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M.Sc. THESIS

ON

ELECTROCHEMICAL DETERMINATION OF ASCORBIC ACID USING A CARBON PASTE ELECTRODE MODIFIED WITH ANTHRAQUINONE

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ELECTROCHEMICAL DETERMINATION OF ASCORBIC ACID USING A CARBON PASTE ELECTRODE MODIFIED WITH ANTHRAQUINONE

BY

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MSc THESIS APPROVAL SHEET

We, the undersigned, member of the Board of Examiners of the final open defense by **TSEGAYE ABERA DABA** have read and evaluated his/her thesis entitled "ELECTROCHEMICAL DETERMINATION OF ASCORBIC ACID USING ANTHRAQUINONE MODIFIED CARBON PASTE ELECTRODE" and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in Chemistry **(Analytical)**

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Declaration

I, the undersigned declare that this is my original work and has not been presented for research in any other University.

Tsegaye Abera

This M.Sc. Thesis has been submitted with our approval as supervisors

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List of Abbreviations

PBS	Phosphate buffer solution
CPE	Carbon paste electrode
CV	Cyclic voltammetry
WHO	World Health Organization
AA	Ascorbic acid
AT	Anthraquinone
DA	Dopamine
UA	Uric acid
RSD	relative standard division
CME	Chemically modified electrode
LOD	Limit of detection
LOQ	Limit of quantification

Abstract

In this work, an electrochemical method for the determination of ascorbic acid using anthraquinone modified carbon paste electrode was developed. The electrode modification has been performed by mixing varying amounts modifier (anthraquinone), carbon paste and the solvent (paraffin oil) for homogenization. Under the optimized conditions. Anthraquinone modified electrode increased the peak currents of ascorbic acid, and greatly lowers the peak potential separation, compared to unmodified counterpart. Optimization of different variables such as pH of the working solution, scan rate, modifier composition and cyclic voltammetry parameters were made to improve the efficiency of the method. Moreover, the data reproducibility and electrode stability and recovery for the analysis were evaluated. The developed method was used for electroanalysis of ascorbic acid from pharmaceutical samples collected from Pharmacies at Jimma town. The peak potential shift with pH in the range 3.0 to 8.0, indicating participation of protons during the oxidation of ascorbic acid. Under optimized conditions and cyclic voltammetric parameters, an excellent linear dependence of the oxidative peak current on concentration of ascorbic acid was observed in the range 5×10^{-6} - 1×10^{-4} M with limits of detection (LOD) and quantification (LOQ) of 1.63 and 5.44 μM, respectively. Percentage recovery results being 98.5% for spiked standard ascorbic acid in pharmaceutical tablets. This method confirmed the potential applicability on the determination of ascorbic acid in real samples

Keywords: Ascorbic acid, cyclic voltammetry, Anthraquinone, Modified carbon paste electrode

1. INTRODUCTION

1.1 Background of study

Ascorbic acid (AA), is water soluble type of organic acid, which is also known widely as Vitamin C. Ascorbic acid is essential in human body due to its importance in antioxidant in food, animal feed, beverages, pharmaceutical formulations and cosmetic applications property.[1]-[2] Besides, ascorbic acid also plays role in metabolisms include collagen synthesis, amino acid metabolism, synthesis of adrenalin, synthesis of anti-inflammatory steroids and certain hormones and neurotransmitters synthesis.[3]Ascorbic acid a vitamin commonly present in many biological systems and in multivitamin formulations, is widely employed to provide an adequate dietary intake and as an antioxidant.[4]It is a power full water-soluble type of organic acid, and clinically used for the treatment and prevention of scurvy, common cold, mental illness, cancer and protecting living cells against oxidative injury.[5]Ascorbic acid is nontoxic, but in some cases an over dose (2-6g/day) can leads gastrointestinal disturbances, such as; abdominal distention, flatulence, diarrhea, transient colic, headache, trouble sleeping, and flushing of the skin.[6]It is a vital component in human diet, among animal organs, the liver, leukocytes, and anterior pituitary lobe show the highest concentration of AA. The human body cannot produce ascorbic acid, and so it must be obtained entirely through one's diet it is widely used in foods and drinks as an antioxidant.[7] AA has the capacity to eliminate toxic free radicals and other reactive oxygen species, formed in cell metabolism, which are associated with several forms of tissue damage and diseases and keeps the membrane-bound antioxidant a-tocopherol in the reduced state. [8]

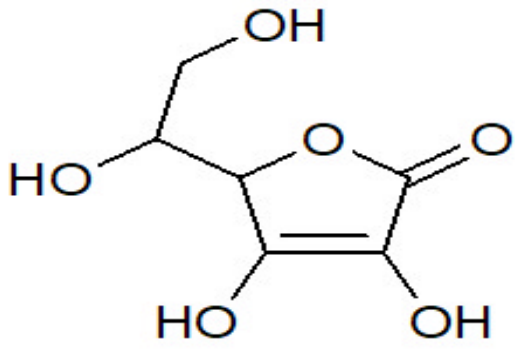

Figure1: Chemical structure of ascorbic acid

Since AA cannot be produced by human body, it has to be supplemented through ingestion via other sources since its deficiency in humans can lead to several diseases[9].Therefore, development of fast, selective and sensitive methods is vital for determination of AA.

Various analytical methods have been reported for detection of Ascorbic acid in foods and pharmaceuticals. These include chromatographic techniques[10]-[11] ,fluorescence[12] and spectroscopy[13]-[14]. However, most of the above methods require time consuming, manipulation steps, low-sensitivity sophisticated instruments and special training.[15]Since AA is an electroactive species, electroanalytical methods are the possible alternative for electroanalysis of AA. As it is well known, electrochemical methods are usually described by inexpensiveness, possess better sensitivity and selectivity and fast analysis.[16]

Electrochemical techniques are known to offer some benefits such as fast analysis, low in cost, higher sensitivity and accuracy. However, the major problem frequently encountered in the electroanalysis of AA is the effect of interferents caused by the substances with similar redox potentials at conventional electrodes which results in poor selectivity. In the presence of coexisting oxidizable species, the level of the vitamin C determined could be overestimated.[17] Thus, it is difficult to detect specifically one substance in the presence of others substances in real biological samples at conventional electrodes. AA exists in anionic form at the physiological pH. Based on this property; different techniques were developed to detect AA selectively. Modification of the working electrode with modifiers like tetrabromo-**p**benzoquinone[18] electronically conductive anion exchange polymers based on polypyrrole[19] and polyaniline[20] showed promising applications in the fabrication of sensors for sensitive and selective detection of AA. The most distinguishing feature of chemically modified electrodes is their modification by a selected substance that is coated onto the electrode surface which provides the electrode certain desirable properties. The use of nanomaterials for nano structuring of electrode surface has aroused the interest of analysts' [21] because nanostructured materials can be tailored to improve selectivity and sensitivity of the sensors. Further investigation on these new materials to fabricate chemically modified electrodes is needed to exploit the system for improvement of selectivity and sensitivity of sensors.

In this work, the working electrode from carbon paste electrode was modified by anthraquinone. We have demonstrated the improvement in selectivity and sensitivity for determination of AA using the electrode

surface modification method we have developed.[22]Anthraquinone have been found important modifying agent for the preparation of modified carbon paste electrodes.[23] Therefore, in this work, we demonstrated electroanalytical technique using AQCPE for electroanalysis of AA. The electrochemical determination of AA is possible due to its electro-reduction at the electrode. In general, it is believed that electro-reduction of AA is a reversible process involving two electrons and two protons (Figure 2).[16]

1.2 Statement of the problems

Drug analysis is an important branch of chemistry and plays an important role in drug quality control. The main aim of the pharmaceutical drugs is to serve the human to make them free from potential illness or prevention. Previously, analytical methods have been developed using different types of modified electrodes to reduce the over potential for the catalytic electro-oxidation of active compounds. To overcome the problems, the study was conducted by introducing a simple electrochemically activated carbon paste electrode without employing any expensive modifier. Hence, this study was focused on the development of alternative analytical method for the determination/analysis of ascorbic acid using carbon paste electrode modified with anthraquinone.

Considering the following basic questions to be answered:

- Does AQMCPE has better sensitivity for determination of ascorbic acid?
- What is the selectivity of the method for the target analyte?
- Is the electrode stable for determination of ascorbic acid?

1.3 OBJECTIVES

1.3.1 General Objective

To develop sensitive electroanalytical method for the determination of ascorbic acid using anthraquinone modified carbon paste electrode.

1.3.2 Specific Objective

- To prepare AQMCPE for the determination of ascorbic acid
- To investigate the electrochemical behavior of ascorbic acid using AQMCPE
- To optimize electrochemical parameters during determination of ascorbic acid
- To validate the developed method for determination of AA in real samples

1.4. Significance of the Study

The developed method is vital to determine pharmaceutical dosage forms. The study provided new information to quantify and qualify pharmaceutical drugs on health care. The result of the study gives information about how the selected technique is less toxic, cheap, ecofriendly but equally sensitive electrochemical method on quantitative determination of AA for regular quality control purpose in laboratories. Additionally, the result of the study provided adequate information those who are interesting to work on similar work with different pharmaceutical drug formulations. Therefore, developing less time-consuming, more-sensitive, more-selective and less expensive electro-analytical method is recently very important

- To assess the quality determination method of AA.
- It serves as baseline information recommend and as reference materials for those who want to study about AA.
- To provide background information about modification of CPE by anthraquinone and determination methods used for analysis of AA.

2. LITERATURE REVIEW

2.1 Ascorbic acid

Ascorbic Acid (AA) is a soluble vitamin present in many biological systems and in multi vitamin preparations which are commonly used to supplement inadequate dietary intake and as anti-oxidants.[24] It is important in forming collagen, a protein that gives structure to bones, cartilages, muscles, and blood vessels. Vitamin C also aids in the absorption of iron, and helps maintain capillaries, bones, and teeth. It is the most common electroactive biological compound and one of the most ubiquitous vitamins ever discovered.[16]Ascorbic acid plays a paramount role as an antioxidant and free radical scavenger and hence is a vital component in human diet with the highest concentrations in animal organs like liver, leukocytes, and anterior pituitary. It is widely used in the treatment of certain diseases including scurvy, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility.[25]AA in biological fluids can be used to access the amount of oxidation stress in human metabolism and excessive oxidative stress has been linked to cancer, diabetes and hepatic disease.[26]-[27]Ascorbic acid (AA) known for its reductive properties is used as an antioxidant agent in foods and drinks, for therapeutic purposes and biological metabolism. The human body cannot produce ascorbic acid, and so it must be obtained entirely through one's diet. Therefore, humans depend on exogenous sources of the vitamin which include fruits and vegetables as well as food supplements and pharmaceutical preparations. The physicochemical and biochemical actions of vitamin C (Figure 2) are accounted for its action as an electron donor.[28]

2.2 Mechanism of electrochemical oxidation of AA

Ascorbic acid is a water-soluble compound consisting of two inter-convertible compounds: L ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L- dehydroascorbic acid. It is an excellent source of electrons and donates electrons to free radicals such as hydroxyl and super oxide radicals and quenches their activity.[29] There have been difficulties in quantifying ascorbic acid due to its instability in aqueous solution or due to its oxidation to dehydroascorbic acid, which is a reversible reaction.[30] The oxidation of AA has been widely agreed to follow the electrochemical method mechanism and the process involves the loss of two electrons at pH (1 -

5), one proton at pH > 5 (figure 2),[31] and the final species is electro inactive, which explains the absence of peak at the reverse scan .[32]

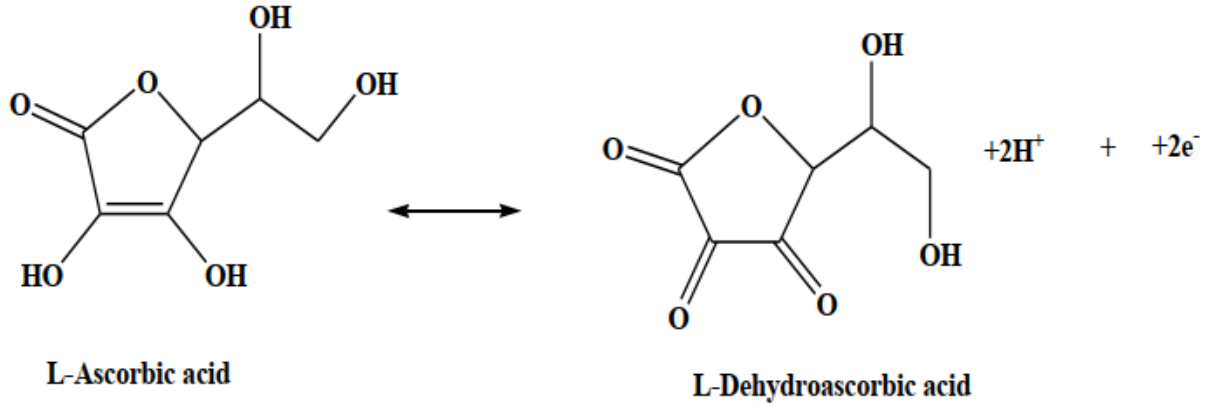

Figure 2. Electrochemical process for the oxidation of ascorbic acid.

2.3 Physicochemical properties of AA

The physicochemical properties of ascorbic acid ($C_6H_8O_6$) are related to its structure (figure 2). The stability of AA decreases with increased temperature, sun exposure and pH. The appearance of $C_6H_8O_6$ is white, odorless, crystalline solid with sharp acidic state.[33] It contains several structural elements that contribute to their chemical behavior: the structure of the lactones and two enolic hydroxyl groups, a primary and secondary alcohol group. Enediol structure motivates their antioxidant properties, as can be oxidized easily enediols to diketones. AA is rapidly interconverting in to unstable diketone tautomer's by proton transfer, though it is most stable in the enol form. The proton of the enol is lost, and again acquired by the electrons from the double bond to produce a diketone.[34]

2.4 Theoretical background electrochemical techniques

Electrochemistry, as the name suggests, studies the relationship between electrical and chemical occurrences. It covers mainly two areas: electrolysis-conversion of chemical compounds by passage of an electric current and electrochemical power sources-energy of chemical reactions transformed into electricity. Electrochemistry is one effective technique to study electron transfer properties. Electrochemical techniques are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as large linear dynamic range, with relatively low-cost instrumentation.[35] The application of electrochemical techniques in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. The renewed interest in electrochemical techniques can be attributed in part to more sophisticated instrumentation and to increase the understanding of the technique themselves.[36] The techniques have shown remarkable advantages in the analysis of drugs in pharmaceutical preparations, and can easily solve many problems of pharmaceutical interest. The most useful electroanalytical techniques are based on the concept of continuously changing the applied potentials to the electrode solution interface and the resulting measured current.[37]

2.4.1 Voltammetric techniques

Voltammetry is an electro-analytical technique conducted by measuring the current flowing through an electrode dipped in solution containing electro-active compounds while a potential is imposed upon it. The common characteristic of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (I) flowing through the electrochemical cell. The electrochemical cell, where the voltametric experiment is carried out, consists of a working (indicator) electrode, a reference electrode, and a counter (auxiliary) electrode.[38] The voltammetric techniques have been applied for the determination of pharmaceutically electroactive compounds in dosage forms (tablets, capsules, injections and suspension) and biological samples (real and spiked urine samples, blood and serum). The short analysis time in these methods makes it very attractive for routine determination of the analytes in different samples. The confirmed analytical advantages of the various voltametric techniques are: enhanced sensitivity, a broad concentration range (10^{-12} to 10^{-1} M), numerous applicable solvents and electrolytes, wide working temperature ranges, fast analysis, simultaneous determination of analytes (organic and inorganic), the capacity to assess kinetic and mechanistic parameters (including reasonable estimation of unknown ones), the facility of different potential waveforms generation yielding low current intensity values, these features being sustained by a strongly developed theoretical background. Electrocatalytic processes in Electroanalytical chemistry play crucial role in a number of contemporary technologies, facing the scientific and engineering community with the necessity of having powerful and versatile techniques for the investigation of these processes. In this sense, voltammetric techniques present irreplaceable tools due to their fastness and ability to provide a vast amount of important thermodynamics and kinetics information. [39] Electrochemical sensors are broad aspects of physical and analytical chemistry, material science biochemistry, solid-state physics, device fabrication, electrical engineering, and even statistical analysis. A typical chemical sensor is a device that transforms chemical information in selective and reversible way, ranging from the concentration of specific sample component to composition analysis, into an analytically useful signal. This work, focus on electrochemical sensors from analytical perspective. A huge research effort has taken place over years to achieve electrochemical sensors with attractive qualities including rapid response, low cost, miniaturisable, superior sensitivity and selectivity, appropriate detection limits.[31-33]

In addition, the analytical advantages of the various voltammetric techniques include excellent sensitivity, a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times, simultaneous determination of several analytes, the ability to determine kinetic and mechanistic parameters, a well-developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential waveforms can be generated and small currents recorded.[35]

2.5 The Electrochemical Cell

The electrochemical cell, where the voltammetric experiment is carried out, consists of a three-electrode system is used in voltammetry, which includes a working electrode, at which the oxidation or reduction process of interest occurs, a reference electrode, saturated silver-silver chloride electrode (Ag/AgCl) and an auxiliary or counter electrode, which carries the bulk of the current (instead of reference electrode). Voltammetry functions by measurement of a current response (I) at a working electrode as a function of the applied potential (E) with respect to a reference electrode by means of a potentiostat. When a potential is applied between the working and reference electrodes, the current that is produced passes between the working electrode and a third auxiliary/counter electrode. Platinum wire is often used as the material for the auxiliary electrode**.** In general, an electrode provides the interface across which a charge can be transferred or its effects felt. Because the working electrode is where the reaction or transfer of interest is taking place, whenever we refer to the electrode, we always mean the working electrode. The reduction or oxidation of a substance at the surface of a working electrode, at the appropriate applied potential, results in the mass transport of new material to the electrode surface and the generation of a current. Even though the various types of voltammetric techniques may appear to be very different at first glance, their fundamental principles and applications derive from the same electrochemical theory.[34-35] A schematic representation of a typical electrochemical cell set up is given in Figure 3

2.5.1 The Working Electrode

The working electrode is at which the investigation process occurs. The working electrode can be bare or modified. Usually in the range of positive potential, platinum, gold, and carbon (graphite, glassy carbon) electrodes are used. The working electrode can be referred to as either cathodic or anodic.[35]

2.5.2 The Function of the Working Electrode

A fixed potential difference is applied between the working electrode and the reference electrode. This potential drives the electrochemical reaction at the working electrode's surface. The current produced from the electrochemical reaction at the working electrode is balanced by a current flowing in the opposite direction at the counter electrode. The reference electrode acts as a reference point for the redox couple. The current resulting from the electrochemical reaction is amplified and, when plotted as a function of time, appears as a peak on the recording device .[40]

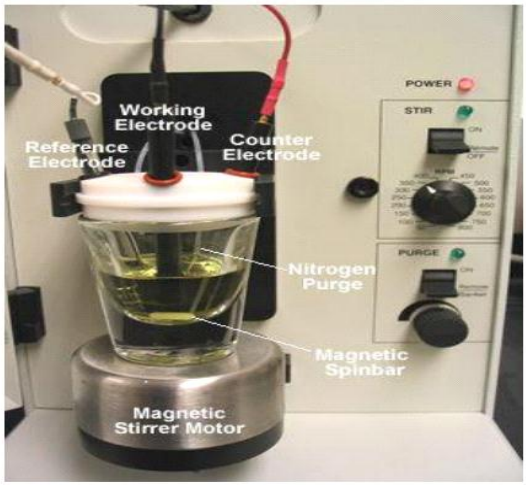

Figure 3: Typical electrochemical cell composed of three electrode systems for voltammetry

2.5.3. Cyclic voltammetry

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions but is rarely used for quantitative determinations, and it is widely used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics processes and the kinetics of heterogeneous electron transfer reactions and also on coupled chemical reactions or adsorption processes.[41] It is often the first experimental technique to be performed. In particular, it offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media on the redox process.[42] Cyclic voltammetry is an electrochemical method carried out by scanning the potential at a controlled rate and measuring the current during oxidations or reductions.[43] The potential is altered linearly with time, in a system composed of three electrodes: a counter, working and reference electrode. The important parameters obtained from a voltammogram are *I*a (anodic peak current), *I*c (cathodic peak current), *E*a (anodic peak potential), and *E*c (cathodic peak potential). According to the Randles-Sevcik equation, the peak current, *I*, for an electrochemically reversible system, is described as follows:

$I_P = (2.69\times10^5)n^{3/2}\,ACD^{1/2}\,V^{1/2}$

Where *I* is the peak current in Amperes, *n* is the electron stoichiometry in equivalents per mole, *A* is the electrode area in square centimeters, *D* is the diffusion coefficient in square centimeters per second, *C* is the concentration in moles per cubic centimeter and *v* is the Potential scan rate in volts per second.[44]

2.5.4 Chemically Modified Electrodes

Chemically modified electrodes are different from other types of sensors as they are a molecular monolayer or micrometer-thick layers or films made from a certain chemical depending on the function of the electrode. The thin film is coated on the surface of the electrode (herein on a carbon paste electrode). The outcome would be a modified electrode with special new properties in terms of physical, chemical, electrochemical, optical, electrical, (electron transport) and other useful properties.[45] Chemically modified electrodes depend on electron transport that is a general

term for electrochemical process where the charge transports through the chemical film to the electrode. The term coverage is used to express the area- normalized in mol/m_2 of a specific type of chemical site in the thin chemical film on the surface of chemically modified electrode.[46]

2.5.5 Purpose of Developing Chemically Modified Electrodes

Advancement in the field of electrochemical sensors kept getting more thorough until chemists in this field found no use of bare surface to continue their investigations. The reason behind that is researchers that involved electrodes required certain chemical and physical properties that did not naturally exist in the materials used as electrical conductors (e.g., bare carbon paste electrode). To solve their problem, they used chemical modification to tailor the materials they used. Atoms, molecules, and nano particles are attached to the surface of materials to modify their electronic and structural properties, leading to changing their functionality .[47]

2.6. Carbon Electrodes

Solid electrodes based on carbon are currently in widespread use in electroanalysis primarily because of their broad potential window, low background current, low cost, chemical inertness, and suitability for various sensing detection applications. In contrast electron transfer rates observed at carbon surfaces are often slower than observed at metal electrodes. A variety of electrode pretreatment procedures have been proposed to increase the electron transfer rates. The type of carbon, as well as the pretreatment method, thus has a profound effect on the analytical performance. The most popular carbon electrode materials are those involving glassy carbon, carbon paste, carbon fiber, screen printed carbon strips, carbon films, or other carbon composites.[48]

2.6.1 Carbon paste electrodes (CPE)

Carbon paste electrodes (CPEs) are among the most popular types of carbon electrodes which have been widely used in electro analysis, mainly due to such interesting properties as chemical inertness, low cost, wide potential window and suitable for a variety of sensing and detection app -lication. The operational mechanism of the carbon paste electrodes depends on the properties of the modifier materials used to import selectivity towards the target species. Modified carbon elec -trodes have been widely used as sensitive and selective sensors in various electro analytical

methods.[49] Carbon paste electrodes which are well suited for the determinations of easily anodically oxidizable substances have been successfully used for the determination of related APAH. Analytical methods employing carbon paste electrodes are quite sensitive.[50] Carbon paste electrodes have demonstrated their potential for use as electrochemical sensors. In addition to these advantages, the sensitivity of the prepared carbon paste electrodes can be increased relatively easily with variety of modified.[47-51]

2.6.2 Modified Carbon Paste Electrode

Chemically modified electrodes (CMEs) represent a modern approach to electrode system. These electrodes rely on the placement of reagents on to the surface to impart the behavior of that reagent to the modified surface. Such deliberate alteration of electrode surfaces can meet the needs of many electroanalytical problems, and may form the basis for new analytical applications and different sensing devices. The immobilization of electrocatalysts has also been done by incorporating an electroactive substance in the electrode matrix and carbon paste electrode spiked with catalysts may be suitable. The construction of electrodes by incorporating an electroactive substance in to a carbon paste matrix was first reported by Kuwana in 1964 and has been extensively applied till now.[52] The development and application of chemically modified CPEs electrodes have received considerable the following processes in Electroanalytical chemistry: Providing selectivity of electrodes, Resist fouling Concentrating species, improving electrocatalytic properties, Limiting of interference in samples and easily manufacture, renewable surfaces, accelerating electron transfer reaction, preferential accumulation or selective membrane permeation thus, imply higher selectivity, sensitivity, or stability on electrochemical reactions.[53-54]

3. METHODS AND MATERIALS

3.1 Chemicals

Ascorbic acid (Sigma Aldrich), anthraquinone (Meric), paraffin oil (Fulka, Switzerland), graphite powder (BDH, UK), K_2HPO_4, KH_2PO_4(Techno Pharmachem), HCl, 37% (Riedel de Haen), KOH (LeSOL laboratory reagent), AA tablet (Addis Pharm. Co., Ltd) and distilled water was used to prepare all aqueous electrolyte solutions throughout the study.

3.2 Instruments

The electrochemical measurement was performed by using Epsilon EC-Ver 1.40.67 voltammetric analyzer (Bioanalytical System, USA), using a standard cell with three electrodes. The three-electrode system consists of UMCPE or Anthraquinone MCPE which was used as working electrode, Ag/AgCl as a reference electrode and a platinum wire as a counter electrode.

3.3 Procedure for Preparation of Solution

3.3.1 Buffer solution

Phosphate buffer solution (PBS) buffer was prepared by dissolving 0.1M K_2HP0_4, and 0.1M KH_2PO_4 in distilled water and adjusting to the required pH value with dilute hydrochloric acid solution 0.1M HCl and 0.1 M KOH.

3.3.2 Standard preparation

Ascorbic acid (AA) was prepared by dissolving 0.1762 g of the component into 100mL of distilled water. Working solutions of AA standards were prepared daily by diluting the stock solutions in supporting electrolyte to the required concentrations

3.4 Preparation of Electrodes

3.4.1 Preparation of UCPE

To prepare carbon paste electrode for this experiment 70% (w/w) of graphite powder and 30% (w/w) of paraffin oil were mixed homogeneously for 20 min.[9-25] with a mortar and pestle. The homogenized mixture was kept in the refrigerator for 24 hrs.' and then the paste was housed in a tip of an insulin syringe by introducing a conducting copper wire

that extends between the tip and the back of the syringe. Finally, the electrode surface was flattened and smoothed against a clean smooth white paper until a polished shiny surface was appeared.

3.4.2 Preparation of anthraquinone Modified CPE

Anthraquinone modified CPE was prepared by the following variable compositions of graphite powder, anthraquinone, and paraffin oil providing homogeneous mixture for 20 min. For the preparation of AQMCPE, 5% (w/w) anthraquinone; 70% (w/w) graphite powder, and 25% (w/w) paraffin was used for 10%(w/w) anthraquinone, 65%(w/w) graphite powder, and 25%(w/w) paraffin liquid was used. Similarly, For 15%(w/w) anthraquinone; 60% (w /w) graphite powders, and 25%(w/w) paraffin liquid mixed. For 20%(w/w) anthraquinone; 60% (w/w) graphite powder, and 20%(w/w) paraffin mixed. After the modified paste was prepared, it was kept for 24 hrs.' in a refrigerator. Then, the electrode surface was flattened and smoothed against a

clean smooth white paper until a polished shiny surface was appeared before electrochemical measurements

3.5 Procedure of Reproducibility, Repeatability and Stability Study of AQCPE

To study the reproducibility of the Anthraquinone modified CPE, was examine by measuring the same concentration 0.1mM AA on three modified electrodes in three successive days (three measurements on each day) with triplicate measurement in each day. The relative standard deviation (RSD) of the measured current signal was calculated to determine reproducibility of the modification strategy among the electrodes.

For the repeatability study, the AQMCPE was prepared and the concentration of AA was measured seven times in one day at AQMCPE. Then, the %RSD of the readings was calculated to evaluate the repeatability of the measurements.

To examine the stability of the modified electrode, three AQMCPEs were prepared on the same day and the current response of AA was recorded on each weak for three months. On each trial, triplicate measurements were taken and the average current signals was compared to those of the last day to evaluate the stability of AQMCPE

3.6 Interference Study

For the applicability of the anthraquinone for the detection of AA was evaluated by studying the selectivity of the method for the determination of AA. Various possible interfering species such as, 1 mM dopamine (DA), uric acid (UA), tartaric acid (TA), starch (STA) and glucose (GL), were added into the solution containing 0.1 mM of AA responses were recorded. Then the percent change in the oxidation current of AA was calculated up on addition of these interfering substances.

3.7 Procedures of Real Sample Preparation

Ascorbic acid (AA) tablets were purchased from local drug stores, from Jimma and it was weighed and powdered in a mortar. A 0.18 g of the powdered tablet was dissolved in to 100 mL volumetric flask and diluted with PBS buffer. It was labeled that one multivitamin tablet contains 10 mg of AA. Therefore, the concentration corresponding to 10 mg of AA in the tablet in 100 mL is 0.266 mM. The diluted solution was directly analyzed by proposed method. Finally, 100 µL of tablet sample solutions was diluted in 10 mL PBS buffer. Triplicate analysis using CV was measured and the mean values were reported. Also, three different solutions of AA solutions were prepared by mixing 100 µL of tablet sample solution with 0.1µM, 0.3 µM and 0.6 µM standards of AA and the %recovery was calculated. The determination of AA in the tablet was taking place using the linear regression equation obtained for the calibration curve.

4. RESULTS AND DISCUSSION

4.1 Electrochemical behavior of AA

The electrochemical behavior of ascorbic acid was studied using cyclic voltammetry (CV) in 0.1 M phosphate buffer at scan rate 100 mV/s. Fig.4 showed the cyclic voltammograms in the potential range from -0.3 to 0.4 V of bare carbon paste electrode with standard ascorbic acid (curve a) and modified CPE with standard ascorbic acid (curve b). The response in the cyclic voltammograms during the experiment revealed that in case of unmodified carbon paste electrode, the oxidation peak was not observed during the anodic sweep. In the reverse scan, the reduction potential peak was also not observed. The peak potential separation, at the unmodified carbon paste electrode, indicating that the electron transfer reaction is slow. Under similar conditions, when anthraquinone modified CPE was used the response potential for of ascorbic acid redox system showed different behavior: The oxidation peak potential 177 mV while the reduction peak potential shifted negatively to 75 mV, which gives separation in potential of 102 mV at the anthraquinone modified carbon paste electrode, suggesting the electrochemical behavior of ascorbic acid with slight reversibility than before, as one utilizes anthraquinone as modifier. This decrease in separation potential using AQMCPE showed the catalytic activity of anthraquinone for ascorbic acid detection and indicated quasi-reversible oxidation reaction. Oxidation mechanism of ascorbic acid, which includes transfer of two electrons and 2 H+ ions, is shown in Fig. 2.

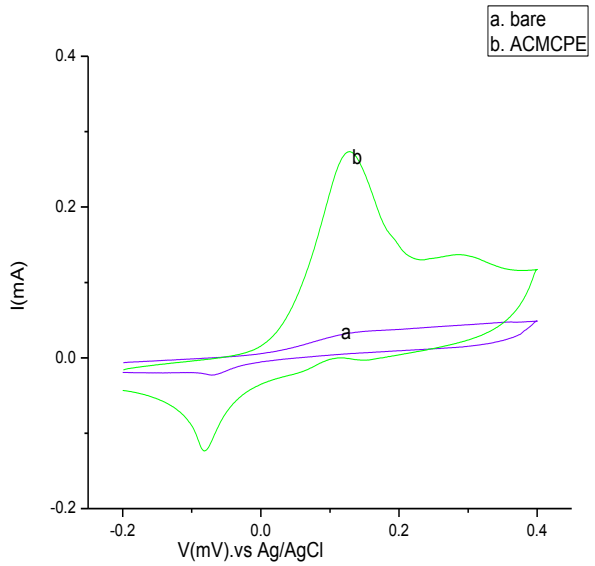

Figure 4. Cyclic voltammogram of a) 0.1mM AA (pH 4.5) UMCPE and b) 0.1mM AA (pH 4.5) ATMCPE, scan rate 100 mVs-1

4.2 Optimization of Experimental Parameters

To obtain the best experimental conditions, some parameters were optimized for CV anthraquinone MCPE

4.2.1 Effects of pH

The change in the value of pH plays important role in the mechanics of the oxidation and reduction reactions of most compounds. Consequently, the effect of pH on the electrochemical oxidation of AA at AQMCPE was studied. Cyclic voltammetry was carried out to examine the effects of pH (PBS, pH 3.0 to 8) on voltammetric determination of 0.1 mM AA at 100 mV/s. The pH value of the solution has a significant influence on the peak current and peak potential because of the involvement of protons in the overall electrode reaction. The effects of pH of the buffer solution on the voltametric behavior of is containing 0.1 mmol L^{-1} of AA was investigated in a wide pH range (3.0–8.0). As shown, the peak potentials shifted towards negative values as the pH increased due to proton involved in electrochemical reaction of AA. In addition, the

oxidation peak current of AA decreased as the pH of the solution changes from 3.0.0 to 8.0. The deviation at higher pH, indicating that deprotonation or no longer an equal number of protons and electrons process and, suggesting that oxidation reactions of AA are kinetically less favorable at higher pH. Furthermore, the decreasing of the peak currents at high solution pH could be due to the electrostatic repulsion between AA and the activated surface.

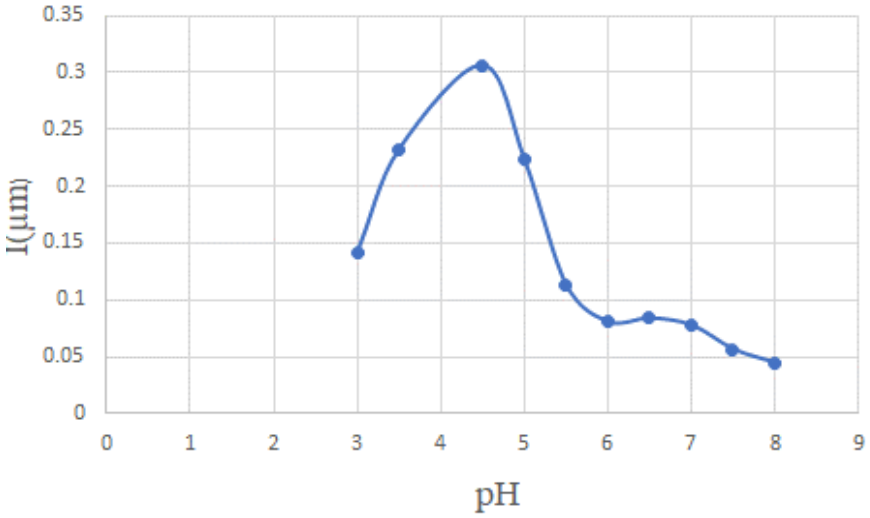

Figure 5. The relationship between the redox peak current of AA and buffer pH values

4.2.2 Effect of Scan Rate

The effect of varying scan rate in the oxidation process of AA was studied. Cyclic voltammograms of 0.1 mM AA in 0.1 M KH_2PO_4 supporting electrode using an anthraquinone modified electrode was obtained for the scan rate from 20-300 mV/s (Fig.6). It was noted from Figure 6 that, with an increasing scan rate, the anodic current peak for the electro-oxidation of AA increased due to more molecules of AA are oxidized, and it is potential shifts to more positive values. The observed increased in the oxidative current of AA is due to heterogeneous kinetics. The obtained slope (0.0412) is in a good agreement with the theoretical slope for a diffusion-controlled process. Good linearity between the anodic peak current and the square root of scan rate.

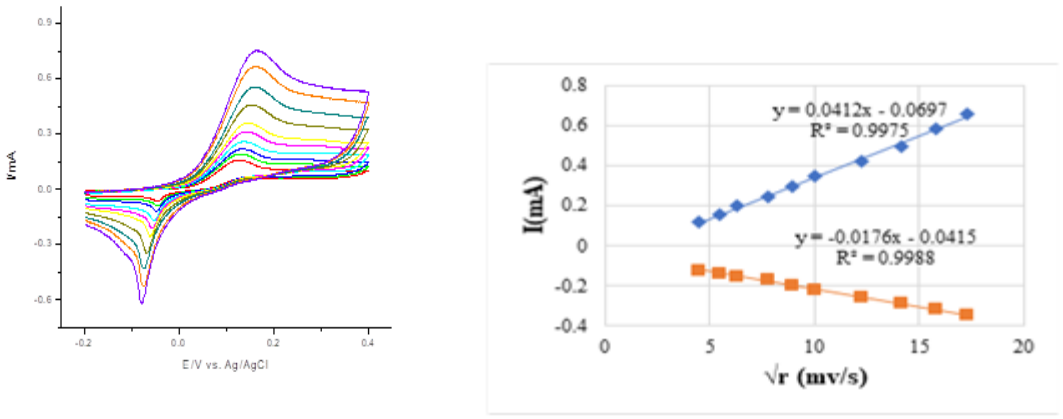

Figure 6. CV voltammograms of 0.1mM AA at ATMCPEs in PBS buffer at pH 4.5 at different scan rates, from 20-300 mVs-1 (Inset: The plot of Ipa and Ipc *vs.* υ1/2)

4.2.3 Effect of modifier composition

The chemistry of anthraquinone has received much attention because of its relevance to some important technological process. Anthraquinone and anthraquinone derivatives have been used in analytical chemistry, mainly as chelating agents and chromophores. They also display interesting electrochemical behavior because of their quinoid structure. Anthraquinone as a modifier considerably enhances the oxidation signal of ascorbic acid although its electric conductivity is poor since it is an organic compound. The paste composition strongly affects the electrode reactivity, with the increase in pasting liquid content decreasing the electron transfer rates Fig. 7 explains the oxidation peak current as function of modifier composition (amount of

anthraquinone (% w/w)). When the content of anthraquinone was increased from 0% to 15%(w/w), with the selected data sets (points) showed an increment. Here, the response did not appear robust so additional data sets should be considered, i.e., points in between 10% and 15%. Whereas with further increasing the modifier amount from 15% to 20 %(w/w), the oxidation peak current decreased. Hence 15% was selected as a working condition for the experiment.

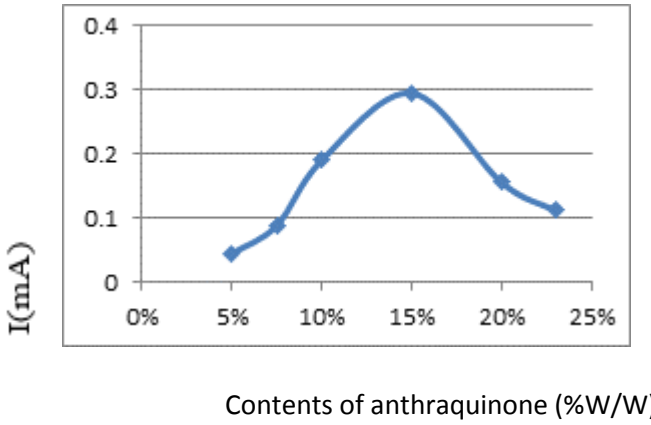

Figure 7. The effect of amount of anthraquinone (modifier) on the oxidation peak current of 0.1mM AA scan rate at 100mv/s.

4. 3 Calibration Plot of Ascorbic Acid

Concentration of AA was determined using Anthraquinone modified electrode as shown in Figure 8. The anodic current increased with an increase concentration of AA from 0.005 mM to 0.1 mM. The calibration graph of AA in different concentrations was obtained by using an AQMCPE as in Figure 8. A linear response was achieved in the concentration range of 0.005 to 0.1 mM, with a sensitivity response obtained from the linear with R^2 = 0.9982 with the regression equation of Y = A + B*C, Where A = -0.0777 B=0.005 equation slope. In analytical practice, calibration graphs frequently give numerical *R*-values greater than 0.99, and *R*-values less than about 0.90 are relatively uncommon. The detection limit for ascorbic acid, considering signal-to-noise ratio of three was found to be 1.63×10^{-6} M.

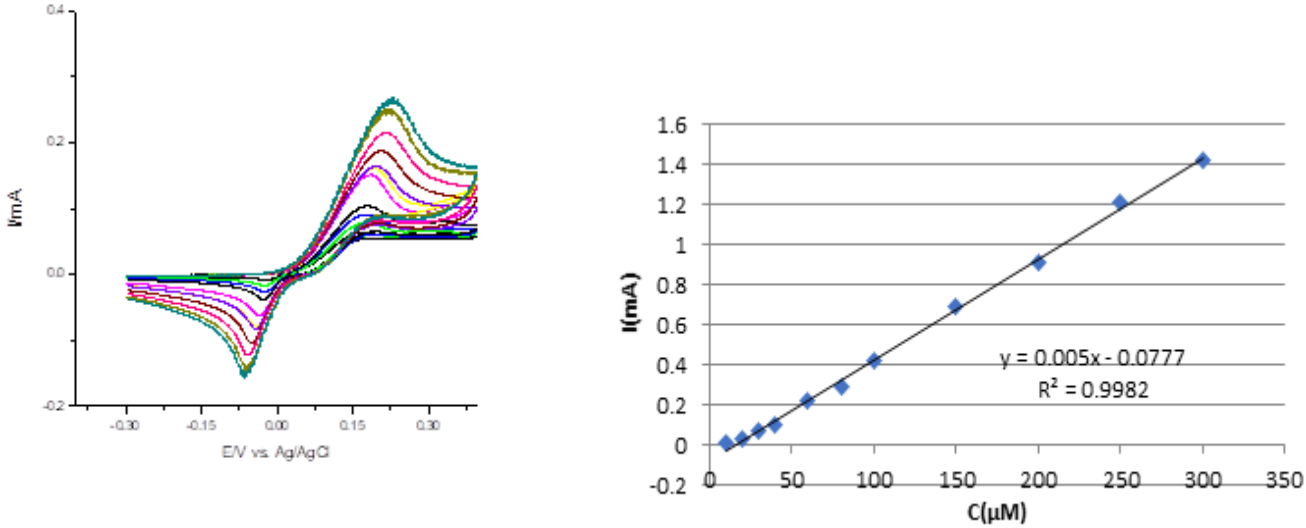

Figure 8. Cyclic voltammetry voltammograms for anthraquinone modified electrode containing different concentration of ascorbic acid scan rate 100mv/s

The limit of detection (LOD) which is three times the standard deviation of the blank divided by the slope of the curve and limit of quantification (LOQ), which refers to ten times the standard deviation of the blank divided by the slope of the curve, were calculated to be 1.63 and 5.44 µM, respectively. These findings thus clearly confirm the reliability of the developed electroanalytical method for determination of the target analyte in the environmental samples.

4.4 Reproducibility, Repeatability and Stability of modified carbon paste electrode

In this experiment, to characterize the repeatability of anthraquinone modified CPE, was obtained by estimating the percent relative standard deviation (%RSD) of triplicate determination of a solution of 0.1mM of AA. The %RSD were obtained to be 3.6%which revealed an excellent repeatability.

Reproducibility was investigated by considering three modified electrodes prepared independently by taking triplicate measurements using the three electrodes. The reproducibility expressed in relative standard deviation was found to be 3.85 % for 0.1mM of AA solution showing excellent reproducibility of the method.

The stability of modified electrode was studied by measuring the current response of 0.1mM AA anthraquinone MCPE after the electrode kept at room temperature for three months. It was observed that, the current response conserved almost 98.6 % of its initial value. This result indicated that there was insignificant peak current change lower than 5%, showing that the anthraquinone modified carbon paste electrode had good stability.

4.5 Interference Study

The influence of other several compounds starch (STA) that can coexists in the pharmaceutical dosages containing vitamin C, and like glucose (GLU), dopamine (DA) and uric acid (UA) which coexists in human fluid may interfere with the determination of AA. Dopamine uric acid, tartaric acid and glucose on the electrocatalytic peak current of ascorbic acid oxidation at the surface of carbon paste electrode/ATMCPE| was examined using cyclic voltammetry. If the tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately 5% relative error, for 1×10^{-4} mol L^{-1} ascorbic acid. The experimental results showed that the presence of these compounds (except dopamine did not significantly influence the determination of ascorbic acid under the experimental conditions. Therefore, this result demonstrated the selectivity of this method for the voltammetric determination of ascorbic acid.

Table: 1 Study of effect of interferences

Interferences	Change in oxidation currents (%)
Dopamine	-16.03
Glucose	1.78
Uric acid	1.9
Tartaric acid	-1.18
Starch	-1.62

The effect of interference for the detection AA at AQCPE was evaluated by studying the selectivity using possible interfering agents. Various possible interfering species were added into the solution containing 0.1mM of AA. The result was shown in Table 1. The change in oxidation current was taken as the difference between the oxidation current of 0.1mM AA alone and the oxidation current after adding 1 mM interferences. Percentage in change in oxidation current was calculated by Eq. (1). It was observed that 1 mM dopamine (DA), uric acid (UA), glucose (GL), tartaric acid, starch has negligible interference with the determination of 0.1 mM AA.

$$\Delta i(^0/_0) = \frac{i_{AA} - iM_i x}{iAA} \times 100 \qquad (1)$$

4.6 Real Sample Analysis and Recovery Study

For practical applicability of Anthraquinone MCPE for the detection of AA, in content in pharmaceutical tablets was used. This sample was prepared as described in experimental section. Briefly, the tablet from Addis Pharm. Co., PLC. Was weighed and powdered in a mortar and pestle. 0.18 g of the powdered tablet was dissolved in to 100 mL volumetric flask and diluted with PBS buffer. It was labeled that one multivitamin tablet contains 10 mg of AA. Therefore, the concentration corresponding to 10 mg of AA in the tablet in 100 mL is 0.266 mM. The diluted solution was directly analyzed by proposed method. Finally, 100 µL of tablet sample solutions was diluted in 10 mL PBS buffer. Also, three different solutions of AA solutions were prepared by mixing 100 µL of tablet sample solution with 1µM, 3µM, 6µM standards of AA and triplicate of CV was measured and the mean values were recorded and the %recovery was calculated. The determination of AA in the tablet was take place using the regression equation of the calibration curve. The percent recovery was performed to evaluate the accuracy of the method and the results are presented in Table 3 and 4

Table 2; Amount of AA detected in the multivitamin tablet with the demonstrated method

Expected (µM)	Detected		Labeled value (mg/tablet)	% recovery
	In µM	mg/tablet		
5.2	5.125	9.86	10.0	98.55

Table 3; Recovery study of AA in real sample.

Present (µM)	Added (µM)	Expected (µM	Found (µM)	%Recovery	%RSD
5.2	1	6.2	6.4	103.84	4.46
	3	8.2	8.26	101.15	4.91
	6	11.2	11.16	99.23	5.18

The developed electroanalytical method for the determination of ascorbic acid (AA) in tablet samples by using anthraquinone modified CPE was comparable with the values reported in literature as shown in (Table 4). The proposed method has shown moderate performance. A limit of detection of 1.63 μM (3σ/m), for the linear ranges (σ is the standard deviation of the lowest concentration and m is the slop of the calibration curve). The analytical performance of the demonstrated electrode was compared with previously reported sensors and results are shown in Table 4. It can be realized from the Table that the proposed electrode herein is comparable in terms of linear range, sensitivity and reproducibility to those attained with other electrodes used for the detection of AA

Table 4: Comparison between the developed method and other reported methods.

Electrode	Method	Linear Range (M)	LOD (M)	Ref.
Pt.	DPV	3.1×10^{-4}- 2×10^{-2}	8.7×10^{-5}	[48]
Bi_2O_3 /GCE	CV	5×10^{-4} - 5×10^{-3}	8.1×10^{-6}	[56]
MWCNT/TTAB/GCE	DPV	5×10^{-7}-1.7×10^{-4}	$1.1 * 10^{-7}$	[46]
CPE	SWV	1×10^{-5} - 6×10^{-4}	1.76×10^{-6}	[29]
Silica gel MWCNTs/CPE	SWV	$5 \times 10^{-8} – 4 \times 10^{-6}$	1.2×10^{-8}	[47]
Anthraquinone MCPE	CV	5×10^{-6} - 1×10^{-4}	1.63×10^{-6}	This work

5. Conclusion and Recommendation

5,1. Conclusion

The present work clearly represents a CPE modified with Anthraquinone to determine AA in PBS using CV. The peak current of AA was increased with increase in scan rate and concentration. The effect of pH at the modified electrode shows the high peak current of AA oxidation at pH 4.5. Relatively an excellent sensitivity and good selectivity were achieved by the anthraquinone modified CPE towards AA. The ease of preparation of the electrode in combination with the relatively low detection limit, better selectivity and sensitivity, very good recoveries illustrated the potential applicability of the developed method as an alternative method for the determination of Ascorbic acid in real samples from pharmaceutical formulations

5.2. Recommendation

Based on the findings of this study, the sensitivity and the analytical performance of the developed method for the determination of ascorbic acid was successfully addressed. However, the selectivity of the method was not studied due to the lack of standard chemicals required for the interference study. So, to determine the level of ascorbic acid in environmental samples other researchers can apply the developed method for further investigation.

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