.

### 2.8.2 Cyclic Voltammetry (CV)

Cyclic Voltammetry technique is the most widely used for acquiring qualitative information about electrochemical reactions because it offers a rapid location of redox potentials of the electroactive species (Skoog et al., 2006). This technique depends on monitoring the current as a function of applied potential to the working electrode both in positive and/or negative direction at a constant scan rate (Kounaves, 1997). In this case the voltage is swept between two values at a fixed rate, however now when the voltage reaches

V2 the scan is reversed and the voltage is swept back to V1 a typical cyclic voltammogram recorded for a reversible single electrode transfer reaction is shown in below. When the scan is reversed, we simply move back through the equilibrium positions gradually converting electrolysis product (Fe2+ back to reactant (Fe3+. The current flow is now from the solution species back to the electrode and so occurs in the opposite sense to the forward seep but otherwise the behaviour can be explained in an identical manner. For a reversible electrochemical reaction, the CV recorded has certain well-defined characteristics.

a. The voltage separation between the current peaks is
b. The positions of peak voltage do not alter as a function of voltage scan rate
c. The ratio of the peak currents is equal to one
d. The peak currents are proportional to the square root of the scan rate

### 2.8.3 Electrochemical impedance Voltammetry

Electrochemical impedance spectroscopy (EIS) is a method of analysis which uses the surfaces of various systems either batteries, photovoltaic systems, and or some life science applications. The system is relying on introduction of a perturbation by using a sine wave current or small amplitude potential. The instrument senses the resulting changes in the form of an impedance diagram that provides useful data. The impedance spectrum is obtained by changing the frequency over a wide range AC (Fang et al., 2008). Electrochemical impedance spectroscopy is divided into two categories which are steady-state methods and non-steady state methods. In a steady-state EIS, a constant perturbation is imposed on the system which then defines the potentiometry and voltammetry as steady-state methods.

Usually the EIS measurements take a prolonged period, i.e., it can take from anywhere between ~10 min to more than several hours to collect an EIS spectrum and this depends on the relaxation processes/stability of the system under study, and the frequency range chosen. Unlike other techniques available which easily separate the bulk membrane charge transport processes from the interfacial reactions; however, EIS is one of 39 very few techniques that is capable of providing this information simultaneously. EIS also provides more prolific information about the barrier properties (Defega& Kwak., 2008). Electrochemical impedance spectroscopy-based biosensors are widely used in biological

applications such as food science, medicine, and environmental analysis, since the instrument can be miniaturized using micro electromechanical systems (MEMS) technology.

Electrochemical impedance spectrum (Nyquist plot, Z" vs. Z') is composed of a semicircle part in a high frequency region and a linear part in a low frequency region, corresponding to the electron transfer process and the diffusion process, respectively. In EIS, the diameter of the semicircle represents the charge-transfer resistance (Rct), which controls the electron transfer kinetics of the redox probe at the electrode interface. In AC impedance, amplitude and a frequency range are applied at open circuit potential (Fang et al., 2008).

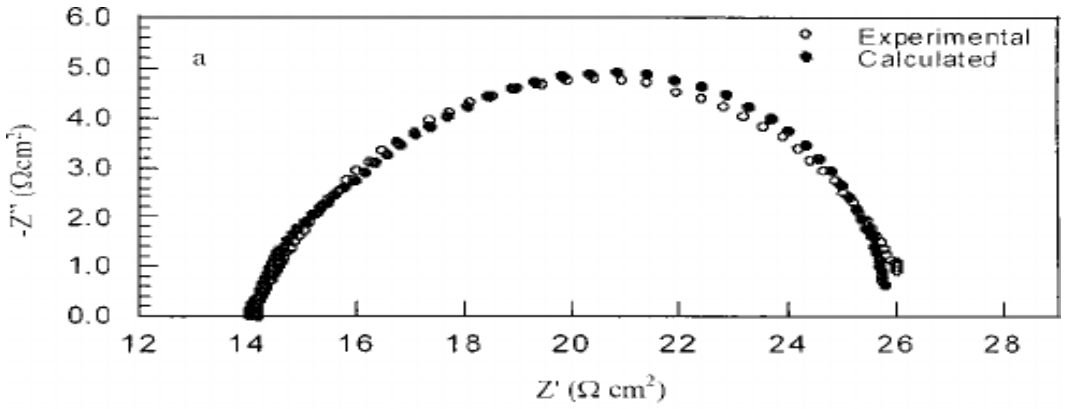



**Figure 2.8:** ANyquist plot

### 2.8.4. Differential pulse Voltammetry (DPV)

DPV is more advantageous to use for analytical measurements in comparison to other electrochemical techniques. This is because DPV as a technique has excellent sensitivity, which when the potential is alters linearly with time (potential linear sweep) superimposed by the 19 potential pulses of the amplitude between 10 and 100 mV for several milliseconds (Sochor et al., 2013). The current is measured at two points at each pulse, where the first point (a) before the application of pulse and the second point (b) at the end of the pulse. The difference between the two sampled currents is plotted against the staircase potential and leads to a peak shaped waveform. The excitation waveform of the differential pulse voltammetry is shown in Figure 9.

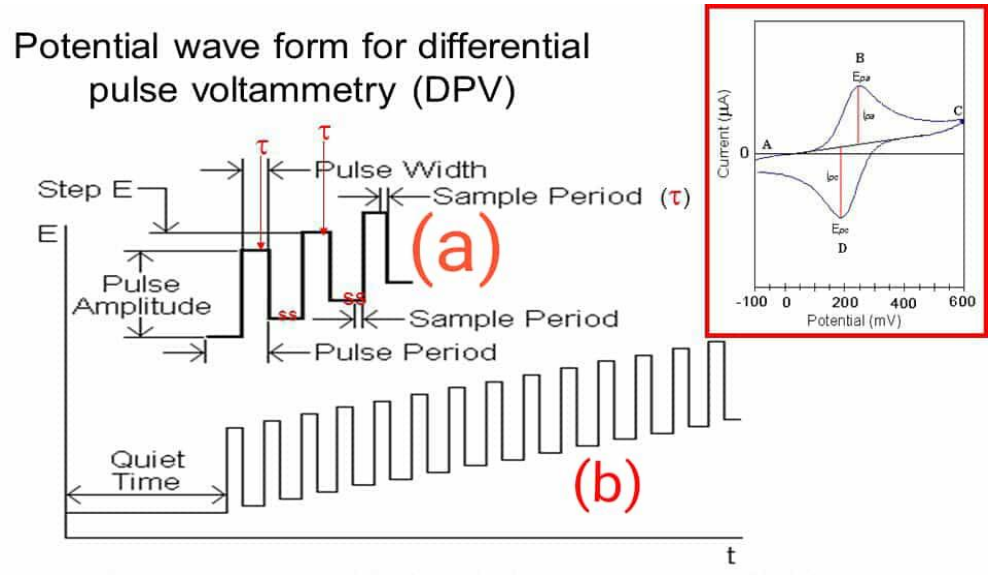



**Figure 2.9:** The excitation waveform of the DPV

# CHAPTER 3

# RELATED RESEARCH

This chapter summarizes the previously conducted studies on ascorbic acid and different quantitative techniques used for its determination.

The DPV technique was employed for the direct quantitative determination of ascorbic acid in tablet dosage form and some fruit juices samples were used. The electrochemical oxidation of ascorbic acid occurred on the surface of a glassy carbon electrode in various aqueous solutions pH range of 0.64-10.15 (in Britton-Robinson, acetate, phosphate buffers and 0.5 mol $L^{-1}$sulphuric acid solutions) by cyclic (CV) and differential pulse (DPV) voltammetry. Quantitatively, a diffusion controlled voltammetric peak was obtained in 0.2 mol $L^{-1}$ acetate buffer (pH 3.49) at 0.342 V by DPV technique. From the results, the linear response was obtained in the concentration range of $6\times10^{-6}$ - $8\times10^{-4}$ mol $L^{-1}$ with a detection limit (LOD) of $5.17\times10^{-7}$ and quantitative limit (LOQ) of $1.72\times10^{-6}$ mol $L^{-1}$ (Yilmaz et al., 2008).

According to Gheibietet al., (2013) cyclic voltammetry (CV) and chronoamperometry were used to investigate the suitability of AP as a mediator for the electrocatalytic oxidation of ascorbic acid in aqueous solution. The oxidation of ascorbic acid occurred at a potential about 320 mV less positive than with the unmodified carbon paste electrode at pH 7.0. The catalytic reaction rate constant, $k_h$ was calculated ($2.257\times10^{3}$ $M^{-1}$ $s^{-1}$) using chronoamperometry. The differential pulsevoltammetric (DPV) peak currents of the electrode increased linearly with the corresponding AA concentration in the range of $2.0\times10^{-7}$ M – $1.2\times10^{-4}$ M with a detection limit of $8.0\times10^{-8}$ M (Gheibiet at., 2013). pH and potential interfering substances on the determination of ascorbic acid were also studied. Finally, the electrocatalytic oxidation of ascorbic acid was studied using p-aminophenol modified carbon nanotubes paste electrode (APMCNTPE) it proved to be selective, simple and precise electrochemical sensor to use in real samples such as fruit juices and fresh vegetable juice for ascorbic acid determination.

A screen-printed electrode (SPE) it is widely used in the electro-analytical measurements (Pardakhty et al., 2016) because of its simplicity to use, portability, cost effectiveness, versatile, reliable, and capable of mass production. In this particular study the SPEs were modified with nanostructures such as carbon nanomaterials, grapheme, and nanostructures metal oxides (Baghizadeh et al., 2015) to improve their electrochemical performance on sensitivity and selectivity, even the detection limit became lower to facilitate the direct electron transfer.

Carbon nanotubes have become the subject of intense researches in the last decades because of their unique properties and the promising applications in any aspect of nanotechnology (Shahmiri et al., 2013). Because of their unique one-dimensional nanostructures, CNTs display fascinating electronic and optical properties that are distinct from other carbonaceous materials and nanoparticles of other types. CNTs are widely used in electronic and optoelectronic, biomedical, pharmaceutical, energy, catalytic, analytical, and material fields (Ensafi and Karimi-Maleh 2010a). Particularly, the properties of small dimensions, functional surfaces, good conductivity, excellent biocompatibility, modifiable sidewall, and high reactivity make CNTs ideal candidates for constructing sensors with high performances. As an example, CNTs have been extensively employed in constructing various electrochemical sensors (Ensafi et al. 2012a, 2013; Mokhtari et al. 2012).

According to Wang (2017), Nanomaterials are used for various applications in electrochemistry and ZnO nanostructures due to wide band gap (3.37 eV), large excitation binding energy (60 eV), non-toxicity, biocompatibility, chemical and photochemical stability, and high electron communication features is preferred for the fabrication of effective sensors, (Pardakhty et al., 2016). Absence of any of these constituting components severely reduces the performance of the catalyst system (Wang, (2017). Therefore, the importance of using $ZnO/Al_2O_3$nanocomposite as catalytic materials is very high. $ZnO/Al_2O_3$/SPE shows advantages in terms of selectivity, reproducibility, and sensitivity.

It has been reported that, (Ganjali1 et al., 2016, Ganjali et al., 2107) a sensitive and selective voltammetric sensor based on graphite screen printed electrode modified by $ZnO/Al_2O_3$nanocomposite for the ascorbic acid detection was developed. The

electrochemical behavior of prepared electrode for the determination of ascorbic acid was systematically investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Under optimum conditions, the modified electrode exhibited the linear responses to ascorbic acid and finally, they successfully developed a sensor which was applied to detect ascorbic in real samples (Ganjali1 et al., 2016).

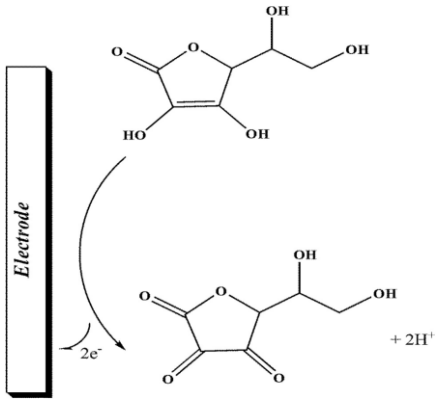



**Figure 3.1:** Electrochemical mechanisms for electro-oxidation of ascorbic acid (Lui et al 2015)

Wei developed a ratiometric electro-chemical sensor to detect AA by using the thionine (Thi)/Ketjenblack (KB) nanocomposites dropped on the surface of (GCE) glassy carbon electrode (Wang et al., 2017). Although the ratiometric electrochemical sensor could selectively detect AA, the Thi and KB were just only physically mixed, which made the components easy fall off from the electrode surface to result in a poor performance (Wang et al., 2017). The ratiometric electrochemical approach is not only a new method for AA detection but also opens a new way for sensitive detection of other analytes.

A high-quality method for one-pot biosynthesis of silver nanoparticles (AgNPs) using onion extracts as reductant and stabilizer was reported. The synthesized AgNPs were characterizedQ2 by ultraviolet-visible spectroscopy (UV-Vis), (Khalilzadeh&Borzoo 2016) X-ray powder diffraction (XRD), and transmission electron microscopy (TEM). UV-Vis spectroscopy results showed that the AgNP absorption band was located at a peak of 397 nm in aqueous solution.

Recently, metal-based nanoparticles have also been incorporated into electrochemical sensors for food compounds and pharmaceutical, (Baghizadeh et al., 2015). Since they

constitute a number of properties similar to some types of carbon nanotubes, they have a number of comparative advantages including enhanced electron transfer, large edge plane/basal plane ratios, and rapid kinetics of the electrode processes. One published paper reported that voltammetric determination of AA and vitamin B6 was done using modified electrodes (Baghizadeh et al., 2014).

Graphene and its derivatives have attracted considerable attention due to their unique properties which includes electronic, optical and mechanical properties. According to Liu et al (2017) in their research, an electrochemical sensor for ascorbic acid was made through a one-step electrochemical approach which reduced grapheme oxide (rGO) and co-polymerizing neutral red (NR) and rGO to hybridize the two to form a pNR/rGO film on the glassy carbon electrode. Effectively, electrochemical data proved that pNR/rGO film can enhance the electron donation between ascorbic acid and electrode (Liu et al 2017), and this would reduce the over potential of ascorbic acid oxidation. Of significance is that the pNR/rGO-GCE gave identical results to that obtained with HPLC when measuring real samples, (Esteve et al., 1995).

Literature reviews that an experiment was carried out using vitamin C concentration as a nutritional marker, and there was no clear or significant difference interns of nutritional content between organically grown and conventionally grown fruit, (oranges, mangoes, kiwi, lemons, gala apples, and red delicious apples). Of the six types of fruits analyzed by cyclic voltammetry only one, lemon, demonstrated a significantly higher vitamin C concentration for organically grown versus conventionally grown fruit. Our results suggest that other factors are more influential on vitamin C levels than whether fruits are organically or conventionally grown.

A simple selective and precise voltammetric method for the determination of ascorbic acid in pharmaceutical preparations and fresh fruit juices(Pournaghi-Azar&Ojani, 1997),the electrocatalytic oxidation of ascorbic acid in homogeneous solution using electrogenerated ferricenium carboxylic acid as mediator. The pH and mediator concentration affecting the performance of the electrocatalytic oxidation of the analyte were optimized. The method was applied to determine vitamin C in deeply colored, viscous and turbid fruit juice

samples with ascorbic acid contents ranging from 15-45 mg per 100 ml, without further dilution, concentration or other pre-treatment of the samples(Pournaghi-Azar&Ojani, 1997). The method was also applied again for pharmaceutical analysis using a calibration graph. For fruit juice samples the standard addition technique was adopted to prevent the matrix affecting the accuracy of the determination. The relative standard deviation for the analysis of vitamin C in fruit juices ranged from 1.5-5%. The reliability of the method was established by parallel determination against the official methods, (Pournaghi-Azar&Ojani 1997).

Colored fruit juices are difficult to work with, but some voltammetric methods using conventional electrodes, a micro disc electrode and a micro band electrode were reported. These methods suffer from interference, loss of response with repeated use because of electrode fouling by oxidation products or a lack of generality(Pournaghi-Azar&Ojani 1997). Ascorbic acid often has to be quantified in complicated matrices where the exploitation of heterogeneous as well as homogeneous electrocatalytic oxidation might be advantageous. This provides selectivity and prevents the fouling of the electrode surface.

Some research works have been reported in this context. A method using a flow-injection system with square-wave voltammetric detection was also reported. Recently literature demonstrated that a polypyrrole/hexacyanoferrate(II)-modified glassy carbon electrode can be used for the catalytic determination of ascorbic acid in a solution of pH 4, (Pournaghi-Azar&Ojani 1997). This same method was used for pharmaceutical analysis but was not suitable for the determination of vitamin C in fruit juices. In addition, it is reported that ascorbic acid can be oxidized catalytically in homogeneous solution by some electrogenerated ferricinium derivatives in buffered solution at pH 4, the method was used successful for the simultaneous determination of ascorbic acid and dopamine in the same sample.

Titrimetric method using N-bromosuccinimide and also by cyclic voltammetry using a glassy carbon as working electrode, Ag/AgCl as reference and platinum as the auxiliary electrode in 0.1 M phosphate buffer, pH 2.0 containing 1mM $Na_2EDTA$ was reported for the determination levels of ascorbic acid in 50 tropical fruit samples. The potential range of 200mV to 1000mV of measurements was made in relation to reference electrode using a

scan rate of 50mV/s. The anodic peak currents for the electrochemical oxidation of ascorbic acid to dehydroascorbic acid were recorded at 580 mV, however no peak current observed on thecathode potential range studied (Okiei et al., 2009). Fruits found to have high levels of ascorbic acid include grape (68.82mg/100cm$^3$), orange (64mg/100cm$^3$), lime (56.57 mg/100cm$^3$), pawpaw (55.8 mg/100cm$^3$), cherry (54.86mg/100g), and guava (51.02mg/100g). These results were obtained by cyclic voltammetric method and titrations with N-bromosuccinimide are generally comparable but large differences are obtained in some fruits (Okiei et al., 2009).

# CHAPTER 4

# MATERIALS AND METHODS

## 4.1 Citrus fruit samples

Samples were collected from Cyprus Guzelyurt commercial horticultural farm. The commercial Citrus fruit samples names are:the bitter orange *Citrus aurantium*, Lemon (*Citrus limon)*, grapefruit*(Citrus paradisi*), mandarine I*(Citrus reticulata)* and mandarine II *(Citrus reticulata).*

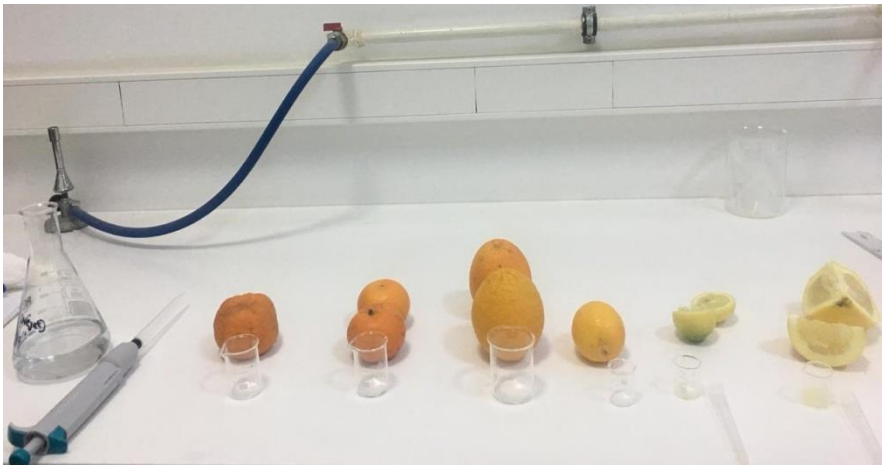


**Figure 4.1:** The citrus fruit samples used in the analysis.

### 4.1.2Vitamin C standards preparation.

Vitamin C pure standard was supplied by SIGMA and it was used for the preparation of stock solution and then calibration solutions. The different concentrations of vitamin C were applied at 1.56mg/100ml, 3.125mg/100ml, 6.25mg/100ml, 12.5mg/100ml, and 25mg/100ml respectively as we calibrated the standard solutions.

## 4.2 Apparatus and electrodes

### 4.2.1 Potentiostat

All the electrochemical differential pulse voltammetric measurements were done using anAUTOLAB PGSTAT 204 (Utrecht, The Netherlands)potentiostat with a conventional three electrode system. The pencil graphite electrode was used as working electrode. An Ag/AgCl with 3.0 M KCl, and platinum wire were used as reference and counter electrodes, respectively. The Potentiostat-galvanostatwith software NOVA 2.12 was linked toan ACER desktop computer for electrochemical measurements and treating of data.

### 4.2.2 pH meter& beakers

353 ATC pH-meter was used to read the pH of the buffered solution. Digital (110g/0.1mg) balance model LA 114 was used for mass measurements. Beakers were used to contain reagents and the juices. Distilled water was used to clean the equipment to be used to prevent contamination.

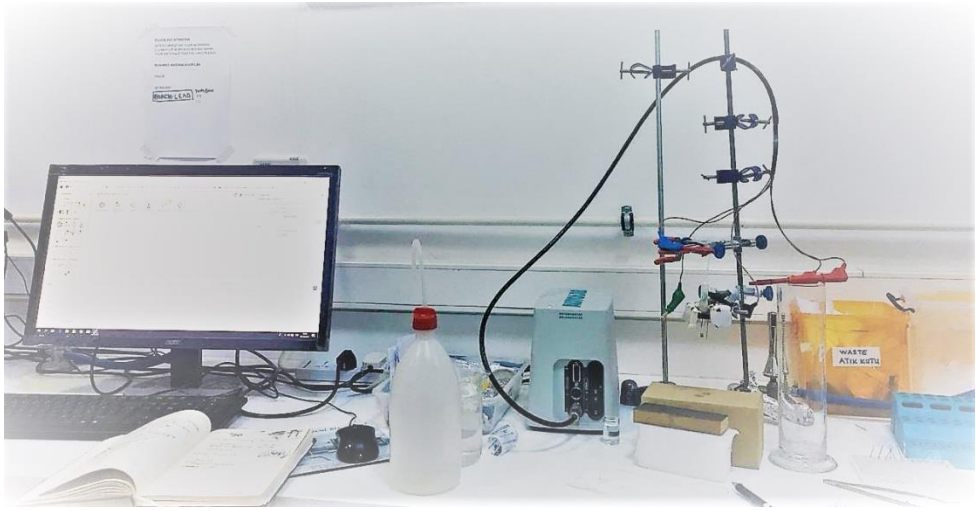


**Figure4.2:**AutolabPotentiostat/Galvanostat 204coupled with an Acer desktop computer.

### 4.2.3 Preparation of pencil graphite

The pencil graphite electrode (PGE) was prepared by using 0.5 mm HB pencil lead of length of 60 mm. Pencil leads are made up of 65% graphite, 30% clay, and a binder (wax, resins or high polymer) The pencil graphite electrode was formed by cutting the leads (Tombow, HB, D: 0.5mm) into 30mm long. The PGE leads were cut into half and were inserted into a mechanical pencil holder to keep out at least 1.5cm of the pencil graphite lead out. The PGE was connected to the instrument by soldering metal wire on the pencil tip holders' metallic top. The one end of the pencil lead was connected to a copper wire to make electrical contact and during analysis 1cm of the pencil graphite lead was inserted into the solution to be analyzed while the holder was kept upright to avoid short circuiting.

### 4.2.4Reagents

**Preparation of 0.5 M acetate buffer solutions:**

Acetate buffer solution was prepared with 14.45 ml of glacial acetic acid dissolved in 250ml of distilled water, and 250ml of 1M NaOH was added in the solution and 0.02 M of NaCl for the electrical conductance. The pH of the buffer was adjusted by a drop wise addition of NaOH. Acetate buffer solution was adjusted to pH 4.8, the potential-controlled pulse voltermetric. The analyses were done at 25 ˚C.

## 4.3 Methods

### 4.3.1Electrochemicalactivation of Pencil graphite electrodes (PGEs)

Chronoamperometry is an electrochemical technique in which the potential of the Pencil graphite electrode (working electrode) is stepped and the resulting current from faradaic processes occurring at the electrode (caused by the potential step) is monitored as a function of time. The functional relationship between current response and time was measured after applying single potential step to the working electrode of the electrochemical system. The pencil graphite electrode (PGE) was inserted in an unstirred solution of acetate buffer for chronoamperometry procedure which was performed under the constant potential of +1.4V for 30s. Electrochemical pretreatment of PGE was carried out by scanning at positive potential between 0.4 and 1.2 V with the scan rate of 100 mV $s^{-1}$ for 50 cycles in 0.5M acetate buffer solution (ABS) of pH 4.8. PGE was used as a working electrode.

### 4.3.2Vitamin C extraction from Fruits

The fruit samples were first washed with water and the juice from each fruit was hand squeezed out, and filtered into the glass beakers to avoid contamination from metal ions which could interfere in the determinations. Only the traditionally consumed portions of the fruit were used in the extraction procedure. The juices obtained were filtered into a beaker, a 5ml portion of the filtrate was transferred into an electrochemical cell the required amount of 5ml of 0.5 M of Acetate buffer solution was added as supporting electrolyteto make 10ml. Vitamin C was extracted, from the fruit, into solution at a pH 4.8 phosphate buffer. The filtrate was degassed with nitrogen for 5 minutes and immediately tested. Since an ascorbic acid solution is unstable, each sample and reference solutions

were made freshly and kept away from light to avoid oxidation prior to any test. This procedure was the same for all the different citrus fruit samples which were analyzed. The ascorbic acid content in the fruit juice samples were determined by measuring the peak current from the calibration curves.

### 4.3.3Differential pulse voltammetry of citrus fruits extracts

The electrochemical behavior was examined using differential pulse voltammetry (DPV). Citrus fruit samples were collected from Cyprus Guzelyurt commercial horticultural farm.Since DPV has a much higher current sensitivity and a far better resolution than cyclic voltammetry, DPV was used for determination of ascorbic acid. These steps were done to the 6 different citrus fruit samples and 3 pencil tips were used for each sample. The reference and the counter electrodes were washed with distilled water after each experiment; DPV was performed with a potential range from 0 to +1.2 V, with 0.005 V step potential, 10 mV/s scan rate, 119s duration time. Responses were linear with ascorbic acid concentrations ranging with three different slops. The peak height of the current response was taken as a measure of the ascorbic acid concentration.

**Figure 4.3** A photo from experiment

# CHAPTER 5

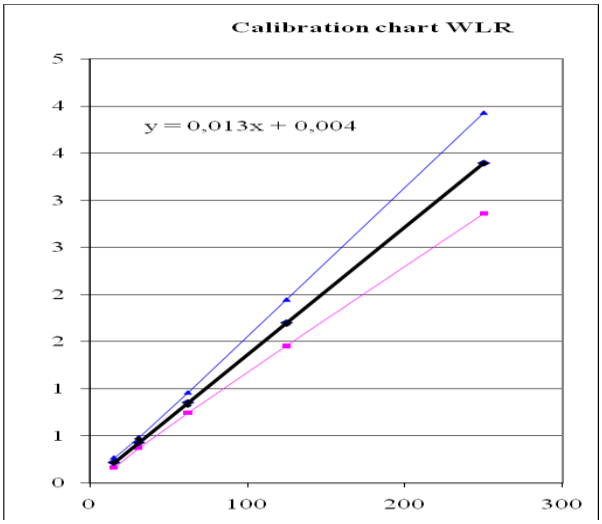
# RESULTS AND DISCUSSION

## 5.1 Construction of calibration Curve

After the investigation of the optimum parameters, the calibration plot of ascorbic acid was constructed by using 5 different concentrations (Table 5.1). The analytical parameters for the quantitative determination of ascorbic acid were given in the Table 5.1. The quantitative evaluation was based on the linear correlation between the peak current and concentration of vitamin C. LOD (limit of detection) was calculated from calibration curve. It was 5.487mg/kg. The $R^2$ was 0.999. Voltammograms of vitamin C were used to measure the amount of ascorbic acid in citrus fruit sampled.Fig 5.1 displays the calibration curve concentrations and corresponding responses. The proposed method has had sufficient detection limit and wide linear range for detection of ascorbic acid in sample sources.

**Table 5.1:**Showing the peak area of the samples

| Cppm | Peak area 10-6 mA |
|---|---|
| **15.6000** | 0.21 |
| **31.2500** | 0.47 |
| **62.5000** | 0.82 |
| **125.0000** | 1.68 |
| **250.0000** | 3.39 |




Concentration (mM)

**Figure5. 1:** Calibration curve for ascorbic acid

*LOD: 5.487 ppm $R^2$=0.999. Calibration equation is Y=0.0136x+0.0046*

Our results on Figure 5.2 illustrate the quantitative measurements of all together vitamin C calibration standards by differential pulse voltammetry.

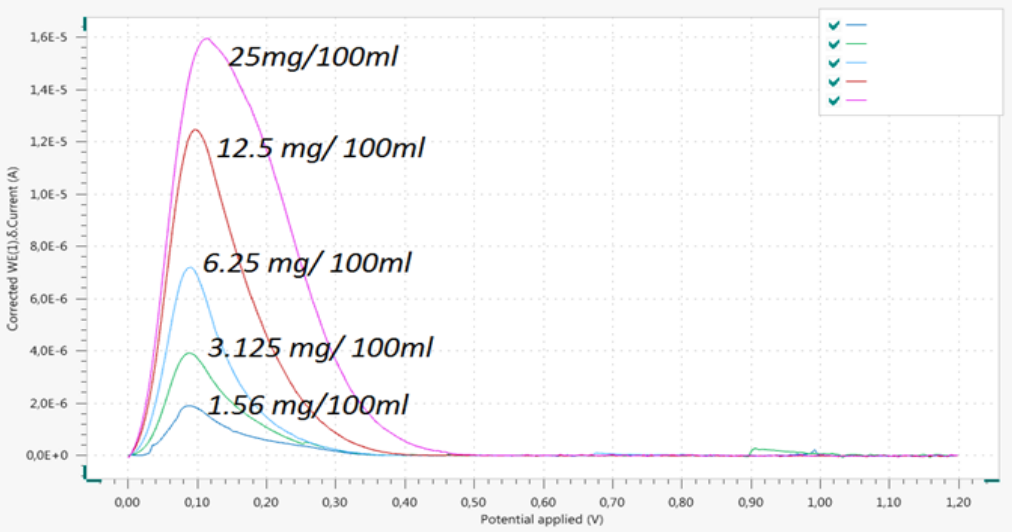



**Figure 5.2:** Shows the summary of different peak heights due to different concentration of vitamin C standard solutionin 0.5M acetate buffer solutionpH 4.8.

Since the differential pulse voltammetry has a higher sensitivity than cyclic voltammetry, it was used for the optimum technique for determination of vitamin C concentration. The influence of concentration of compounds was studied by DPV with vitamin C prepared in triplicated under the selected concentration between15.60 and250.0. The current (A was plotted versus concentration.Peak area of vitamin C peaks were used in quantitation. The correlation coefficient and the detection limit were found as 0.999 and 5.487mg/kg respectively. Good linearity was obtained for vitamin C.

### 5.2 Confirmation of vitamin C peak position with standard addition

First, we obtained vitamin C standard solution and fruit samples voltammograms. It was observed that there was a shift in peak position of vitamin C in fruit samples. Then to be sure we added some vitamin C standard into fruit samples and re-analysed. Figure 5.3-5 shows voltammograms of these measurements in different fruit samples. As a result, vitamin C peak was shifting in sample matrix. Then this peak position of 0.34 was used as a qualitative parameter for further sample analysis.

#### 5.2.1DPV voltammograms for bitter orange

Figure 5.3illustrates different voltammograms ofbitter orange, standard added bitter orange and the standard. From the graphical representation of vitamin C concentration in Fig. 5.3 the peak position voltage for the vitamin C ranged from 0.2 -0.4 (V). The peak position at the highest peak point was 0.34 V.

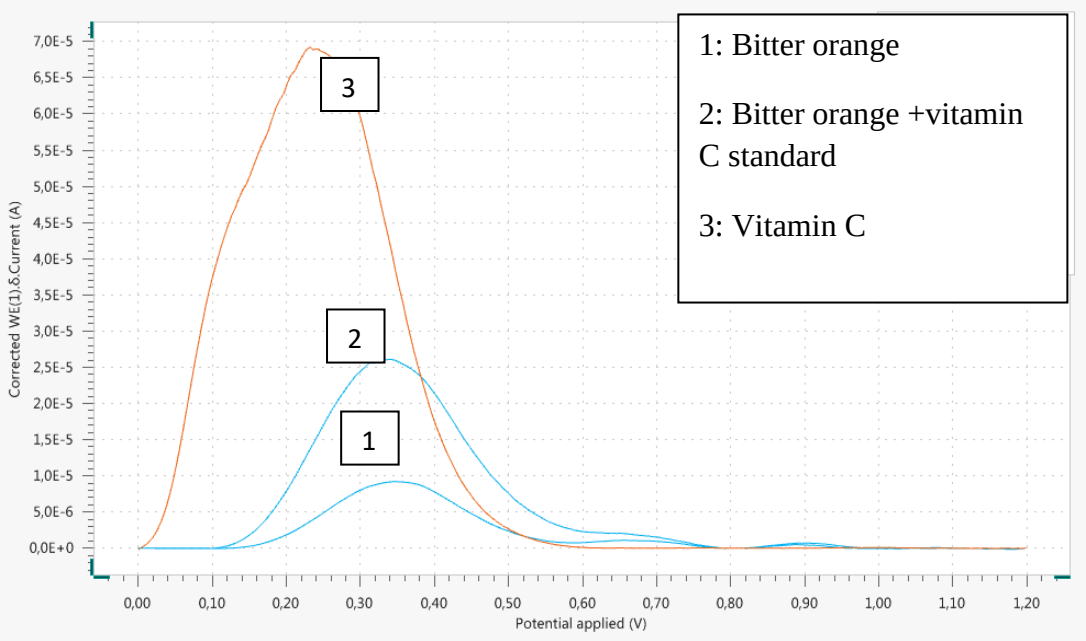
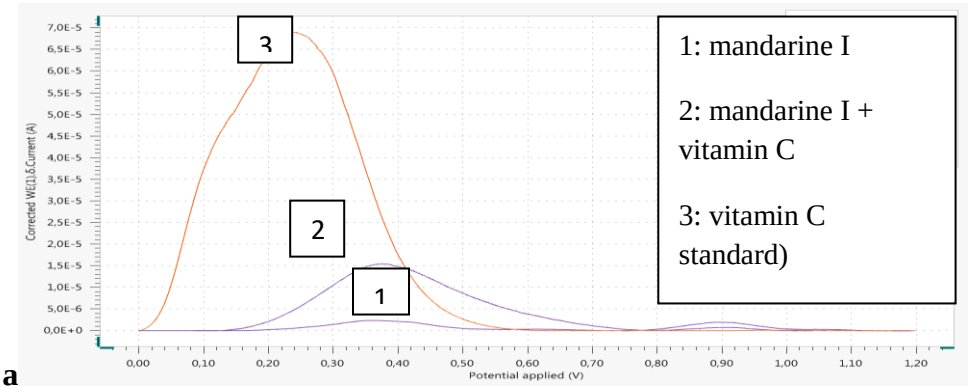


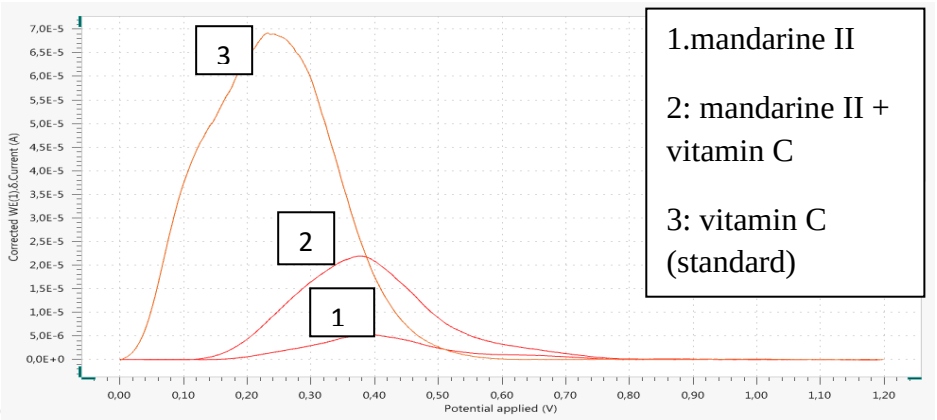

**Figure5.3:**DPV voltammograms recorded for fresh juices and with added standards: 1. bitterorange + vitamin C; 2. bitterorange 3. vitamin C (standard)in 0.5M acetate buffer solution (pH 4.8).

### 5.2.2 DPV voltammograms for mandarine I and II

The same procedure was employed as before for mandarine samples. The potential voltage for the peak height ranged from 0.2 – 0.4 (V).




a



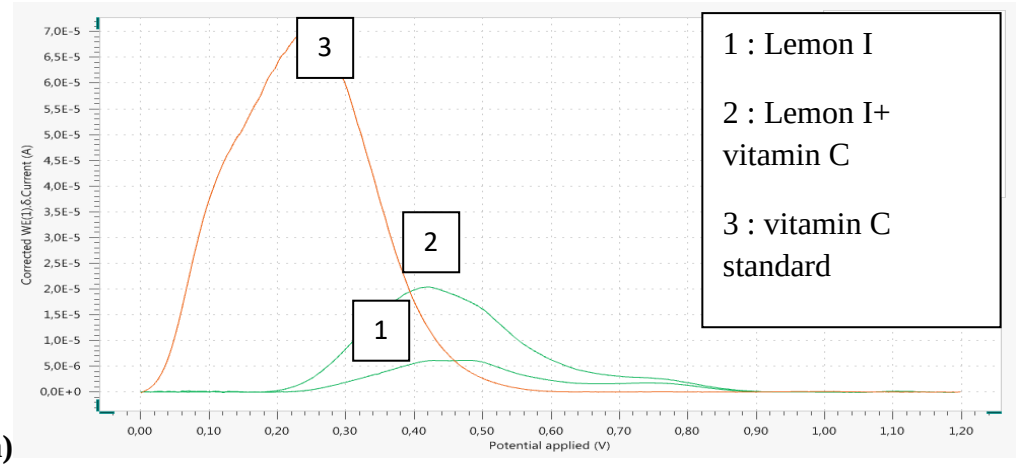

b

**Figure 5.4:** DPV voltammograms recorded ofmandarine I (a)andmandarine II(b)with added standardsin 0.5M acetate buffer solution (pH 4.8).

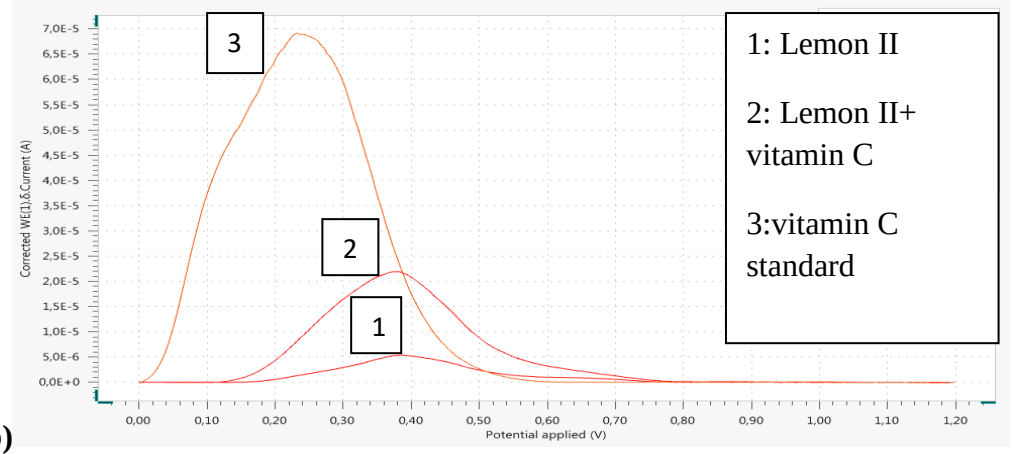
Figure 5.4,(a) shows the DPV voltammograms recorded in mandarine I with added standards: 1) mandarine I; (2) mandarineI + vitamin C (standard) and (3) vitamin C standardwhereas, (b)shows DPV voltammograms recorded for mandarine II, (1).mandarine II;(2).mandarine II + vitamin C (standard)and (3). Vitamin C standard; the potential voltage for the peak height ranged from 0.2 – 0.4 (V). This in interpretation means mandarineI & II juice constitutes vitamin C but in low concentration as compared to the standard.

### 5.2.3DPV voltammograms for Lemon

Figure 5.5 shows the voltammograms of lemon samples examined using DPV method.




**(a)**




**(b)**

**Figure 5.5:**DPV voltammograms recorded for Lemon I(a) and Lemon II (b).with added standards in 0.5M acetate buffer solution (pH 4.8).

Fig 5.5 (a), Illustrates different voltammograms oflemon, standard added lemon and the standard. (1)Lemon I; (2)Lemon I + vitamin C (standard);and (3) vitamin C standard and (b): shows the DPV voltammograms of lemon II with (1)Lemon II;(2) Lemon II + standard; (3) Vitamin C (standard).Figure 5.5 shows the different peak positions of samples examined using DPV method. The potential voltage for the peak height ranged from 0.2 – 0.4 (V).

### 5.3 Vitamin C in various citrus fruits

The determination of ascorbic acid in fruit samples was carried out without pretreatment of the sample by using DPV (Figure 5.6). The results are summarized in Table 5.2.

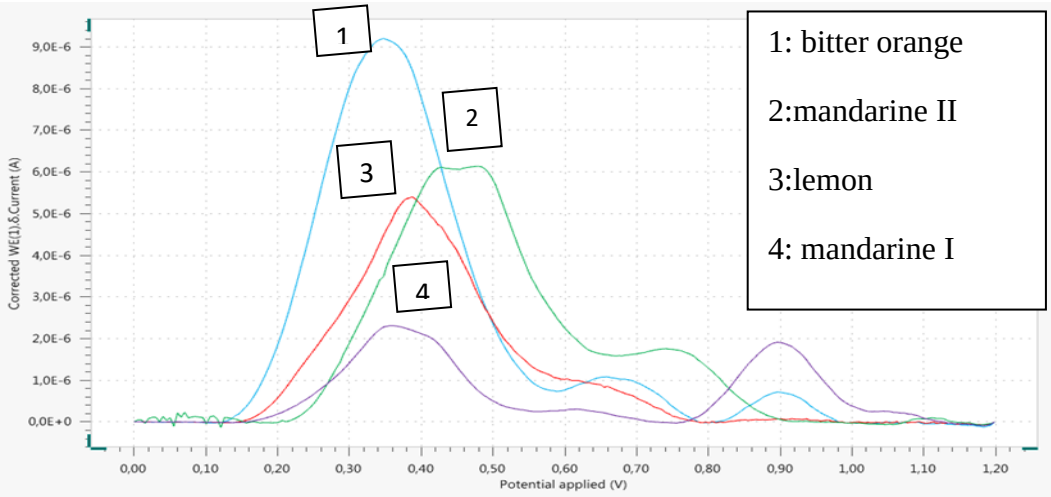



**Figure 5.6:** DPV voltammograms obtained from citrus fruit samples in 0.5M acetate buffer solution (pH 4.8).

**Table 5.2:**Vitamin C concentration of different citrus fruits and their respective standard deviations (n = 3)in 0.5M acetate buffer solution (pH 4.8).

| Sample | Peak position (V) | Vitamin C amount, (mg/kg) | Standard deviation |
|---|---|---|---|
| **Orange** | 0.347 | 214.059 | 16.768 |
| **Bitter orange** | 0.377 | 210.369 | 14.888 |
| **Grapefruit** | 0.428 | 185.279 | 14.710 |
| **Mandarine I** | 0.403 | 46.057 | 5.619 |
| **Mandarine II** | 0.387 | 167.077 | 14.560 |
| **Lemon I** | 0.408 | 204.467 | 16.713 |
| **Lemon II** | 0.383 | 91.809 | 13.83 |

Orange sample was containing 214mg/kg vitamin C, bitter orange constituted 210mg/kg and grapefruit had 185mg/kg. Vitamin C amount in mandarine samples was different depending on the variety.In caseof lemon samples, we found similar results like in mandarine, namely different lemon varieties were containing different amounts of vitamin C. Generallythe results were good in comparison with results obtained using other conventional methods. For example, from our study oranges were found to have a vitamin C content of 214mg/kg, which were comparable to the literature result where vitamin C content for orangeswas found to be 280mg/kg, (Wu et al.,2016).

As a result, the amounts of ascorbic acid in the samples were successfully determined without precipitation, evaporation or extraction steps on PGE electrode by DPV technique.The method used during the study was simple, easy, fast, cost effective, and environmentally friendly since we did not use any organic solvent.Of importance to note, growingseason, storage, and post-harvest handling conditions may have a more important influence on vitamin C content. Warman and Harvard (1997) found that storage influenced the vitamin C content in cabbage and carrots respectively. It can be suggested that other factors such as the environment,growing season and conditions, storage, and varieties may have a more impact on vitamin C content. The levels of vitamin C have also been found to vary in the fruit from a single variety.

# CHAPTER 6

# CONCLUSIONS

Many analytical methods have been reported for the determination of ascorbic acid. These conventional methods which are titrimetric, fluorimetry, spectrometry, chemiluminiscence, enzymatic methods, capillary electrophoresis, electrochemical methods, amperometric methods and HPLC are the most common. Spectrophotometric, enzymatic and chemiluminiscence analysis are time consuming methods due to the analysis duration, and in consequence of ascorbic acid degradation the determined results are then overestimated. For the determination of ascorbic acid (vitamin C) in different matrices, novel electrochemical methods (EM) are lately more widely used as they are simple, sensitive and moderate methods. However, voltammetry has become the popular technique for the ascorbic acid determination. The method is quick, easy, cost effective and also environmentally friendly since we did not use any organic solvent. A linear curve was obtained within a concentration range of 15.69-250mg/kg. Limit of detectionwas calculated from calibration curve as (5.487mg/kg). The results showed that the method might be used to determine vitamin C in food samples. However, additional experiments should be conducted to compare our results with existing conventional methods and proposed method should be validated.

## REFERENCES


Allen, S., Chen, X., Davies, J., Davies, M. C., Dawkes, A. C., Edwards, J. C., ... & Williams,P. M. (1997). Detection of antigen− antibody binding events with the atomic force microscope. *Biochemistry*, *36*(24), 7457-7463.

Alves, N. S., Ribeiro, L. F., Caires, V., Mendes, T. S., &Spínola, R. O. (2014, September). Towards an ontology of terms on technical debt. In *2014 Sixth International Workshop on Managing Technical Debt* (pp. 1-7). IEEE.

Ardjmand, M., & Rad, A. S. (2009). Electrochemically Activation of Pencil Graphite Electrode for Determination of Ascorbic Acid and Uric Acids. *Asian Journal of Chemistry*, *21*(5), 3500-3508.

Ardjmand, M., & Rad, A. S. (2009). Electrochemically Activation of Pencil Graphite Electrode for Determination of Ascorbic Acid and Uric Acids. *Asian Journal of Chemistry*, *21*(5), 3500-3508.

Alkire, R. C., Kolb, D. M., Lipkowski, J., & Ross, P. N. (Eds.). (2009). Chemically modified electrodes (Vol. 22). John Wiley & Sons.

Babaei, A., Aminikhah, M., & Taheri, A. R. (2013). A Multi-Walled Carbon Nano-Tube and Nickel Hydroxide Nano-Particle Composite-Modified Glassy Carbon Electrode as a New Sensor for the Sensitive Simultaneous Determination of Ascorbic Acid, Dopamine and Uric Acid. *Sensor letters*, *11*(2), 413-422.

Baghizadeh, A., Karimi-Maleh, H., Khoshnama, Z., Hassankhani, A., &Abbasghorbani, M. (2015). A voltammetric sensor for simultaneous determination of vitamin C and vitamin B 6 in food samples using ZrO 2 nanoparticle/ionic liquids carbon paste electrode. *Food analytical methods*, *8*(3), 549-557.



Baghizadeh, A., Karimi-Maleh, H., Khoshnama, Z., Hassankhani, A., &Abbasghorbani, M. (2015). A voltammetric sensor for simultaneous determination of vitamin C and vitamin B 6 in food samples using ZrO 2 nanoparticle/ionic liquids carbon paste electrode. *Food analytical methods*, *8*(3), 549-557.



Barcelo-Barrachina, E., Moyano, E., Galceran, M. T., Lliberia, J. L., Bagó, B., & Cortes, M. A. (2006). Ultra-performance liquid chromatography–tandem mass spectrometry for the analysis of heterocyclic amines in food. *Journal of Chromatography A*, *1125*(2), 195-203.Bürzle, M., &Hediger, M. A. (2012).

Functional and physiological role of vitamin C transporters. In *Current topics in membranes* (Vol. 70, pp. 357-375). Academic Press.

Chen, J., Guo, L., Zhang, L., Wu, H., Yang, J., Liu, H., ... & Liu, J. (2013). Vitamin C modulates TET1 function during somatic cell reprogramming. *Nature genetics*, *45*(12), 1504.

David, I. G., Popa, D. E., &Buleandra, M. (2017). Pencil graphite electrodes: a versatile tool in electroanalysis. *Journal of analytical methods in chemistry*, *2017*.

Degefa, T. H., & Kwak, J. (2008). Electrochemical impedance sensing of DNA at PNA self-assembled monolayer. *Journal of Electroanalytical Chemistry*, *612*(1), 37-41.

Derakhshi, M., Jamali, T., Elyasi, M., Bijad, M., Sadeghi, R., Kamali, A., ... & Mokhtari, S. (2013). Synthesis and characterization of NiO nanoparticle as a highly sensitive voltammetric sensor for vitamin C determination in food samples. *Int. J. Electrochem. Sci*, *8*, 8252-8263.

Elgailani, I. E. H., Gad-Elkareem, M. A., Noh, E. A., Adam, O. E., & Alghamdi, A. M. (2017). Comparison of two methods for the determination of vitamin C (ascorbic acid) in some fruits. *American Journal of Chemistry*, *2*(1), 1-7.

Ensafi, A. A., & Karimi-Maleh, H. (2010). Modified multiwall carbon nanotubes paste electrode as a sensor for simultaneous determination of 6-thioguanine and folic acid using ferrocenedicarboxylic acid as a mediator. *Journal of Electroanalytical Chemistry*, *640*(1-2), 75-83.

Esch, J. R., Friend, J. R., & Kariuki, J. K. (2010). Determination of the vitamin C content of conventionally and organically grown fruits by cyclic voltammetry. *International Journal of Electrochemical Science*, *5*(10), 1464-1474.

Esteve, M. J., Farré, R., Frigola, A., López, J. C., Romera, J. M., Ramirez, M., & Gil, A. (1995). Comparison of voltammetric and high-performance liquid chromatographic methods for ascorbic acid determination in infant formulas. *Food Chemistry*, *52*(1), 99-102.

Gheibi, S., Karimi-Maleh, H., Khalilzadeh, M. A., & Bagheri, H. (2015). A new voltammetric sensor for electrocatalytic determination of vitamin C in fruit juices and fresh vegetable juice using modified multi-wall carbon nanotubes paste electrode. Journal of Food Science and Technology, 52(1), 276-284.

Gerard, M., Chaubey, A., & Malhotra, B. D. (2002). Application of conducting polymers to biosensors. Biosensors and bioelectronics, 17(5), 345-359.

Gazdik, Z., Zitka, O., Petrlova, J., Adam, V., Zehnalek, J., Horna, A., ... &Kizek, R. (2008). Determination of vitamin C (ascorbic acid) using high performance liquid chromatography coupled with electrochemical detection. Sensors, 8(11), 7097-7112.

Hu, M., Yao, Z., & Wang, X. (2017). Graphene-based nanomaterials for catalysis. *Industrial & Engineering Chemistry Research*, *56*(13), 3477-3502.

Kissinger, P. T., & Heineman, W. R. (1983). *Cyclic voltammetry*. *Journal of Chemical Education*, 60(9), 702.

Jamalan, M., Rezazadeh, M., Zeinali, M., &Ghaffari, M. A. (2015). Effect of ascorbic acid and alpha-tocopherol supplementations on serum leptin, tumor necrosis factor alpha, and serum amyloid A levels in individuals with type 2 diabetes mellitus. *Avicenna journal of phytomedicine*, *5*(6), 531.

Jampasa, S., Wonsawat, W., Rodthongkum, N., Siangproh, W., Yanatatsaneejit, P., Vilaivan, T., &Chailapakul, O. (2014). Electrochemical detection of human papillomavirus DNA type 16 using a pyrrolidinyl peptide nucleic acid probe immobilized on screen-printed carbon electrodes. *Biosensors and Bioelectronics*, *54*, 428-434.

Khalilzadeh, M. A., &Borzoo, M. (2016). Green synthesis of silver nanoparticles using onion extract and their application for the preparation of a modified electrode for determination of ascorbic acid. *journal of food and drug analysis*, *24*(4), 796-803.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest biology and technology*, *20*(3), 207-220.

Lim, S. A., & Ahmed, M. U. (2016). Electrochemical immunosensors and their recent nanomaterial-based signal amplification strategies: a review. *RSC Advances*, *6*(30), 24995-25014.

Luo, X., Zhang, W., Han, Y., Chen, X., Zhu, L., Tang, W., ... & Li, Z. (2018). N, S co-doped carbon dots-based fluorescent “on-off-on” sensor for determination of ascorbic acid in common fruits. *Food chemistry*, *258*, 214-221.

Malhotra, B. D., &Chaubey, A. (2003). Biosensors for clinical diagnostics industry. *Sensorsand Actuators B: Chemical*, *91*(1-3), 117-127.

Masamba, K. G., & Nguyen, M. (2008). Determination and comparison of vitamin C, calcium and potassium in four selected conventionally and organically grown fruits and vegetables. African Journal of Biotechnology, 7(16).

Mello, L. D., & Kubota, L. T. (2002). Review of the use of biosensors as analytical tools in the food and drink industries. *Food chemistry*, *77*(2), 237-256.

Muguruma, H., &Karube, I. (1999). Plasma-polymerized films for biosensors. *TrAC Trends in Analytical Chemistry*, *18*(1), 62-68.

Moyo, P., Mugadza, T., Mehlana, G., &Guyo, U. (2016). Synthesis and characterization of activated carbon–ethylenediamine–cobalt (II) tetracarboxyphthalocyanine conjugate for catalytic oxidation of ascorbic acid. Research on Chemical Intermediates, 42(8), 6511-6529.

Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *Rsc Advances*, *5*(35), 27986-28006.

Nolan, M. A., Tan, S. H., &Kounaves, S. P. (1997). Fabrication and characterization of a solid state reference electrode for electroanalysis of natural waters with ultramicroelectrodes. *Analytical Chemistry*, *69*(6), 1244-1247.

Norfun, P., Arqueropanyo, O., Liawruangrath, S., &Ounnunkad, K. (2016). Electrochemicalflow injection determination of ascorbic acid in fruit samples employing a graphene-polyaniline electrode. *International Journal of Chemical Engineering and Applications*, *7*(2), 142.

Nováková, L., Solich, P., &Solichová, D. (2008). HPLC methods for simultaneous determination of ascorbic and dehydroascorbic acids. *TrAC Trends in Analytical Chemistry*, *27*(10), 942-958.

Okiei, W., Ogunlesi, M., Azeez, L., Obakachi, V., Osunsanmi, M., &Nkenchor, G. (2009). The voltammetric and titrimetric determination of ascorbic acid levels in tropical fruit samples. *Int. J. Electrochem. Sci*, *4*(9), 276-287.

Olana, B. N., Kitte, S. A., &Soreta, T. R. (2015). Electrochemical determination of Ascorbicacid at p-phenylenediamine film–holes modified glassy carbon electrodes. *J. Serb. Chem. Soc.*, *80*(9), 1161-1175.

Pardakhty, A., Ahmadzadeh, S., Avazpour, S., & Gupta, V. K. (2016). Highly sensitive and efficient voltammetric determination of ascorbic acid in food and pharmaceutical samples from aqueous solutions based on nanostructure carbon paste electrode as a sensor. *Journal of Molecular Liquids*, *216*, 387-391.

Peterson, J. I., &Vurek, G. G. (1984). Fiber-optic sensors for biomedical applications. *Science*, *224*(4645), 123-127.

Pisoschi, A. M., Pop, A., Serban, A. I., &Fafaneata, C. (2014). Electrochemical methods for ascorbic acid determination. *Electrochimica Acta*, *121*, 443-460.

Pournaghi-Azar, M. H., &Ojani, R. (1997). A selective catalytic voltammetric determination of vitamin C in pharmaceutical preparations and complex matrices of fresh fruit juices. *Talanta*, *44*(2), 297-303.

Purushothama, H. T., & Nayaka, Y. A. (2017). Electrochemical study of Hydrochlorothiazide on electrochemically pre-treated pencil graphite electrode as a sensor. *Sensing and bio-sensing research*, *16*, 12-18.

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). *Principles of instrumental analysis*. Cengage learning.

Sochor, J., Dobes, J., Krystofova, O., Ruttkay-Nedecky, B., Babula, P., Pohanka, M., ... & Kizek, R. (2013). Electrochemistry as a tool for studying antioxidant properties. *Int. J. Electrochem. Sci*, *8*(6), 8464-8489.

Tarrago-Trani, M. T., Phillips, K. M., &Cotty, M. (2012). Matrix-specific method validation for quantitative analysis of vitamin C in diverse foods. *Journal of Food Composition and Analysis*, *26*(1-2), 12-25.

Wang, J., Liu, J., Chen, L., & Lu, F. (1994). Highly selective membrane-free, mediator-free glucose biosensor. *Analytical Chemistry*, *66*(21), 3600-3603.

Wang, L., Gong, C., Shen, Y., Ye, W., Xu, M., & Song, Y. (2017). A novel ratiometric electrochemical biosensor for sensitive detection of ascorbic acid. Sensors and Actuators B: Chemical, 242, 625-631.

Warman, P. R., &Havard, K. A. (1997). Yield, vitamin and mineral contents of organically and conventionally grown carrots and cabbage. Agriculture, ecosystems & environment, 61(2-3), 155-162.

Wring, S. A., & Hart, J. P. (1992). Chemically modified, carbon-based electrodes and their application as electrochemical sensors for the analysis of biologically important compounds. A review. Analyst, 117(8), 1215-1229.

Wu, W., Sun, Z., & Zhang, W. (2016). Simple and rapid determination of Vitamin C in vegetables and fruits by a commercial electrochemical reader. Food analytical methods, 9(11), 3187-3192.