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# VOLTAMMETRIC DETERMINATION OF ASCORBIC ACID CONTENT IN GARLIC(ALLIUM SATIVUM ) USING CARBON PASTE ELECTRODE IN SOME SELECTED AREA IN NORTH GONDAR

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**Bahir Dar, Ethiopia**

***August 2017***

# VOLTAMMETRIC DETERMINATION OF ASCORBIC ACID CONTENT IN GARLIC(ALLIUM SATIVUM ) USING CARBON PASTE ELECTRODE IN SOME SELECTED AREA IN NORTH GONDAR

A Thesis Submitted to the department of Chemistry

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry **(Physical Chemistry)**

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**BAHIR DAR UNIVERSITY**

**COLLEGE OF SCIENCE**

**CHEMISTRY DEPARTMENT**

**Bahir Dar, Ethiopia**

***August 2017***


# Thesis approval sheet

The Thesis entitled "**Voltammetric Determination of Ascorbic Acid Content In Garlic (Allium Sativum) Using Carbon Paste Electrode In Some Selected Areas in North Gondar**" by **Yalew chekol** is approved for the Degree of Master of Science in Chemistry**.**

## Board of Examiners

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# Declaration

I hereby declare that the thesis entitled **"**Voltammetric determination of ascorbic Acid Content In Garlic (Allium Sativum) using carbon paste electrode in Some Selected Area in North Gondar", that I submitted to the department of Chemistry, Bahir Dar University in partial fulfillment of the Master Degree in chemistry is a record of bona fide and original research work carried out by me under the guidance and supervision of Dr. Alemu Tesfaye, Assistant Professor, Department of chemistry, Bahir Dar University .To the best of my knowledge no part of this thesis has been submitted to any other university or institution for the award of any degree or diploma.

Name: Yalew chekol

Signature:_____

Place: Science Collage

Bahir Dar University

Date of Submission: August 2017

## Acknowledgement

Research is expected to be full of intense works which needs strong collaboration between people. That is exactly what I have experienced during whole of my work. That is why I am here to give credit to whom it deserve.

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## Abstract

*Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used for characterizing and determining Vitamin C concentration in fresh Garlic collected in some selected kebeles of North Gondar administrative zone, Ethiopia. In this study, Ascorbic acid (AA) content in Garlic was determined by SWV technique. The effect of Ascorbic acid concentration on the oxidation response of CPE, pH of phosphate buffer solution and scan rate on the response characteristics of the electrode were investigated by CV. It was found that the anodic peak current of AA is directly related to the concentration and scan rate, whereas during pH optimization, the anodic peak current was increased with the pH value ranges 1 – 3. However, the peak current was found to decrease after pH 3. Hence, pH 3 is chosen as the optimum value for this work. On the other hand, the anodic peak potential is shifted to less positive as a function of pH values used. A calibration curve was obtained by the square wave voltammetry at CPE after square wave frequency, step potential, and square wave amplitude were optimized. The optimized parameters chosen for the whole work were a wave frequency of 25 HZ, a step potential of 8 mV, and wave amplitude of 35 mV, respectively. The Vitamin C content determined in this work using SWV was 22.8 mg/100 g and 19.45 mg/100 g of Debark and Gondar Garlic respectively. From the results obtained, the ascorbic acid content of Debark Garlic was higher than Gondar Garlic. The recoveries ranging between 99.99-99.98% were achieved indicating the method for the determination of vitamin C in real samples was more valid.*



***Key words:*** *Ascorbic acid, cyclic voltammetry, square wave voltammetry and Garlic.*

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# List of abbreviation/acronyms

**SHE** Standard hydrogen electrode

**AA** Ascorbic acid

**HPLC** High performance liquid chromatography

**CV** Cyclic Voltammetry

**SCE** Saturated Calomel Electrode

**SWV** Square Wave Voltammetry

**HMDE** Hanging mercury drop electrode

**AE** Auxiliary electrode

**UV-VIS** Ultra violet visible

**WE** Working electrode

**LOD** Limit of detection

**LOQ** Limit of quantification

**RSD** Relative standard deviation

**CPE** Carbon Paste Electrod

# 1. INTRODUCTION

## 1.1. GARLIC (ALLIUM SATIVUM)

Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure forms or crude extracts to treat many diseases[1]. Garlic (Allium sativum L.) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases [2]. The taxonomic position of garlic and related genera had been a matter of controversy for long period of time. The most recent classification scheme of garlic was class Liliopsida, subclass Liliidae, superorder Liliianae, order Amaryllidales, family Alliaceae, subfamily Allioideae, tribeAllieae and genus Allium which is mainly based on these quences of nuclear ribosomal DNA [3].

The early Egyptians used garlic to treat diarrhea and its medical power was described on the walls of ancient temples and on papyrus dating to 1500 BC .[4]It was used by Greek physicians Hippocrates and Galen to treat intestinal and extra-intestinal diseases; ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections [5]. In Europe and India, it was used to treat common colds, high fever and asthma [6]. Garlic is nicknamed as Russian penicillin for its widespread use as a topical and systemic antimicrobial agent; it is commonly used in many cultures as an excitement and reputation of healing power [6].

## 1.2. CHEMISTRY OF GARLIC

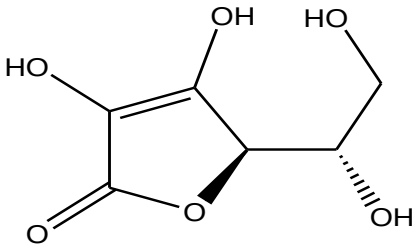
Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids to be found in garlic: lysine, It has a higher concentration of sulfur compounds than any other Allium species which are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfinate or diallyldisulfide). The most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 and 30 mg/g in

fresh and dry garlic, respectively [7].Typical garlic food preparation such as chopping, mincing and crushing disturbs S-allyl cysteine sulfoxide and exposed it to the allinase enzymes, then quickly converted it to diallyl thiosulfinate, which give off garlic's characteristic aroma.

### 1.3. Vitamin C (Ascorbic Acid)

Vitamin C is white crystalline powder with a molecular formula of $C_6H_8O_6$, melting point of 192-193 $^0$C, PH=3 (5% solution) and a formula weight of 176.12 g/mol. It is water soluble, antioxidant vitamin, important in forming collagen, a protein that gives structure to bones, cartilages, muscles, and blood vessels[8]. Much of the current in vitamin C is focused on its ability, as reducing agent, to quench free radicals. All of these effects may promote optimal health through support for the immune system and defense of tissue against the oxidative challenges associated with chronic disease. It prevent tissue damage and used in treatment of certain diseases such as scurvy, anemia , common cold ,diabetes, hemorrhagic disorder, wound healing , cough, influenza, sores, gingivitis, skin disease, diarrhea, malaria, bacterial infections, plug poisoning, liver disease, allergic reactions, arteriosclerosis as well as infertility in males[9]. Vitamin C also aids in the absorption of iron, immune response activation and helps maintain capillaries, bones, and teeth. It is the most common electro active biological compound and one of the most ubiquitous vitamins ever discovered. Ascorbic acid is known for its reductive properties. Hence, it is used on large scale as antioxidant in the pharmaceutical, chemical, cosmetics and food industry. It is also important for theraptic purposes and biological metabolism [10].

Ascorbic acid (AA) is a labile substance, as it is easily degraded by enzymes and atmospheric oxygen .Its oxidation can be accelerated by excessive heat, light, and heavy metal cations. That is why AA content of foodstuffs and beverages represents relevant indicator of quality which has to be carefully monitored, regarding its variation during manufacturing and storage [11, 12].




ascorbic acid

Figure1.1 .The chemical structure of ascorbic acid

There is need to find an accurate, reliable, rapid and easy method to implement for measuring the amount of ascorbic acid in a sample. However there have been difficulties in quantifying ascorbic acid due to its instabilities in aqueous solution. The instability of ascorbic acid due to its oxidation to dehydroascorbic acid, which is irreversible reaction and subsequently to 2,3-diketo-L-gulonic acid[13].

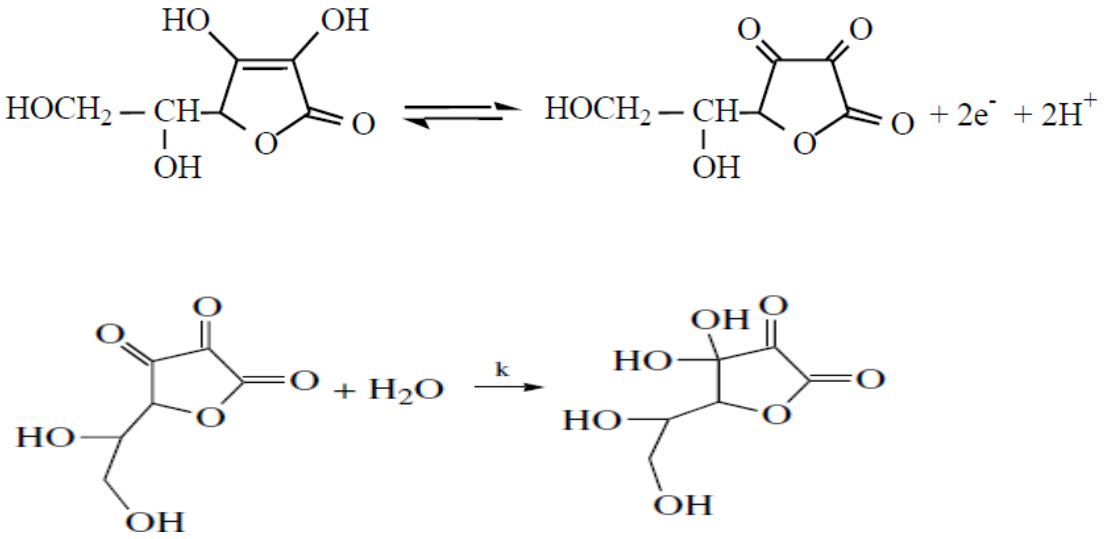



Figure1.2.The instable ascorbic acid oxidation to dehydroascorbic acid and subsequently to 2,3diketoL-gulonic acid [13].

### 1.3.1. Deficiency of vitamin C

A severe form of vitamin C deficiency, known as scurvy, is characterized by general weakness, anemia, gum disease (gingivitis), and skin hemorrhage. Smokers have higher vitamin C

requirements because of smoking's ability to increase oxidative stress, and. deficiencies are more common in smokers than in nonsmokers. Vitamin C deficiency was also directly related to low fruit and vegetable intakes. Symptoms of vitamin C deficiency include dry and splitting hair, gingivitis, rough, dry skin, decreased wound healing rate, weakened tooth enamel, anemia, and a decreased ability to fight infections [14, 15, 16].

### 1.3.2. Excess of vitamin C

AA excess can lead to gastric irritation, conditioned scurvy, oxalate kidney stones, increased uric acid excretion , excessive iron absorption or release, vitamin B12 destruction , erosion of enamel and gastro intestinal distress .In some cases, excessive quantity of AA may result in the inhibition of natural processes occurring in food and can contribute to taste deterioration[17].

## 1.4. Statement of the problem

Ascorbic acid more commonly known as vitamin C is an essential nutrient. The amount of vitamin C required in a healthy diet varies with age and gender. According to Natural Health product Monograph, children 1-3 age requires 15 mg/day, adult females 75 mg/day and adult males 90 mg/day [18]. At intake of vitamin C about 60 mg/d in both genders, ascorbate begins to appear in the urine [19]. However, intakes of 250 mg/d and higher are required to substrate ascorbate concentrations in plasma and contents of white blood cell [17].

As it is mentioned under 1.31 and 1.3.2, the excess and deficiency of ascorbic acid has a series health problems and even the amount of AA that intake by human is different based on age and gender. Knowing all this is very crucial and must be studied. However, with the best of my knowledge, the amount of AA in Garlic that cultivated in different localities of North Gondar administrative zone, Ethiopia is not studied yet. Hence, this work is aimed to determine the content of ascorbic acid in garlic cultivating in different places in North Gondar (debark and Gondar) urban areas.

## 1.5. Significance of the study

Human body requires AA for normal physiological functions. Infections deplete the body stores of AA, which in turn making the body immune system weak. AA plays an important role in the treatment and preventation of infections and infectious diseases. The purpose of the present study is to assess the level of ascorbic acid from some fresh edible locally grown garlic. Therefore, this study can help people to know the content of ascorbic acid in garlic, to show which garlic is good source of ascorbic acid for consumers and to inform how much garlic can intake per a day.

## 1.6. Objective of the study

### 1.6.1. General objective

The general objective of this study is:-

- To make quantitative analysis of ascorbic acid in Garlic sample using electroanalaytical techniques at carbon paste electrode (CPE).

### 1.6.2 .Specific objectives

The specific objectives are to:

- Determine the amount of ascorbic acid in garlic sample using cyclic voltammetry and square wave voltammetric method at carbon paste electrode.
- Compare the amount of AA in garlic cultivated in different place and also compare with another findings.
- Study the influences of some factors such as pH of buffer solution, concentration of ascorbic acid, and the common instrumental parameters (SW frequency, amplitude, and step potential) on the oxidation response of AA using CPE.

# 2. LITERATURE REVIEW

## 2.1. Methods of Determination of Vitamin C

Numerous analytical techniques are available for the determination of vitamin C in different matrices. Some of the techniques include: direct titration [23, 24], Fluorimetric methods [25], chromatographic methods [26-28], Electrochemical [29]. However, some of these methods are time-consuming, some are costly, some need special training operators, or they suffer from the insufficient sensitivity or selectivity [20]. Due to its selectivity and sensitivity, an electrochemical method to determine of ascorbic acid has been a subject of considerable interest [22, 24]. Because of low costs of their quire equipment as well as the simplicity of the employed procedures, voltammetry appears to offer an attractive alternative method to determine ascorbic acid, particularly during the routine quality control of some food products [25]. Cyclic voltammetry (CV) is a versatile tool that allows the electrochemical characterization of a wide variety of materials. It offers a rapid location of redox potentials of the electroactive species [30]. Carbon paste electrodes have been used as the working electrode in cyclic voltammetry experiments aimed to the identification, characterization and quantification of antioxidants, including ascorbic acid, phenolic and polyphenolic compounds, glutathione and synthetic antioxidants [33,34]. The use of carbon-paste matrix, besides renewability by a simple polishing, offers several other advantages including easy preparation, uniform distribution of the catalyst into the paste, better reproducibility and stability, adequate robustness in aqueous solutions, low background current, wide potential window and versatility [21, 22]. Recent advances in the food and pharmaceutical industries and a need for nutritional assessment have necessitated the development of a selective, simple and accurate method to determine ascorbic acid [23].

Square-wave voltammetry was used to determine ascorbic acid, based on its oxidation at a zeolite modified carbon paste electrode [28]. Cyclic and differential pulse voltammetry were used for electrocatalytical ascorbic acid determination, at a carbon paste electrode, modified with 2,7-bis fluoren-9-one [29]. Cyclic voltammetry at a bare Pt electrode was applied to ascorbic acid content estimation in citrus juices and soft drinks [30].The electrochemical oxidation and selective determination of ascorbic acid in pharmaceutical dosage forms and in some *Rosa*

species was investigated by cyclic, differential pulse and square-wavevoltammetry [31]. The linear response was obtained in the range 3.52-176.1 μg $mL^{-1}$, with a detection limit of 0.88 μg $mL^{-1}$ for DPV and 0.52 μg $mL^{-1}$ for SWV.

Cyclic voltammetry studies performed on Pt electrodes proved that the growth of Pt surface oxides and the anodic response of a variety of interferrents (glucose, cystine, oxalate) was greatly surprised by the use of fluorosurfactant-modified Pt electrodes [34]. Ascorbic acid was determined in the presence of $SO_2$ and acetaldehyde by pulsed voltammetry at interdigitated Pt microelectrodes [35].Ascorbic acid, uric acid and dopamine were simultaneously determined by differential pulse voltammetry, performed on a glassy carbon electrode modified with a film of poly (3-(5-chloro-2-hydroxyphenylazo)-4,5-dihydroxynaphtalene-2,7-disulphonic acid [36]. Differential pulse voltammetry was used for the assessment of ascorbic acid, at poly (3,4-ethylendioxythiophene)-modified electrodes [37].Simultaneous determination of vitamins C, B6 and PP in pharmaceuticals formulations was performed using differential pulse voltammetry at a glassy carbon electrode [38]. Differential pulse voltammetry at a glassy carbon electrode was also employed for the analytical characterization and measuring of predominant flavonoids and `phenolic acids [39].Dopamine was determined in the presence of ascorbic and uric acids by differential pulse voltammetry at a bare glassy carbon electrode [40]. Simultaneous determination of vitamin C and uric acid was also possible with a ferrocenium-thioglycollate modified electrode. Under the optimal conditions and within the linear range of $1 \times 10^{-6}$ M to $5 \times 10^{-4}$ M, the achieved detection limits for ascorbic acid and uric acid were $2 \times 10^{-7}$ M and $1 \times 10^{-7}$ M, respectively [41].A differential pulse voltammetric method at a glassy carbon working electrode was also developed for the determination of Silymarin/vitamin E acetate mixture in pharmaceuticals [42]. The linear analytical response was obtained in the range 0.1-4.0 mg $L^{-1}$, with a detection limit of 0.03 mg $L^{-1}$ for Silymarin, and 0.05-4.0 mg $L^{-1}$ with a detection limit of 0.01 mg $L^{-1}$ for vitamin C acetate [42]. Amulti-walled carbon nanotubes tetradecyltrimethylammonium bromide film coated graphite electrode was used to study the electro oxidation of ascorbic acid in differential pulse, cyclic and square-wavevoltammetry [43]. A linear voltammetric response for vitamin C was obtained for the concentration range $5 \times 10^{-7}$-$1.7 \times 10^{-4}$ M, with a detection limit for ascorbic acid of $1.1 \times 10^{-7}$ M, using DPV [43].The electro catalytic oxidation of ascorbic acid (cyclic and differential pulse voltammetry) was investigated

with a carbon nanotubes paste electrode modified with 2,2'-[1,2-ethanediylbis(nitriloethylidine)]-bis-hydroquinone [45]. Using DPV, the calibration curves for ascorbic acid and uric acid were obtained over the ranges 0.1-800 μM and 20-700 μM, respectively [44]. Differential pulse voltammetry with a poly(sulfonazo III) modified glassy carbon electrode enables the highly selective determination of ascorbic acid, dopamine and uric acid [45].

Cyclic voltammetry and differential pulse voltammetry at a binuclear copper complex modified glassy carbon electrode were also applied to determine ascorbic acid and dopamine [46]. Linear analytical curves were obtained in the ranges 2.0-120.0 μM for dopamine and 5.0-160.0 μM for ascorbic acid, using DPV. The detection limits were $1.4 \times 10^{-6}$ M for dopamine and $2.8 \times 10^{-6}$ M for ascorbic acid [46]. The modified electrode was used for ascorbic acid and dopamine determination in medicine and foodstuffs [46].Ascorbic acid and dopamine were determined simultaneously by differential pulse voltammetry performed at a boron-dropped diamond film electrode or on the surface of electrodes modified with self-assembled gold nano particles film [47,48]. The linear analytical curves were obtained in the ranges 0.3-1.4 mM for ascorbic acid and 0.2-1.2 mM for dopamine. The detection limit ($3\sigma$) was $9.0 \times 10^{-5}$ M for both dopamine and ascorbic acid [48].

The simultaneous assessment of ascorbic acid and acetaminophen was investigated by differential pulse voltammetry and cyclic voltammetry, performed on a boron-doped diamond electrode, using sodium sulphate as supporting electrolyte [49]. Fouling of the electrode was not reported. Relative standard deviations of 2-3%, high sensitivity values and low detection limits ($10^{-6}$ M order of magnitude) were obtained [49].

## 2.2. Recommendations of ascorbic acid

The best way to get the daily requirement of essential vitamin C, is to eat a balanced diet that contains a variety of foods from the food guide pyramid. Vitamin C should be consumed every day because it is not fat-soluble and, therefore, cannot be stored for later use. WHO,

recommends the following amounts of vitamin C. Women who are pregnant or breastfeeding and those who smoke need higher amounts [32].

Table-2.1.**WHO**, recommended intake of ascorbic acid, by group [32].

| **Life stage** | **Age** | **RNIs(mg/day)** | **RNIs for only Females(mg/day)** |
|---|---|---|---|
| Infants | 0-6(months) | 25 | |
| Infants | 7-12(months) | 30 | |
| Children | 1-3yeares | 30 | |
| Children | 4-6yeares | 30 | |
| Children | 7-9yeares | 35 | |
| Adolescent | 10-18yeares | 40 | |
| Adults | 19-65yeares<br><br>>65 | 45<br><br>45 | |
| Pregnancy | - | - | 55 |
| Lactation | - | - | 70 |

## 2.3. Voltammetric techniques

Voltammetry belongs to a class of electrochemical methods which can be used to study solution composition through current-potential relationships at the electrode surface. The potential is varied in some systematic manner to cause electroactive chemical species to be reduced or oxidized at the electrode. This produces a current (I) that is proportional to the concentration of chemical species. This resulting current potential plot is known as a voltammogram [50-51].

The common characteristic of all voltammetric techniques is that they involve the application of potential (E) to an electrode and the monitoring of the resulting current (i) flowing through the electrochemical cell. In many cases the applied potential is varied or the current is monitored over a period of time (t). Thus, all voltammetric techniques can be described as some function of

E, i, and t. They are considered active techniques (as opposed to passive techniques such as potentiometry) because the applied potential forces a change in the concentration of an electroactive species at the electrode surface by electrochemically reducing or oxidizing it [52].The analytical advantages of the various voltammetric techniques include excellent sensitivity and selectivity with a very large useful linear concentration range for both inorganic and organic species ($10^{-12}$ to $10^{-1}$ M), fast response, as well as simplicity of the analytical procedure, low cost, a large number of useful solvents and electrolytes, and simultaneous determination of several analytes. That is why; they were applied to the qualitative and quantitative assessment of food additives, ingredients and contaminants.[53,54].

### 2.3.1. Instrumentation

Voltammetry techniques makes use of three electrode namely, a working electrode (WE), a reference electrode (RE) and auxiliary electrode (AE). The whole system consists of a voltammetric cell with a various volume capacity, magnetic stirrer and gas line for purging and blanketing the electrolyte solution [55].

The arrangement of the electrodes within the cell is important. The RE is placed close to the WE and the WE is located between the RE and the AE. Using the three electrode cell concept, a potentiostats monitors the voltage over the WE and AE which is automatically adjust to give the correct applied potential. This is obtained by continuously measuring the potential between the WE and the RE by comparing it to the set voltage and by adjusting the applied voltage accordingly if necessary.

The WE is the electrode where the redox reaction of electro active species takes place it is made of several different materials including mercury, platinum, gold, siliver, carbon, chemically modified and screen printed electrodes. The ideal characteristics of a working electrode are wide potential range, low resistance, reproducible surface and be able to provide a high signal to noise response. The WE must be made a material that will not react with the solvent or any component of the solution over a wide potential range. The potential window of such electrode depends on the electrode material and the composition of the electrolyte. The majority of electrochemical methods use hanging mercury drop electrode(HMDE) and mercury film electrode(MFE) for use

in the cathodic potential area, where as solid electrode such as gold, platinum ,glassy carbon ,carbon paste , are used for examining anodic processes.

The limited anodic potential range of mercury electrodes and its toxicity has precluded their utility for monitoring oxidizable compounds [56].As an inert electrode material, carbon is useful for both oxidation and reduction in both aqueous and non-aqueous solutions. Several different form of carbon have been used to make satisfactory electrodes including Spectroscopy-grade graphite, carbon paste, graphite dispersed in epoxy or silicon rubber and Vitreous or glassy carbon [57].

In voltammetric studies the current flows between the working electrode and auxiliary electrode. If the electrode system consisting of only reference and working electrodes is used, then current flow through the reference electrode will cause a change in its potential. So a three electrode system, incorporating a third electrode called the auxiliary electrode is used. The main condition for an electrode to act as auxiliary electrode is that it should not dissolve in the medium of the electrochemical cell and that the reaction product at the auxiliary electrode should not react at the working electrode. It does not need special care, such as polishing. The electrode area of the auxiliary electrode must also be larger than that of working electrode to ensure that the area of electrode dose not controls the limit current. Platinum electrodes in the form coils or thin foil are the most widely used auxiliary electrodes in aqueous, non-aqueous and molten salt media [58].

The third electrode used in voltammetric technique is the reference electrode (RE).A RE provides a stable potential so that any change in cell potential is attributed to the working electrode. The major requirement for the RE is that the potential does not change during the recording voltammetric curve at different applied voltage. The common REs are standard hydrogen electrode (SHE), calomel electrode (SCE) and silver/silver chloride electrode (Ag/AgCL).The SHE is used to establish standard-state potential for other half reaction. It consists of platinum electrode immersed in a solution in which the hydrogen ion activity is 1.00 and in which hydrogen gas is bubbled at a pressure of 1atm. The standard state potential for the reaction;

$$2H^{+}(aq) + 2e^{-} \longrightarrow H_2(g) \qquad (1)$$

Is 0.00V for all temperatures. It is rarely used because it is difficult to prepare and inconvenient to use.

The second reference electrode is the Standard calomel electrode (SCE) which is based on the redox couple between mercury chloride ($Hg_2Cl_2$) and Hg as shown below;

$$Hg_2Cl_2 + 2e^- \longrightarrow 2Hg(l) + 2\,Cl^-(aq) \quad (2)$$

The potential of this electrode is determined by the concentration of chloride ion. It is constructed using an aqueous solution saturated with KCl which has a potential of +0.2444 V at 25$^o$C. It consists of an inner tube packed with a paste of Hg, $Hg_2Cl_2$ and saturated KCl. A small hole connects the two tubes; an asbestos fiber serves as a salt bridge to the solution in which the other types of reference electrode is the Ag/AgCl electrode which is the most common RE since it can be used at higher temperature. This electrode is based on the redox couple between AgCl and Ag AS illustrated below;

$$AgCL(s) + 2e^- \longrightarrow Ag(s) + Cl^- \quad (3)$$

The potential of this electrode is determined by the concentration of $Cl^-$. For saturated KCl the potential is +0.197V whereas for 3.5M KCl the potential electrode is +0.205V at 25$^o$C [58].

### 2.3.2. Solvent and Supporting electrolytes

Electrochemical measurements are commonly carried in a medium which consists of solvent containing supporting electrolyte .The choice of the solvent is primarily by:-

i. the solubility of the analyte,

ii. Its redox activity and

iii. Also by solvent properties such as electrical conductivity, electrochemical activity and chemical reactivity.

The solvent should not react with the analyte and should not undergo electrochemical reaction over a wide potential range. In aqueous solution the cathodic potential is limited by the reduction of hydrogen ions resulting in hydrogen evolution current:

$$2H^{+}(aq) + 2e^{-} \longrightarrow H_2(g) \quad (4)$$

The more acidic the solution the more positive is the potential of this current due to the reaction expressed by ;

$$E = E^{o}_{H2/H+} - 0.059PH \quad (5)$$

The composition of the electrolyte may affect the selectivity of voltammetric measurements. The ideal electrolyte should give well separated and well shaped peaks for all the analytes sought, so that they can be determined simultaneously [58].

Because of the sensitivity of the voltammetric method, certain impurities in supporting electrolytes can affect the accuracy of the procedures. It is thus necessary to prepare the supporting electrolyte from highly purified reagents and should not easily be oxidized nor reduced. To obtain an acceptable ionic strength of supporting electrolyte, certain concentrations should be prepared which usually about 0.1M. This level is a compromise between high conductivity and minimum contamination.

### 2.3.3. Current in Voltammetry

When an analyte is oxidized at the working electrode, current passes through the external circuit to the auxiliary electrode, where reduction of a solvent or other components of the solution matrix occurs. Reducing an analyte at the working electrode requires a source of electrons, generating a current that flows from the auxiliary electrode to the WE. In either case, a current resulting from redox reaction at the working electrode and auxiliary electrode is called a faradic current. Current due to the analyte's reduction is called a cathodic current. Anodic currents are due to oxidation reaction. The magnitude of the faradic current is determined by the rate of the oxidation or reduction at the electrode surface. Two factor contribute to the rate the

electrochemical reactions which are the rate at which the reactants and the products are transported to and from the electrode, and the rate at which electrons passes between the electrode and the reactants and the reactants and products in a solution [59].

There are three modes mass transport that influence the rate at which reactant and product are transported to and from the electrode surface which are diffusion, migration and convection. Diffusion from a region of high concentration to a region of low concentration occurs whenever the concentration of an ion or molecule at the surface the electrode is different from that in bulk solution. When the potential applied to the WE is sufficient to reduce or oxidized the analyte at the electrode surface, a concentration gradient is established. The contribution of diffusion to the rate of mass transport is time-dependent.

Convection occurs when a mechanical means is used to carry reactants toward the electrode and to remove products from the electrode. The most common means convection is to stirr the solution using a stir bar. Other method includes rotating the electrode and incorporating the electrode in to a flow cell.

Migration occurs when charged particle in a solution is attacked or repelled from an electrode that has a positive or negative surface charge. Unlike diffusion and convection, migration only affects the mass transport of charged particles. Migration is eliminated by adding a high concentration of an inert supporting electrolyte to the analytical solution. The large excess of inert ions ensures that few reactant and product ions will moves as a result of migration.

The rate of mass transport is one factor that influences the current. When electron transfer kinetics is fast, the redox reaction is in equilibrium, and the concentration of the reactants and products at the electrode are those specified by the Nernst equation. Such systems are considered electrochemically reversible. In other system, when electron transfer kinetics is sufficiently low, the concentration of reactants and products at the electrode surface, and thus the current, differ from that predicted by the Nernst Equation. In this case the system is electrochemically irreversible**.**

Other current that may exist in electrochemical cell are those unrelated to any redox reaction. They are nonfaradic and residual currents. The nonfaradic current must be accounted for if the faradic component of the measured current is to be determined. This current occurs whenever the electrode's potential is changed. Another type of nonfaradic current is charging current which occurs in electrochemical cell due to the electrical double layer's formation. Residual current is a small current that inevitably flows through an electrochemical cell even in the absence of analyte.

### 2.3.4. Quantitative and Qualitative aspects of Voltammetry

Quantitative information is obtained by relating current to the concentration of analyte in the bulk solution and qualitative information is obtained from the voltammogram by extracting the standard-state potential for redox reaction. The concentration of the electro active species can be quantitatively determined by measurement of limiting current which is linear function of the concentration of electro active species in bulk solution [58].

Half potential serves as characteristics of a particular species which undergoes reduction or oxidation process at the electrode surface in a given supporting electrolyte, and it is independent of the concentration of that species.

### 2.3.5. Cyclic voltammetry

Cyclic voltammetry (CV) offers a qualitative approach to study behavior of an electrochemical system. Cyclic voltammetry is often the first experiment performed in an electroanalaytical study. In particular, it offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media upon the redox process [62]. Its primary advantage comes from the fact that it gives insight into both the half-reactions taking place at the working electrode, providing at the same time information about the chemical or physical phenomena coupled to the studied electrochemical reaction. In cyclic voltammetry, starting from an initial potential $E_i$, a staircase potential sweep (or linear sweep in older potentiostats) is applied to the working electrode. After reaching a switching potential $E_f$, the sweep is reversed and the potential returns to its initial value [63].Cyclic voltammetry is carried out in quiescent solution to

ensure diffusion control. The important parameters in a cyclic voltammogram are the peak potentials ($E_{pc}$, $E_{pa}$) and peak currents ($i_{pc}$, $i_{pa}$) of the cathodic and anodic peaks, respectively. If the electron transfer process is fast compared with other processes (such as diffusion), the reaction is said to be electrochemically reversible, and the peak separation is

$$\Delta E_P = \left| E_{Pa} - E_{Pc} \right| = 2.303RT/nF \quad (6)$$

Where n is the number of electrons transferred and $E_{pa}$and $E_{pc}$ is the anodic and cathodic peak potentials, respectively, in Volts. Thus for a reversible redox reaction at 25 $^{o}$c with n electrons

$\Delta E_p$ should be 0.0592/nV or about 60 mV for one electron.

The formal reduction potential ($E^0$) for a reversible couple is given by

$$E^0 = \frac{E_{pa} - E_{pc}}{2} \quad (7)$$

Randles-Sevcik equation is an equation that correlates the peak current (Ip) with concentration

(C), $I_p$ = k C, where k is a constant that includes different cell parameters such as transfer coefficient, number of electrons involved in the reaction, electrode area, diffusion coefficient and scan rate.

$$i_p = 2.69x10^5 n^{3/2} ACD^{1/2} v^{1/2} \quad (8)$$

where $i_p$ is the peak current in amps, A is the electrode area ($cm^2$), D is the diffusion coefficient ($cm^2 s^{-1}$), Cis the concentration in mol $cm^{-3}$,n is the number of the electrons exchanged and v is the scan rate in V $s^{-1}$[52].

### 2.3.6. Square-Wave Voltammetry (SWV)

Square-wave voltammetry is a large-amplitude differential technique in which a waveform composed of a symmetric square wave, superimposed on a base staircase potential, is applied to the working electrode. A potential, consisting of symmetrical square-wave pulses superimposed on a staircase-wave form is applied to the working electrode. The current is sampled twice during each square-wave cycle, once at the end of the forward pulse and once at the end of the reverse pulse. The difference between the two measurements is plotted versus the base staircase potential .The peak height is directly proportional to the concentration of the electroactive species and direct detection limits as low as $10^{-8}M$ is possible[64].

Square-wave voltammetry has several advantages. Among these are its excellent sensitivity, speed and the rejection of background currents.

Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels, and its use with electrochemical detection in HPLC [52].

#### 2.3.6.1. Most Important Parameters in Square Wave Voltammetry includes:-

##### 2.3.6.1.1. Square Wave Frequency

An increase in square wave frequency results in an increase in the scan rate which in turn increases the peak current. However, at very high frequency, the peak current may become unstable and be obscured by a large residual current. On the other hand very low frequency gives a low but narrow signal, and increases the total analyses time. Hence, the selection of frequency usually requires a compromise among sensitivity, resolution and speed [65].

##### 2.3.6.1.2. Square Wave Amplitude

In square wave voltammetry, the peak currents usually increase with increasing amplitude. However, the width of the peak also increases as the square wave amplitude grows larger and

normally one refrains from increasing the square wave amplitude, because resolution may be degraded unacceptably [65].

#### 2.3.6.1.3. Square Wave Step Potential

The square wave voltammetric peak current usually increases as the step potential increases with an accompanying peak broadening. At higher step heights, too few points are sampled, thus affecting the reproducibility of the detection [65].

## 2.4. Carbon paste electrode

Carbon paste electrodes (CPEs) belong to promising electrochemical or bioelectrochemical sensors of wide applicability. CPEs are widely applicable in electrochemical studies due to their low background current (compared to solid graphite or noble metal electrodes), long-time stability, low cost, easy preparation, simple renewal of their surface (providing a fresh surface unaffected by electrode history), individual polarizability, easy to apply modifications (suitable for preparing an electrode material with desired composition) and possibilities of miniaturization. The disadvantage of CPE is the tendency of the organic binder to dissolve in solutions containing an appreciable fraction of organic solvent [54].

## 2.5. Analytical parameters

### 2.5.1. Limit of detection(LOD)

Limit of detection or detection limit, is the lowest concentration level that can be determine to be statistically different from a blank[55].

$$LoD = 3\ S/m \qquad (9)$$

### 2.5.2. Limit of quantification (LOQ)

Limit of quantification or lower limit of quantification is the level above which quantitative results may be obtained. The LOQ is mathematically defined as equal to ten times the standard

deviation of the results for a series of replicates used to determine a justifiable limit of detection. Limit of quantification are matrix, method, and analyte specific [61].

$$LOQ = 10S/m \quad (10)$$

### 2.5.3. Percent recoveries

One of the drawback of the method detection limit is that it doesn't take in to account the effect of high or low bias in a serious of measurements. Bias can be measured by the average percent recovery of a serious of samples. In order for the method detection limit to be realistic, the average percent recovery for the sample should be reasonable [60].

$$R_A = \frac{\boldsymbol{Q_A\ (O+S) - QA(O)}}{\boldsymbol{QA(S)}} X100\% \quad (11)$$

Where QA(S) is the quantity of analyte A added (spike value) and QA (O + S) the quantity of A recovered from the spiked sample and QA (O) from the original sample.

# 3. EXPERIMENTAL

## 3. 1 Apparatus and Instruments

The voltammetric experiments were performed using electrochemical analyzer [bioanalytical systems (BAS), made in USA] connected to a personal computer. A three electrode system (carbon paste as a working electrode, platinum coil as a counter electrode and Ag/AgCL (3.0 M NaCL) as a reference electrode) was used for voltammetric measurements. The pH of the buffer solutions was measured with a pH meter (AD 8000, made in Romania). An electronic balance (Nimbus, ADAM) used for measuring the mass of the chemicals used. Rotary evaporator (RE-25VD, Bejing, China), shaker (Heidolph UNIMAX, 2010, made in England), blender, cold storage chamber (freeze) (Lec, Refrigrater, PLC England).

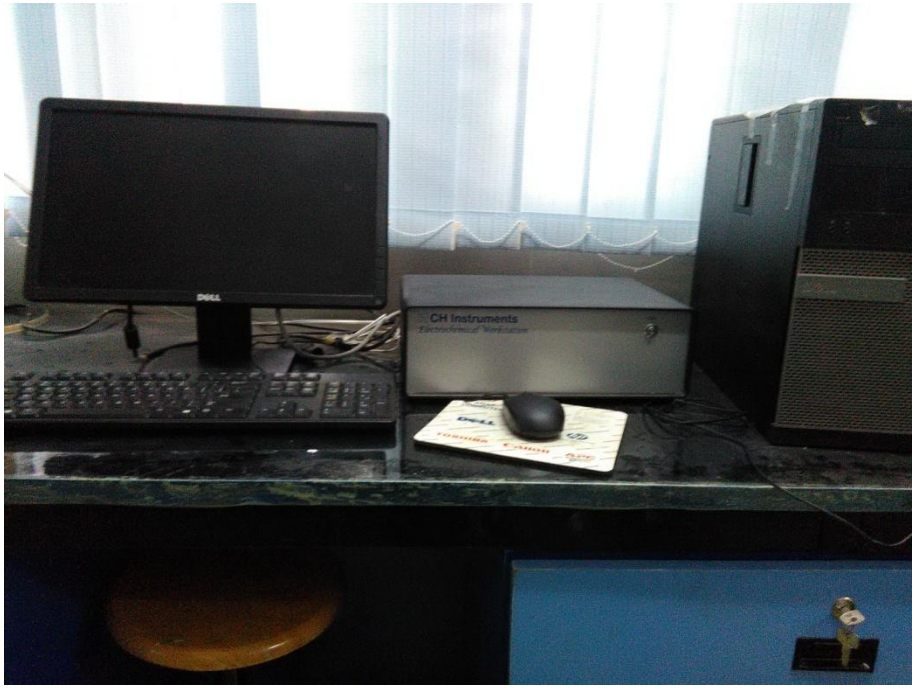



Figure: 3.1 A complete cell stands for Electrochemical Analytical technique

## 3.2 Chemicals and Reagents

Chemicals and reagents used for this work include are L-ascorbic acid(Blulux,INDIA), Sodium phosphate monobasic(Blulux,INDIA), Sodium phosphate dibasic(Blulux,INDIA), Nitric acid(Blulux,INDIA), Hydrochloric acid(Blulux,INDIA), ethanol(UN-chem. 1170), Distilled water, Deionized water, Graphite powder(Blulux,INDIA), and Paraffin oil(CARELABMED, INDIA) was used.

## 3.3. Samples selected for analysis

Fresh Garlic (Allium sativum) sample were purchased that obtain from the two selected areas of North Gondar (Gondar town, and Debark wereda) urban agricultural center and analyzed.

## 3.4. Working Procedure

### 3.4.1. Preparation of supporting electrolyte

Supporting electrolyte of 0.1M of phosphate buffers ($NaH_2PO_4$ –$Na_2HPO_4$) in pH range 1-5 was prepared from 0.1 M $NaH_2PO4$(7.1 g) and 0.1 M $Na_2HPO_4$ (7.384 g) in Deionized water and mix in 1000 ml of volumetric flask. The drops of 1 M HCl and 1 M NaOH Solution were used to adjust the pH of the buffer solution.

### 3.4.2. Preparation of standard solution

A stock solution of 20 mM prepare by dissolving 0.88 g of AA in 250 mL of 0.1 M phosphate buffer at optimum pH solution. From stock solution, serial dilution with phosphate buffer(at optimum PH=3) were obtained 10 mM ,5 Mm,4 Mm,3 mM,2 Mm, 1 mM,0.6 mM and 0.2 mM ascorbic acid solutions to the final volume of 100 ml and10 mM ,5 Mm,4 Mm,3 mM,2 Mm, 1 mM,0.6 mM a 0.16 mM and 0.08 mM ascorbic acid solutions to the final volume of 100 ml, these solutions used for calibration for CV and SWV studies respectively. 15 $cm^3$ each of standard ascorbic acid solution prepared above transferred to the electrochemical cell. The potential of each solution was scan for CV and SWV measurements. The influence of the

operational parameters investigated on the oxidation signal, the plus amplitude, the frequency and step potential varies at SWV.

### 3.4.3. Sample preparation

The garlic deeply washed with running tap water followed by distilled water. Then peel and chopped with stainless steel knife before being grounded the wet form. Half of the chopped garlic store in a deep freezer at -80 °C for two weeks. It has been reported that the amount of AA in dried samples is higher than in fresh samples because in fresh samples enzymatic actions are able to degrade these bioactive compound [66]. The dried garlic then ground to a fine powder using a pestle and mortar.

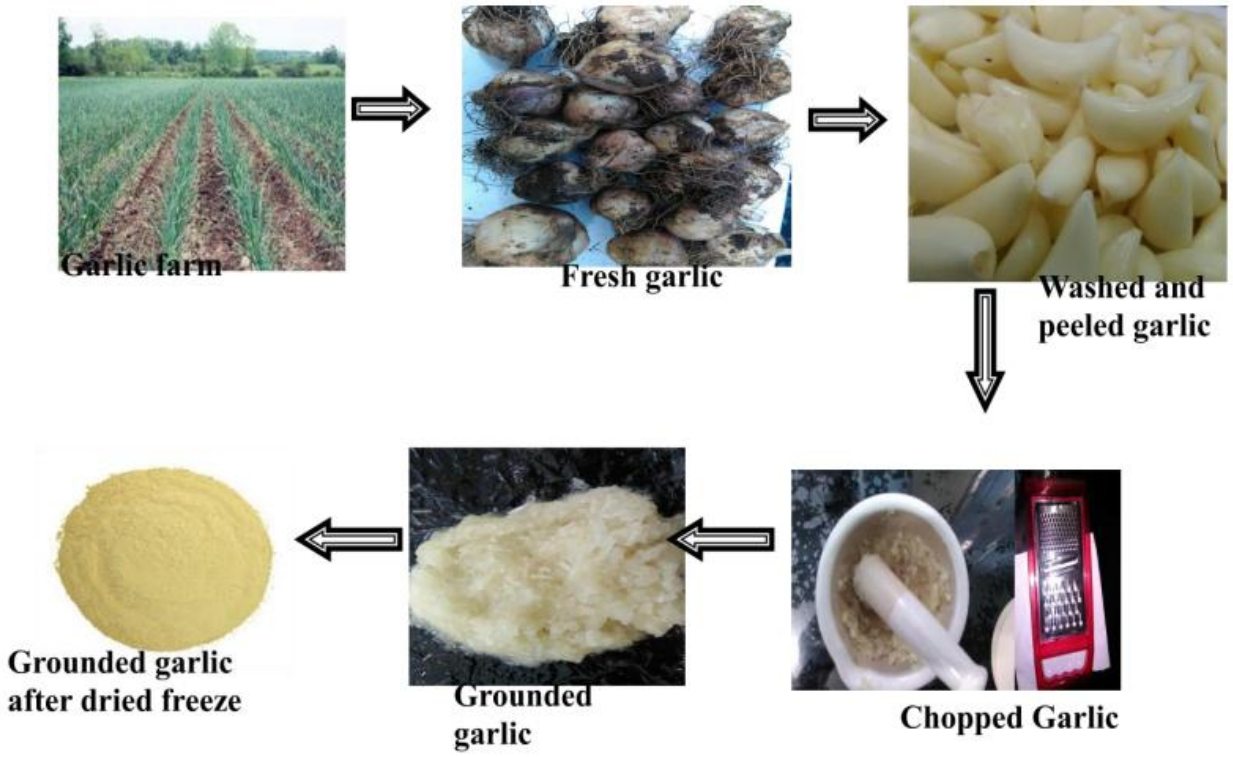



Figure3.2.Preparation of garlic

### 3.4.4. Sample extraction

100 g of grounded garlic placed on conical flask before being added of 796 ml of ethanol [67].Then the top of the flask will cover with aluminum foil before being placed on an orbital shaker at 200 revolutions per minute for 48 hours. The solution was then passed through Whitman filter paper and was evaporated using a rotary evaporator under law pressure at 45 $^{o}$C. The extracts were removed and solvent-free aqueous garlic was place into falcon tubes. Finally trace amount of the solvent was evaporated by storing at 4 $^{o}$C freeze. For SWV 5 $cm^3$ of the aliquot was immediately added to the 0.1 M phosphate buffer (PH =3) to make the final volume of 5 $cm^3$.The potential of all solution were scanned as described in the standard ascorbic acid solutions.

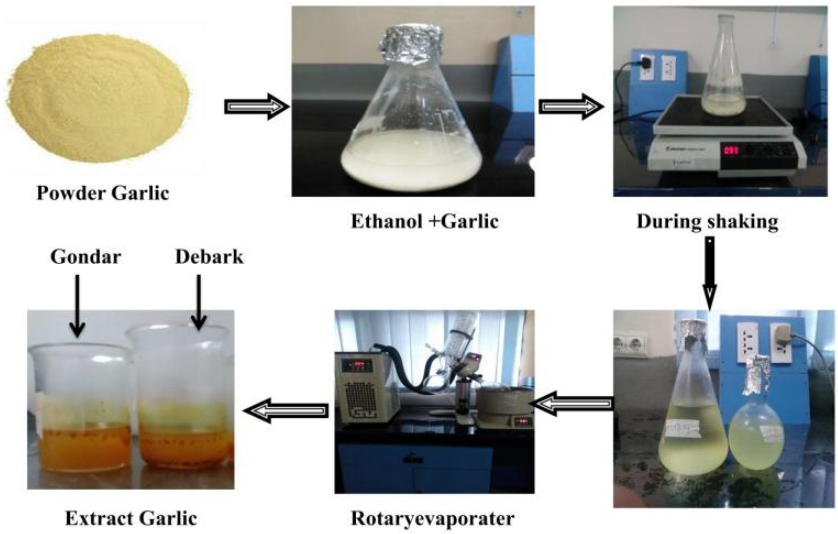



Figure 3.3. Extraction of Garlic

### 3.4.5. Preparation of Working Electrode

Carbon paste electrode was prepared by mixing 7 g of graphite powder with 3.53 mL of paraffin oil. The composition of the paste was 70 % (w/w) graphite powder and 30 % (w/w) paraffin oil. The mixture was homogenized with mortar and pestle for 30 minutes and allowed to rest for 24 hours. The homogenized paste was packed in to tip of a plastic tube (chewing gum stick, with a diameter of 4 mm). A copper wire was inserted from the back side of plastic tube. The surface of

the electrode was smoothed manually against a smooth white paper until a shiny surface was emerged [68].

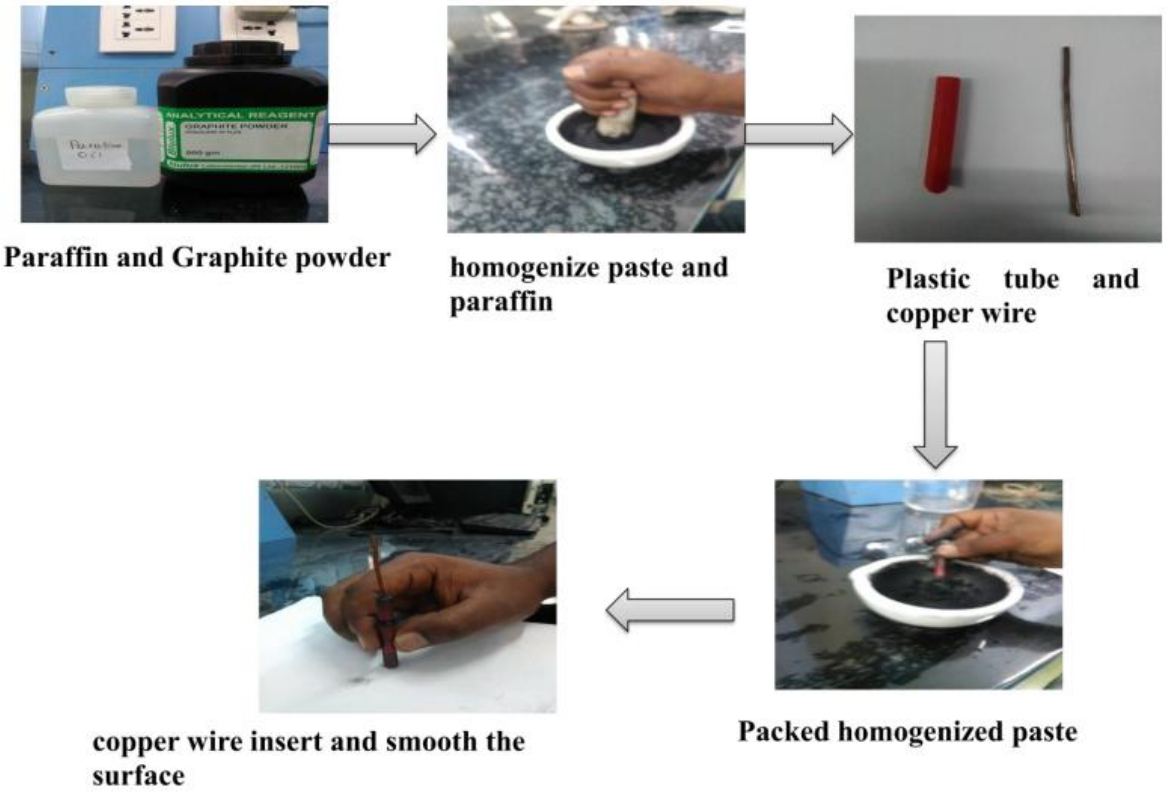



Figure 3.4.Preparation of carbon paste electrode

# 4. Result and Discussion

## 4.1 Cyclic voltammetry behavior of ascorbic acid

In order to understand the electrochemical process of ascorbic acid at carbon paste electrode, the CV technique was used. Figure.4.1(a) shows the cyclic voltammogram for 0.1 M phosphate buffer and (b) the oxidation of 1 mM of ascorbic acid in a 0.1 M phosphate buffer with scan rate of 100 $mVs^{-1}$. No anodic peak current was observed in the voltammogram of buffer phosphate (Figure 4.1.a) but was observed for 1mM ascorbic acid solution (figure 4.1.b) at 734 mV. The voltammogram indicates the absence of a well-defined reduction peak, implying that the electrochemical process of ascorbic acid is irreversible.

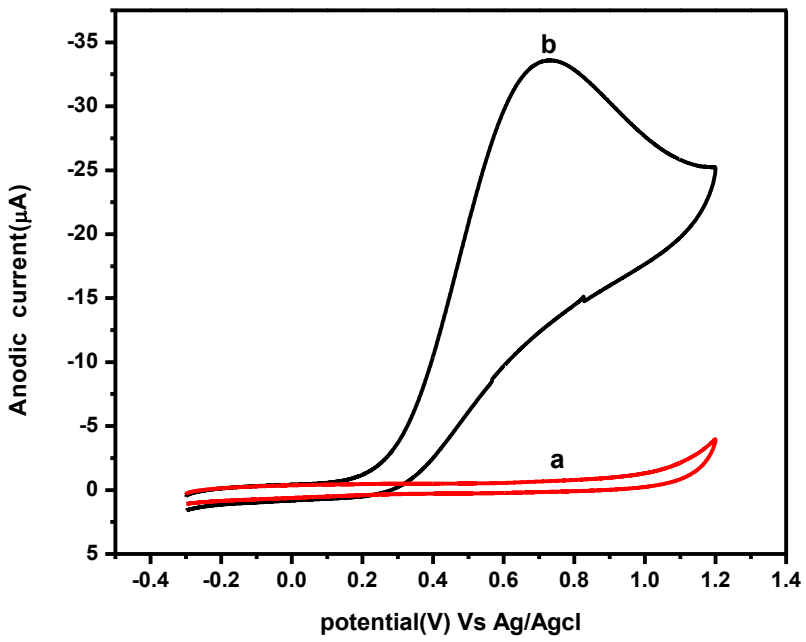



Figure.4.1..Cyclic voltammogram of a) phosphate buffer b) 1 mM ascorbic acid in 0.1 M of phosphate buffer at scan rate of 100 mV.

### 4.1.1. Effect of pH

The effect of pH on the oxidation of ascorbic acid at CPE was studied. To determine the effect of pH on the oxidation property of ascorbic acid, 1 mM ascorbic acid at different pH values (pH 1 – 5) were investigated using a CPE. Varying the pH of the supporting electrolyte would bring different current response of AA. As can be seen from the figure, it is observed that the voltammetric response is strongly pH dependent.

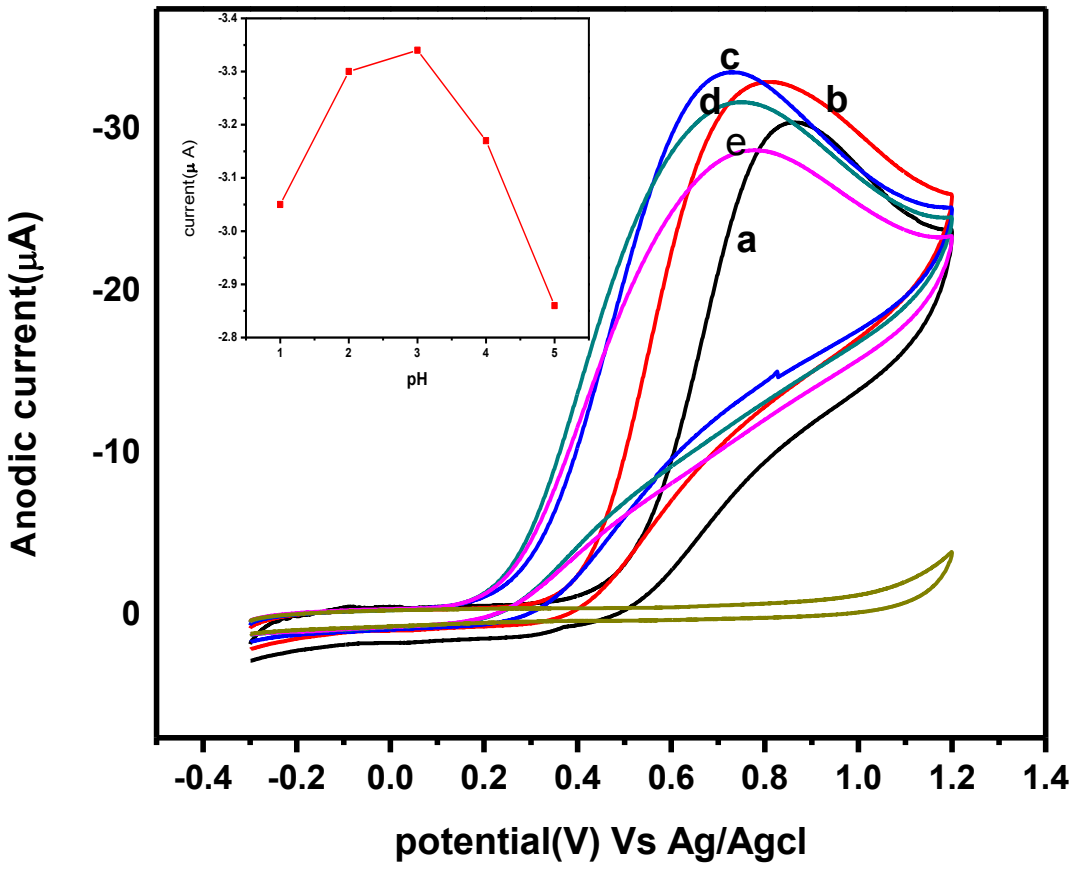



Figure4.2 .A) Cyclic voltammogram of 1 mM ascorbic acid in 0.1 M phosphate buffer for pH values a) 1, b) 2, c) 3, d) 4 and e) 5 at scan rate of 100 $mVs^{-1}$. inset: Plot of peak current Vs pH

As can be seen from the inset, the anodic peak current response for AA is increased when the pH value increases from 1 to 3.However, the peak current decreases beyond pH 3 up to 5. The

maximum peak current response was observed at pH 3. Hence, pH 3 was chosen as the optimum pH value. The results also show the dependence of peak potential on pH value. The peak potential, Ep, is shifted to less potential values with increasing pH (figure 4.3). This implies oxidation of AA is promoted under acidic media that facilitate deprotonation [69].

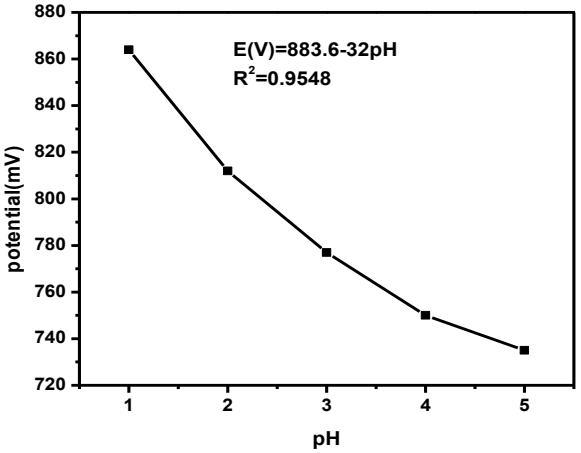



Figure.4.3: Plot of peak potential as a function pH for 1 mM AA in 0.1 M phosphate buffer.

A linear relationship between the peak potential and solution pH was obtained with a linear equation and correlation coefficient of Epa(V) = 883.6-32pH and $R^2$=0.9548 respectively.

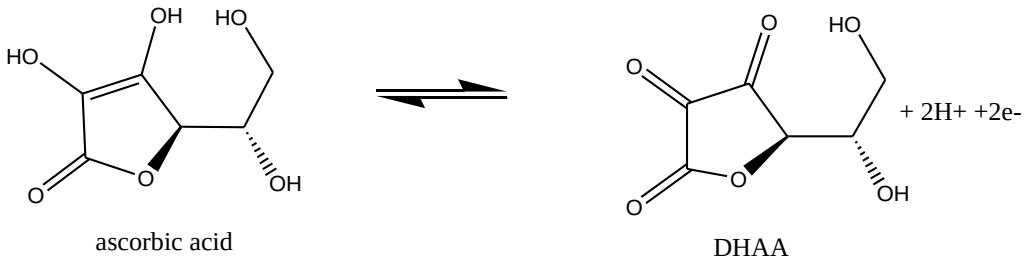



**Figure 4. 4:** The schematic oxidation representation of AA to DHAA at CPE

### 4.1.2. Effect of scan rate on oxidation peak current

In the present study, the effect of scan rate on the peak current of ascorbic acid at carbon paste electrode was studied at scan rates ranging from 25 - 225 mV/s while maintaining other parameters constant. Accordingly, the results showed that the peak current increased with increasing scan rate (Figure 4.5). And the anodic peak potential change with the variation of scan rate. The relation of anodic peak current and the square root of scan rate is shown in Figure 4.6. It can be given by equation 12 with its correlation coefficient of 0.9914.

$$Ip(\mu A) = 17.298 + 0.1603\upsilon \qquad (12)$$

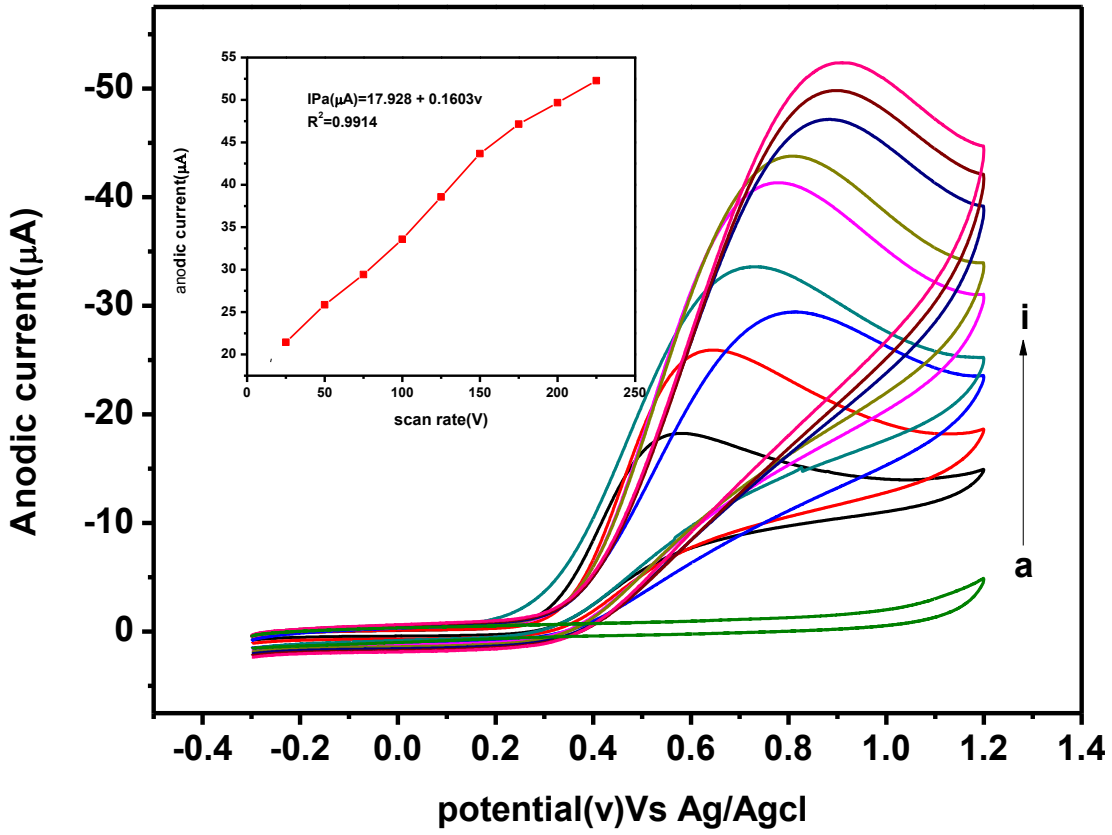



Figure 4.5: A)Cyclic voltammogram of 1 mM ascorbic acid in 0.1 M phosphate buffer solution (pH 3.0) at various scan rates: (a) 25, (b) 50, (c) 75, (d) 100, (e) 125, (f) 150 g)175,h)200 and i)225 mV $s^{-1}$. Inset: plot of peak current Vs scan rate.

In order to investigate whether the oxidation process of ascorbic acid at CPE is predominantly diffusion controlled or surface confined process, the dependence of peak current on the scan rate and square root of scan rate was as shown in figure 4.5 (inset) and figure 4.6.rspectively. The peak current was more linearly dependent on the square root of scan rate ($R^2$=0.992) than on the scan rate ($R^2$=0.9914) showing that the oxidation reaction of AA at carbon paste electrode is predominantly diffusion controlled.

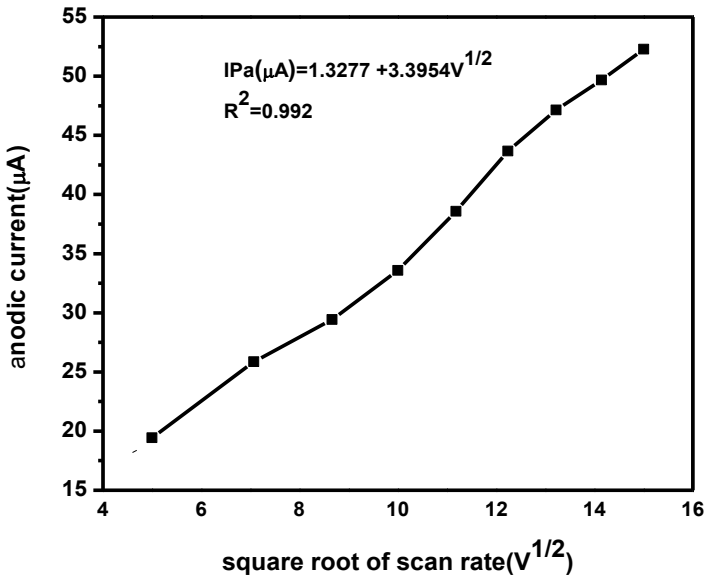


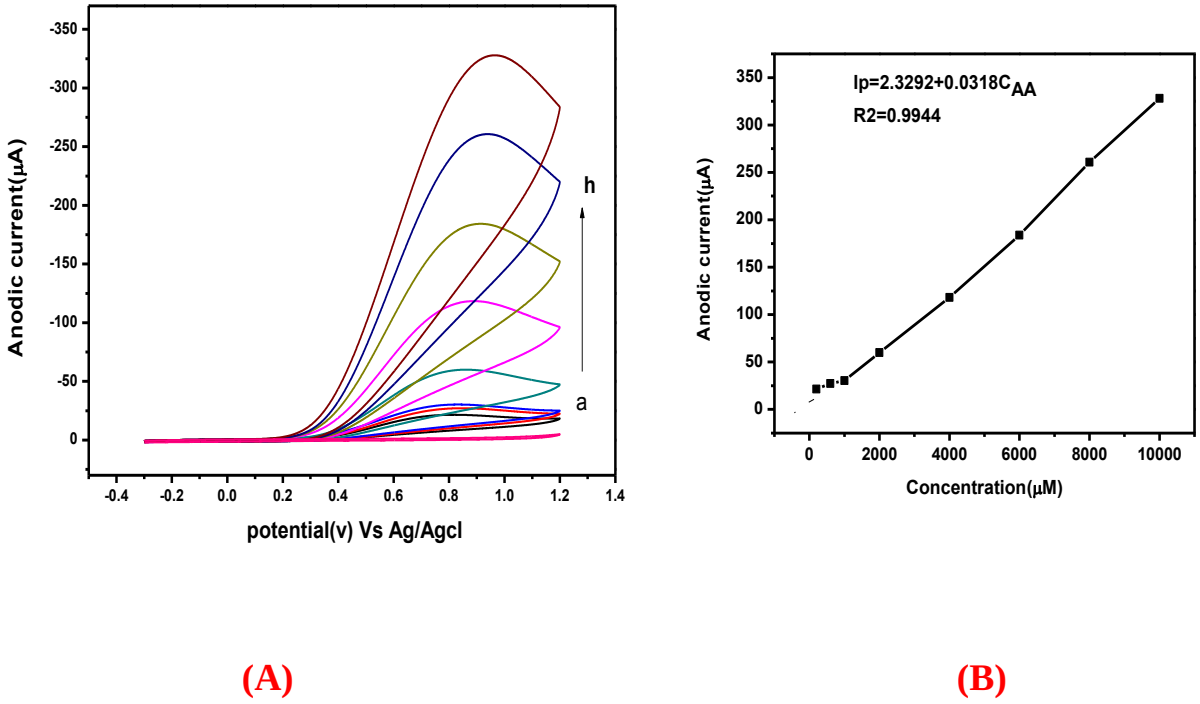

**Figure 4.6.** Plot of peak current Vs square root of scan rate

### 4.1.3. Effect of varying ascorbic acid concentrations

Voltammograms show that peak current increases linearly with increasing concentration of ascorbic acid from 0.2 mM to 10 mM, Figure 4.7. The calibration graph of ascorbic acid concentrations in 0.1 M phosphate buffer, pH 3.0 was determined as shown in Figure 9. The linear regression equation is given in Equation 13:

$$Ip = 2.3292 + 0.0318C_{AA} \quad (13)$$

where Ip represents the anodic peak the current, the background current value was subtracted and $C_{AA}$ is the ascorbic acid concentration The regration value is found to be $R^2$ = 0.9944.




**(A)** **(B)**

**Figure.4.7.** A)Cyclic Voltammograms of ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at pH 3.0 for concentrations of 0.2 (a), 0.6 (b), 1 (c), 2(d), 4 (e), 6 (f), 8 (g) and h)10 at a scan rate of 100 mVs-1. **B)** Calibration graph for ascorbic acid at various concentrations.

## 4.2. Square wave voltammetric behavior of ascorbic acid

The electrochemical oxidation of ascorbic acid at CPE was studied using square wave voltammetry in the potential range from -0.4 V to 1.4 V. Figure 4.8 presents the square wave Voltammograms of CPE in pH 3 phosphate buffer in the absence (a) and presence (b) of 1 mM AA. The voltammogram in the presence of AA showed a sharp oxidative peak (curve b) at a peak potential of 436 mV where as there is no peak observed for the blank solution. (curve a).

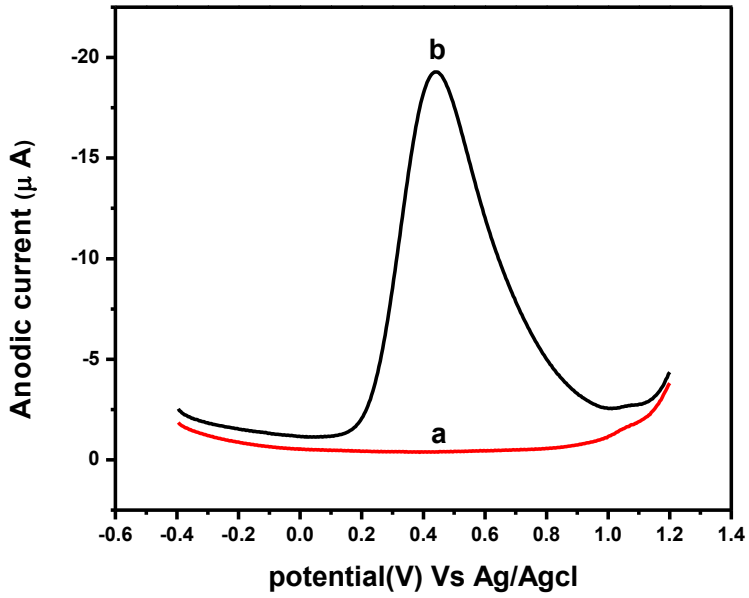



**Figure4.8.**Square wave Voltammograms of (a) 0.1 M phosphate buffer (b) a + 1 mM ascorbic acid at pH 3.0. Experimental conditions: f = 15 Hz, Es = 8 mV.

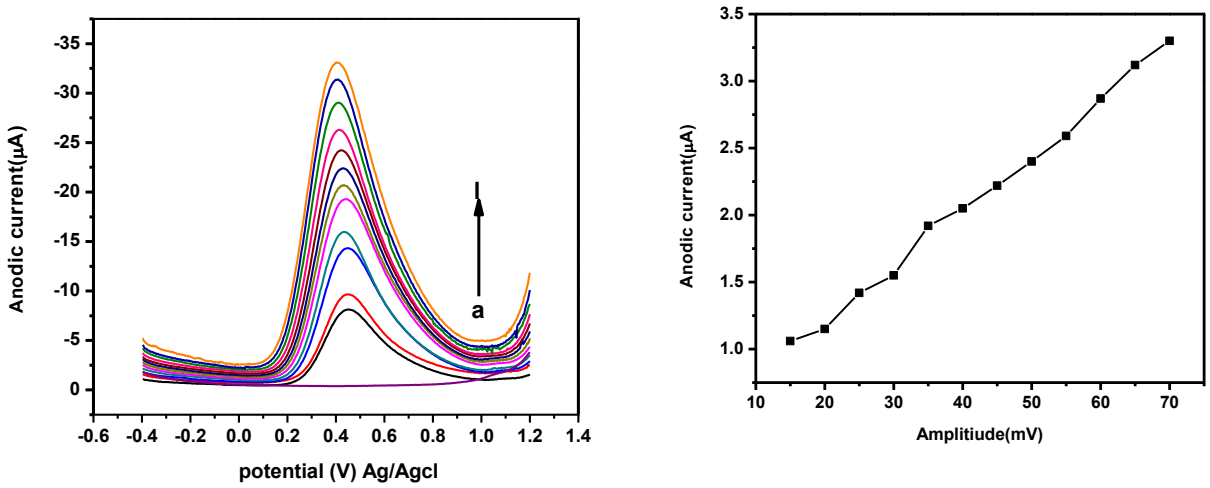
### 4.2.1. Effect of square wave voltammetric parameters

The electroanalaytical method for the determination of ascorbic acid using square wave voltammetry, which is an effective and well-established pulse voltammetric technique and suitable for determination of organic compounds [70]. The response obtained by square-wave voltammetry was dependent on parameters such as frequency, square wave Amplitude and scan increment or step potential, which have a combined influence on the peak current. Hence, optimizing of these parameters for determination of ascorbic acid is very crucial. The square wave parameter optimization was carried out 1 mM ascorbic acid solutions in a 0.1 M phosphate buffer at pH 3.

#### 4.2.1.1. Effect of pulse amplitude

To observe the influence of pulse amplitude on the anodic peak current of ascorbic acid, the investigation was done by varying the value of pulse amplitude in the range of 15 mV and 70

mV by taking, a 4 mV step potential and 15 Hz frequency as it is shown in Figure 4.9. The value of the measured peak current is increased with the applied pulse amplitude. An optimum amplitude must be found to maximize the peak current, and with resolution of small peak width [65]. Therefore 35 mV was chosen for further studies and for real sample analysis. Greater values of the pulse amplitude were not employed, in order to avoid the decrease of resolution.

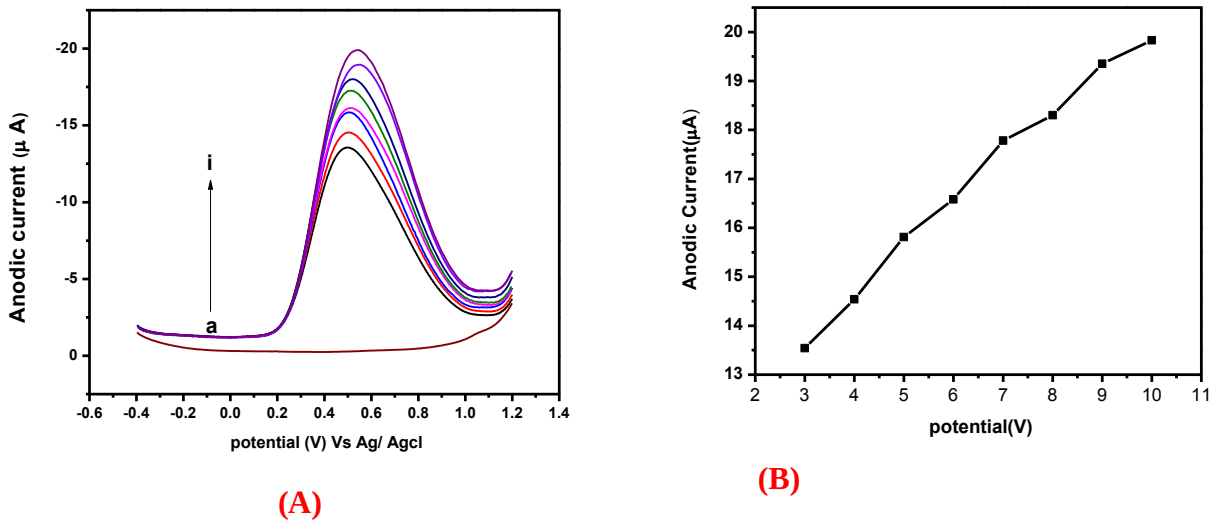



(A) (B)

**Figure.4.9: A)** Influence of the pulse Amplitude on the oxidation response at ascorbic acid determination by square wave voltammetry; (a) 15 mV, (b) 20 mV, (c) 25 mV, (d) 30 mV ,e) 35 mV, f) 40 mV, g) 45 mV, h) 50 mV, i) 55 mV, j) 60 mV, k) 65 mV and l) 70 mV. Experimental conditions: step potential = 4 mV, f= 15 Hz. B) peak current Vs pulse amplitude.

### 4.2.1.2 Effect of step potential on peak current

The influence of step potential, the amount of potential change between data points in the experiment, was investigated from 3 mV to 10 mV at fixed f and Es.




**(A)** **(B)**

**Figure: 4.10.** A). Influence of the step potential on the oxidation response at ascorbic acid determination by square wave voltammetry; (a) 3 mV, (b) 4 mV, (c) 5 mV,(d)6 mV, e)7 mV, f)8 mV ,g) 9 Mv and h) 10 mV. Experimental conditions; frequency 15 Hz, amplitude 35 mV B) Plot of peak current Vs step potential.

The peak height increased because of the effective scan rate was increased due to increment of step potential, so at higher values of step potential, the peak was highly broadened. Accordingly, step potential of 8 mV was chosen for whole work in this study.

### 4.2.1.3. Effect of Frequency

The effect of square wave frequency on peak current and peak potential of ascorbic acid was studied in the range of 10 - 45 Hz at constant amplitude and step potential as shown in Figure 4.11. The peak current was found to increase linearly with square wave frequency as shown in Figure 4.12

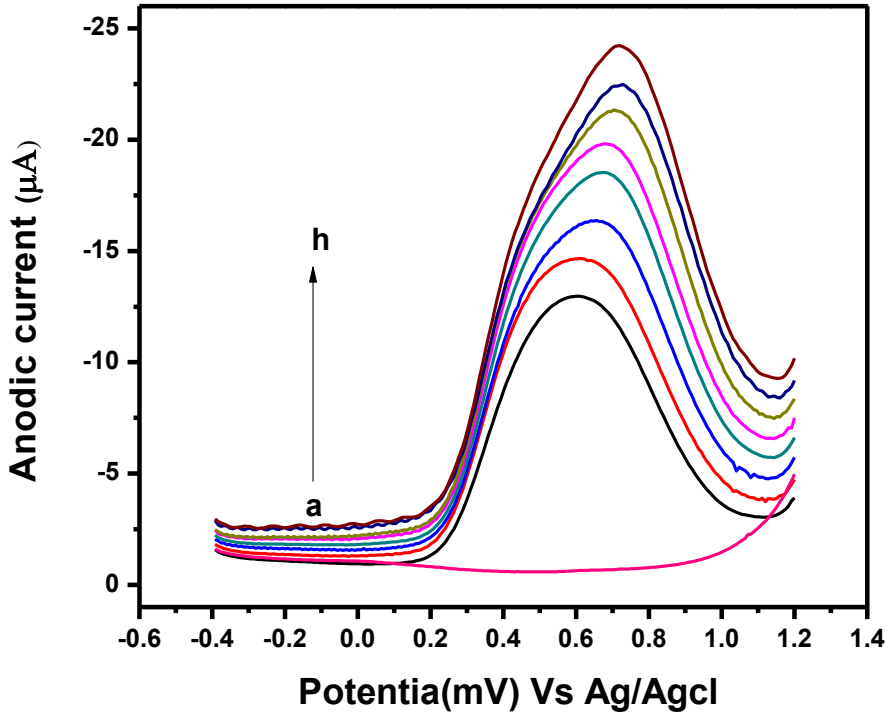



**Figure.4.11**. Square-wave voltammogram of 8 mM ascorbic acid in 0.1 M phosphate buffer solution (pH 3.00) at various frequencies: (a) 10, (b) 15, (c) 20, (d) 25, (e) 30 (f) 35 ,g) 40, and h) 45 Hz.. Experimental conditions:, Es = 4 mV and amplitude of 35 mV

The peak current increased with the frequency due to the increase in the effective scan rate but the slope changes to lower value and at very high frequency the peak current is unstable and baseline are distorted. This was attributed to the greater contribution of the capacitive current at higher frequencies [71]. Therefore, 25 Hz was selected as optimum frequency for the whole work of this study.

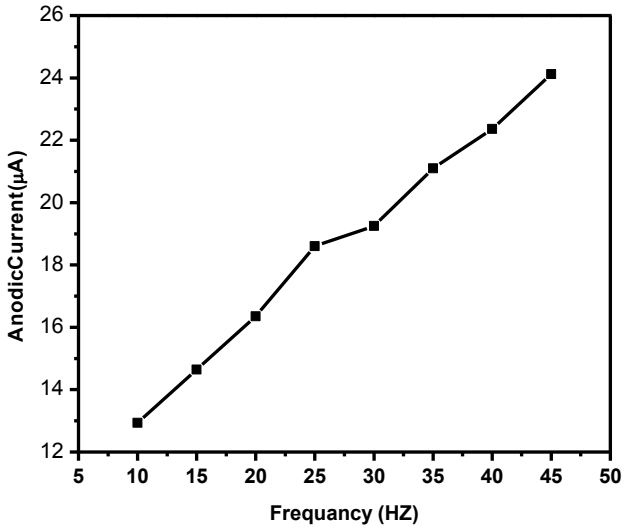



**Figure.4.12** Plot of peak current Vs frequency. Experimental conditions: step potential = 8 mV and amplitude of 35 mV.

### 4.2.2. Optimum Experimental Condition

The effects of experimental parameters have been studied to obtain optimum experimental condition for square wave voltammetric determination of ascorbic acid at CPE. The optimum parameters used for the experiment are summarized in table 2 below**.**

**Table 4.1** Optimum values of the experimental parameters of the determination of ascorbic acid by SWV technique at CPE.

| **Parameters** | **Optimized values** |
|---|---|
| pH of the buffer solution | 3 |
| Square wave frequency (HZ) | 25 |
| Square wave amplitude (mV) | 35 |
| Square wave potential (mV) | 8 |

### 4.2.3. Linear range and Limit of Detection

Under the obtained optimum conditions the calibration graph for determination of ascorbic acid was obtained in the concentration range of 0.08 - 10 mM The Voltammograms for different

concentrations of ascorbic acid are shown in Figure 4.13. The regression equation can be represented by Equation. 14 with a correlation coefficient of 0.9953 as shown in figure 4.14.

$$Ip(\mu A) = 0.0\ 203C_{AA(\mu M)} + 13.47 \quad (14)$$

The limit of detection (LOD) and limit of quantification (LOQ) calculated using equation (15) and (16) are 0.363 mM and 1.209 mM, respectively.

$$LOD = 3\ s/m, \quad (15)$$

$$LOQ = 10\ s/m \quad (16)$$

Where s is the standard deviation of the peak currents (n=9) and m is the slope of the calibration curve from Figure 4.14 for this work respectively.

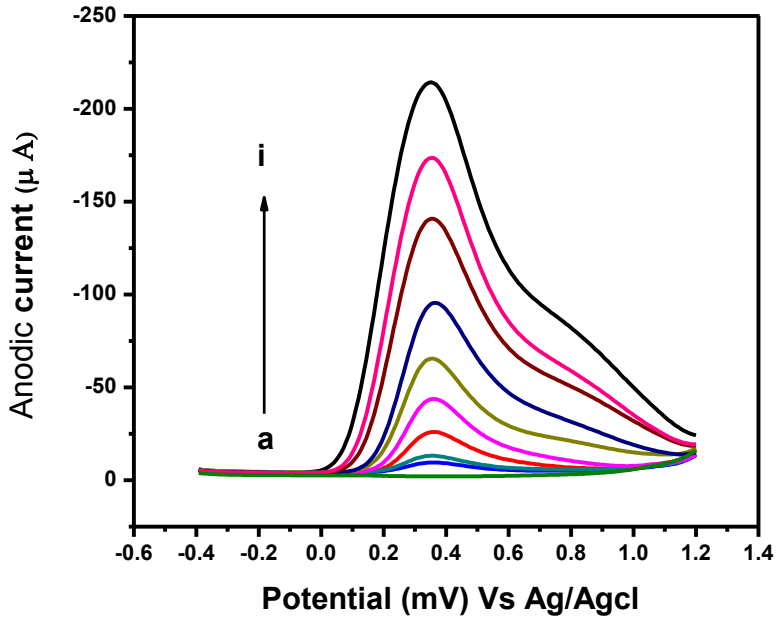



**Figure.4.13.**Square wave Voltammograms of ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at pH 3.0 for various concentrations: (a) 0.08 mM, (b) 0.16 mM, (c) 0.6 mM, (d) 1 mM, (e) 2 mM, (f) 4 mM, (g) 6 mM. h) 8 mM, and i)10 mM,. Experimental condition of: frequency=25 v HZ, step potential=8 mV, SW Amplitude=35 mV.

Figure 4.14 shows the variation of the peak current with concentration. It can be observed that the shift of potential to more positive values also can be explained by the increase of ascorbic acid molecule on the surface of the electrode. The more positive the oxidation potential shows higher facility in oxidizing this species. This can be explained by the fact that the electrode needs high potentials to oxidize other undesired species, interfering with the result [72].

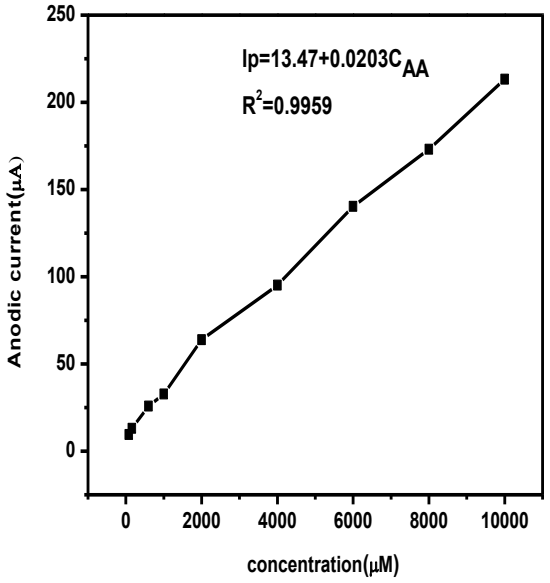



**Figure.4.14.**A calibration plot of peak current Vs concentration under the optimized parameters. Experimental condition of: f=25 HZ, Es=8 mV, SW Amplitude=35 mV

The LOD achieved using the method is comparable with other reported results as shown (Table 3).

**Table 4.2.** .Comparison between the method in this work and other reported methods

| **Electrode** | **Method** | **Linear range(mM)** | **Detection Limit(mM)** | **Reference** |
|---|---|---|---|---|
| GCE | SWV | 0.008 - 0.08 | 0.0115 | 59 |
| GCE | DPV | 0.3-1.4 | 0.09 | 48 |
| CPE | SWV | 0.08-10 | 0.363 | This work |

## 4.3. Real sample Analysis

The determination of AA content was demonstrated by applying square wave voltammetry technique at CPE in fresh Garlic sample. These samples are prepared, extracted, diluted and then added to the electrochemical cell for analysis as briefly described in sections 3.4.2. and 3.4.3. In order to analyze the determination of AA in Garlic cultivated in different place, the standard addition method was applied.

The following figures(figure:4.15 and 4.16) show the square wave Voltammograms of Garlic samples (a), a +4 mM(b) and b+4 Mm(c) standard ascorbic acid solution under the optimized parameters. The voltammogram of the real sample and the spike shows a peak shift towards the positive potential, this is because of when the concentration increases the analyte deposited to the electrode and the electrode need high potential to oxidize the analyte.

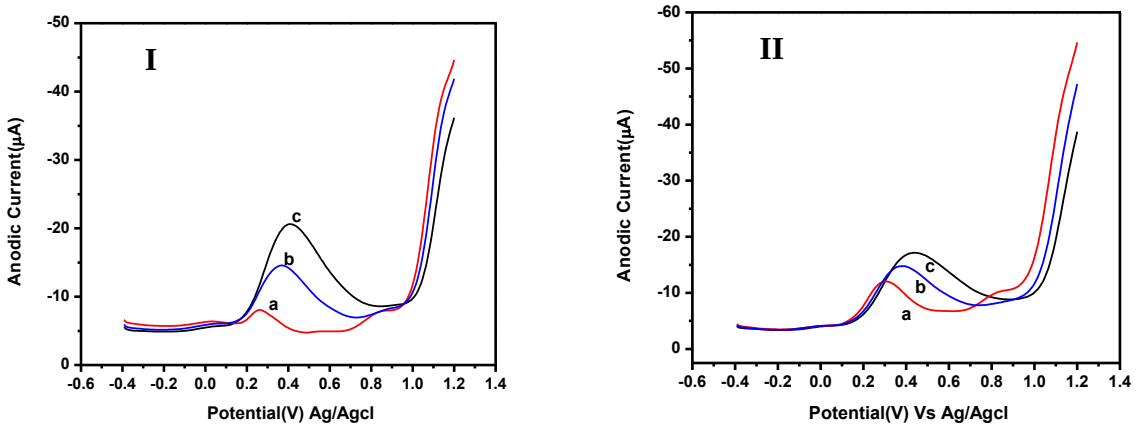



Figure: 4.15 Square wave voltammogram of garlic collected from (I) Gondar and (II) Debark: in each figure (a) is voltammoram of garlic (b) a+4 mM AA (c) b+4 mM AA at pH=3, f=25 HZ, ΔEs=8 mV, and SW Amplitude=35 mV.

### 4.3.1. SWV determination of degree of recovery for each sample

The degree of recovery for each samples were determined from the above figures as shown in table 4 with an excellence recovery value of 99.99%.

Table 4. 3 Degree of recovery for each sample.

| No. | Samples | Concentration before addition of standard AA(mg/100 g) | Amount of AA Added (mg/100 g) | % recovery |
|---|---|---|---|---|
| 1 | Gondar (Maura) Garlic | 19.45 | 70.448 | 99.98 |
| 2 | Debark (Segie) Garlic | 22.8 | 70.448 | 99.99 |

Table 4.4: Comparisons of ascorbic acid concentrations in Garlic samples of this study, other reported works.

| No. | Samples | Method | Electrode type | Concentration of AA from the sample (mg/100 g) | Reference |
|---|---|---|---|---|---|
| 1 | Garlic | CV | GCE | 22.1 | 59 |
| | | SWV | GCE | 30.00 | 59 |
| | | CV | GCE | 38.68 | 73 |
| 2 | Gondar Garlic | SWV | CPE | 19.45 | The present work |
| 3 | Debark Garlic | SWV | CPE | 22.8 | |

As can been seen from the table (Table-5),The level of vitamin C determined from each Garlic in this work was 19.45 mg/100 g and 21.8 mg/100 g of Gondar and Debark Garlic respectively. The values are higher than the one reported paper in CV [59], but close to the other two reported works [59, 73] and the international standard database, United States Department of Agriculture (USDA), which is 31.2 mg/100 g. This may be due to soil fertility of growing area; the plant may be infected by disease, improper management of storing time, temperature of the environment, transportation of the sample, and the extraction process of this sample may loss the content of vitamin C level.

# 5. Conclusion and recommendation

## 5.1. Conclusion

The electrochemical oxidation of ascorbic acid was successfully studied by SWV and CV with carbon paste electrode. Square wave voltammetric method was applied for quantitative determination of ascorbic acid content in some freshly prepared edible portion of Garlic that are cultivated in selected areas of North Gondar, Ethiopia. The effect of square wave frequency, square wave amplitude, step potential, pH, and scan rate on the oxidation response of ascorbic acid is investigated and it is found that the anodic peak current was increased with frequency, amplitude, scan rate, and step potential. Parameter optimization was done by varying the one while keeping constant all the other. The ease preparation of the electrode in combination with relatively low detection limit, better selectivity and sensitivity represent important factor for use of the electrode in quantitatively analysis of ascorbic acid and make it use full device for determination for ascorbic acid in different samples. The detection limit and limit of quantification were found to be 0.363 mM and 1.209 mM, respectively.

The level of vitamin C determined in Gondar and Debark Garlic was found to be 19.45 mg/100 g and 22.8 mg/100 g, respectively, indicating that vitamin C content of Debark Garlic was higher than that of Gondar. According to national database United States Department of Agriculture (USDA) the standard amount of vitamin C was 31.2 mg/100 g, which is somehow close to this study. The degree of accuracy of the investigated voltammetric method is confirmed by the values obtained for the degree of recovery, which range between 99.98 and 99.99%. This result provide suitable guide to the population in their choice of Garlic having high level of vitamin C.

## 5.2. Recommendation

Finally I would like to recommend:-

- Everybody who is interested to study the content of vitamin C in vegetables at higher potential should modify the surface of carbon paste electrode using a material that increases its catalytic effect towards the oxidation of ascorbic acid.
- Further work should be conducted to study other contents of Garlic, specially other benefits of Garlic for human health.
- Since ascorbic acid is easily sensitive to air, light, and temperature, care should be taken while conducting the experiments so as to minimize the exposure of a sample to open air, light and temperature.

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