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Research Article

A comparative study on electrochemical determination of vitamin C in liver and tomato using platinum and glassy carbon electrodes

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Abstract

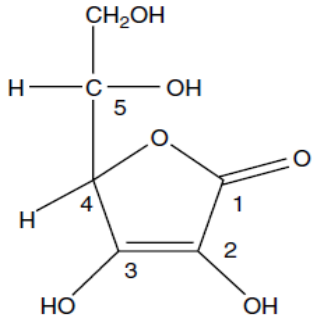
Ascorbic acid, a water-soluble vitamin, is the most common electroactive biological compound found in most biological species. The electrochemical oxidation of vitamin C was investigated at GCE and Pt electrodes in various aqueous solutions in the pH range of 1 to 5 (0.1 M KCl as a supporting electrolyte) by CV and DPV. Experimental conditions, for CV :Scan rate of 50 mV/s, Initial potential -100 mV and Final Potential 1000 mV, for DPV: Scan Rate 50mV/s, Pulse amplitude 50mV,Pulse period 125 ms, Initial potential -100mV and Final Potential 1000 mV. For cyclic voltammetry, Regression equation of y=23.4611X + 13.2489 for GCE and y=5.19714X + 13.7071 Pt; LOD of 0.0035294 mM for GCE and 0.0176 mM for Pt; LOQ of 0.025519 mM for GCE and 0.085066 mM Pt; R.S.D of %2.76% for GCE and 4.42% for Pt. And for DPV Regression equation, y = 1.201X + 0.530393 for GCE and y = 0.0521393X + 0.506857 for Pt, R.S.D % 0.391% for GCE and 4.969% for Pt ,LOD 0.12412 mM for GCE and 0.22497 mM for Pt and LOQ 0.4137 mM for GCE and 0.7499 mM for Pt. The oxidation peak potential of ascorbic acid were 270 mV and 370 mV for GCE in CV and DPV respectively but for Pt electrode 490 mV for CV and 370 mV for DPV (versus Ag/AgCl reference electrode). The influence of the operational parameters like scan rate, pulse amplitude, pulse period, concentration and pH on the analytical signal was investigated. The method developed by standard was applied to ascorbic acid assessment in liver and tomato samples. The results of ascorbic acid assessment by DPV were compared to those obtained by CV on both GCE and Pt electrodes.

Keywords: Vitamin C, Cyclic voltammetry, Differential pulse voltammetry, Glassy carbon electrode, Platinum electrode, Liver, Tomato

INTRODUCTION

Vitamin C is a water-soluble vitamin, which means it cannot be stored and humans need a constant, daily supply of it for normal growth and development. Vitamin C's primary function is that of an antioxidant and quencher of a variety of reactive oxygen species. It is necessary for the formation of skin, scar tissue, tendons, ligaments, and blood vessels. Vitamin C is essential for the healing of wounds and for the repair and maintenance of cartilage, bones, and teeth[1,2,3].

The term 'vitamin C' refers to both ascorbic acid and dehydroascorbic acid, since the latter oxidation product is reduced back to ascorbic acid in the body[4]. Vitamin C is the generic descriptor for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The terms L-ascorbic acid and ascorbic acid are both trivial designators for the compound 2,3-didehydro-l-threo-hexano-1,4-lactone, which was formerly known as hexuronic acid. The oxidized form of this compound is called L-dehydroascorbic acid or dehydroascorbic acid[5].



L–Ascorbic acid
2-oxo-threo-hexono-1,4-lactone-2,3-enediol
Vitamin C

Figure 1. Structure of l-ascorbic acid[5]

Vitamin C plays a role as a redox cofactor and catalyst in a broad array of biochemical reactions and processes. Vitamin C is designated as ascorbic acid because of its ability to cure and prevent scurvy. Ascorbic acid comes from the Scandinavian terms, skjoerberg or skorbjugg, and from the English, scarfy or scorby[6]. Vitamin C (chemical names: ascorbic acid and ascorbate) is a six-carbon lactone which is synthesized from glucose by many animals. Vitamin C is synthesized in the liver in some mammals and in the kidney in birds and reptiles. However, several species including humans, non-human primates, guinea pigs, Indian fruit bats, and Nepalese red-vented bulbuls are unable to synthesize vitamin C. When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy. Humans and primates lack the terminal enzyme in the biosynthetic pathway of ascorbic acid, l-gulonolactone oxidase, because the gene encoding for the enzyme has undergone substantial mutation so that no protein is produced[3,7,8].

Physical properties of Vitamin C

Ascorbic acid exists as colorless, or white or almost white solid crystals but impure samples can appear yellowish. It is odorless or almost odorless. It has a pleasant, sharp acidic taste. It is freely soluble in water and sparingly soluble in ethanol. It dissolves well in water to give mildly acidic solutions. It is practically insoluble in ether and chloroform. Its salts have higher water solubility[52]. All commercial forms except fatty acid esters such as ascorbyl palmitate are insoluble in fats and oils. Ascorbic acid has pKa values of 4.2 and 11.6.Ascorbic acid has a melting temperature of 190°C-192^{o}C with decomposition[4]. Its crystalline form is monoclinic with mixture of platelets and needles[9].

Chemical Properties of vitamin C

Ascorbic acid molecule contains four hydroxyl groups and is extremely sensitive to light, heating and the action of oxidizing agents and metal ions. Vitamin c is readily oxidized, especially in aqueous solutions, by reducing with atmospheric oxygen, and behaves as a two-electron donor l-Ascorbic acid can oxidize through one- or two-electron transfers. One-electron reductions utilize the transition through the l-ascorbic acid free radical (semidehydroascorbic acid or monodehydroascorbic acid). Reducing agents and glutathione dehydrogenase convert l-dehydroascorbic acid back to l-ascorbic acid, completing the oxidation-reduction cycle. Classic free-radical termination occurs by reduction of a free radical with l-ascorbate. An electron is transferred to the free radical from ascorbate, producing an ascorbate radical, which acts as a redox agent. The ascorbate radical interacts with itself, forming a 1:1 mixture of l-ascorbic acid and dehydroascorbic acid. Two-electron reductions occur when transition metals catalyze l-ascorbic acid oxidation[4, 11].

The most important chemical property of ascorbic acid is the reversible oxidation to semidehydro-L-ascorbic acid and oxidation further to dehydro-L-ascorbic acid. In addition, the proton on oxygen-3 in figure 1 is acidic (pK_1 = 4:17), which contributes to the acidic nature of ascorbic acid. Degradation reactions of L-ascorbic acid in aqueous solutions depend on a number of factors such as pH, temperature, the presence of oxygen, or metals. Ascorbic acid is not very stable in aqueous media at room temperature[9].

In addition to redox and acid–base properties, ascorbic acid can exist as a free radical. The ascorbate radical is an important intermediate in reactions involving oxidants and ascorbic acid's antioxidant activity. The physiologically dominate ascorbic acid monoanions and dianions have pKs of 4.1 (pK_1) and 11.79 (pK_2), respectively. Rate constants

for the generation of ascorbate radicals vary considerably, for example, 104–108 s^{-1}. When ascorbate radicals are generated by oxyanions, the rate constants are on the order of 104–107 s^{-1}, when generated by halide radicals, 106–108 s^{-1}, and when generated by tocopherol and flavonoids radicals, 106–108 s^{-1} [9].

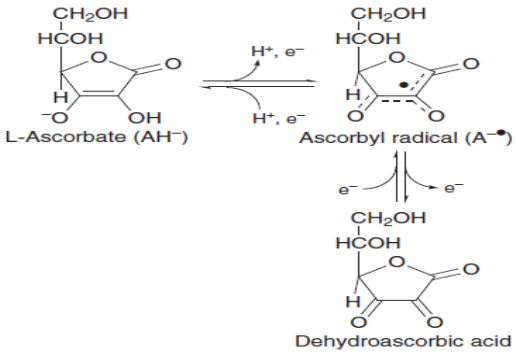


Figure 2. Oxidation of Ascorbate [5]

The spectral properties of l-ascorbic acid E1% 1 cm values for l-ascorbic acid are 695 at pH 2.0 and 940 at pH 6.0. Above pH 5.0, l-ascorbic acid exists predominantly as the monoanion and has maximal absorption at 265 nm. Undissociated, at more acid pH levels, maximal absorption occurs at 244–245 nm. Fully dissociated, above pH 12.0, maximal absorption occurs at 300 nm. l-Ascorbic acid does not fluoresce[4].

Ascorbic acid, a reductone, behaves as a vinylogous carboxylic acid wherein the electrons in the double bond, hydroxyl group lone pair, and the carbonyl double bond form a conjugated system. Because the two major resonance structures stabilize the deprotonated conjugate base of ascorbic acid, the hydroxyl group in ascorbic acid is much more acidic than typical hydroxyl groups. In other words, ascorbic acid can be considered an enol in which the deprotonated form is a stabilized enolate[8, 9].

Electrochemistry of vitamin C

Vitamin C is the only water-soluble vitamin not assayed microbiologically. Methodology has advanced from the bioassay to instrumentally advance spectrophotometric, fluorometric, electrochemical, and chemiluminescence methods. Chromatographic procedures, primarily liquid chromatography, and capillary electrophoresis provide excellent means to resolve l-ascorbic acid, l-dehydroascorbic acid, and d-isoascorbic acid. These separation techniques used with ultraviolet/visible (UV/visible), fluorescence, or electrochemical detectors provide selective and sensitive means to quantify l-ascorbic acid and its isomers from complex biological matrices. Liquid chromatography coupled to mass spectrometry (LC-MS) has been used less frequently for vitamin C analysis compared to its use in other water-soluble vitamin studies[4].

Numerous analytical techniques have been reported in the literature for the determination of vitamin C in different samples. These include titrimetric[12,13], fluorometric[14], Flow Injection Photoamperometric[15], Flow electrochemical determination[16], direct injection liquid chromatography[17], high-performance liquid chromatography[18],spectrophotometric[19,20,21,22],Photoelectrochemical[23],amperometric[24-30], Sonovoltammety[31],Electrocatalytic Determination[32-37], Electrochemical[38-43]. The vitamin C levels in some biological samples have been reported by several investigators. Although titrimetric methods are simple to use in the determination of vitamin C, difficulties are encountered with commonly used titrants and interferences often occur with colored samples. Electrochemical methods can be useful in the determination of vitamin C levels in biological samples because ascorbic acid is easily oxidized to dehydroascorbic acid. Electrochemical methods traditionally have found important applications in sample analysis and in organic and inorganic synthesis. Cyclic and differential pulse voltammetry methods are employed in the determination of ascorbic acid levels in different biological samples including fruits, honey, Juices, Tablets, cosmetics, drinks, blood plasma[44-48].

Electrochemical determination of ascorbic acid can be conducted by bare glassy carbon[49], carbon paste[10], gold or platinum[10,50] electrode or chemically modified electrode[51-54] either by cyclic voltammetry or differential pulse voltammetry[45,57].

OBJECTIVE OF THE STUDY

General objective

The general objectives of the study is to examine and compare the electrochemical oxidation of vitamin C in Liver and Tomato on GCE and Pt electrodes.

Specific objective

The specific objectives are to:

Determine vitamin C content in some selected biological samples using CV and DPV.

Observe the effect of concentration, pH, scan rate, pulse amplitude and pulse period on the electrochemical oxidation of ascorbic acid.

Compare the electrochemical results of vitamin C in liver and Tomato using GCE and Pt electrodes.

EXPERIMENTAL PART

Materials and Instrumentation

BAS 100B, electrochemical analyzer [bioanalytical systems (BAS), USA] connected to a computer was used for voltammetric measurements with three electrode system consisting of working electrode (Glassy carbon, 3 mm in diameter and Platinum, 2 mm in diameter), platinum coil wire for auxiliary electrode and Ag/AgCl reference electrode. All pH measurements were made with a pH meter model Metrohm 3305 at ambient temperature of the laboratory. Weight was measured using analytical balance (OHAUS E11140, Switzerland). Other materials include; Spoon, suction filter paper, wash bottle, Mortar with pestle, volumetric flask of different size, graduated test tube and Graduated cylinder.

Chemicals and reagents

All chemicals and reagents were of analytical grade and were purchased from, Ascorbic acid and KCl (blulux chemicals ltd., India-New Delhi), Sodium Hydroxide and hydrochloric acid (Fluka-Switzerland), distilled water. Liver sample was purchased from Eyoha Cultural Restaurant and Tomato Sauce from open market in Gondar Town.

Electrode polishing and electrode activation

Both working electrodes were polished prior to each run. Polishing involved using extra powdered alumina powder on the polishing pad and form paste using distilled water followed by a thorough rinse with distilled water. The rinsed electrodes were polished again on a polishing pad to remove any film left and again rinsed thoroughly with distilled water and placed immediately in the electrochemical cell. On the other hand platinum auxiliary electrode was immersed in concentrated hydrochloric acid solution in order to remove or dissolve any deposit formed on the counter reaction that decreases the surface area of reaction then rinsed thoroughly with distilled water before use[61].

Working Procedure

0.1 M KCl was used as supporting electrolyte which is prepared by dissolving weighted amount of KCl in distilled water. A stock solution of ascorbic acid (12 mM) was prepared by dissolving an accurate mass of the pure ascorbic acid in appropriate volume of 0.1 M KCl solution. The working solutions ranging between 2 mM to 12 mM for the voltammetric investigations were prepared by dilution of the stock solution using 0.1 M KCl supporting electrolyte solution. 0.1 M solutions of hydrochloric acid and sodium hydroxide were prepared by simple dilution process of stock solutions using distilled water, which were used for pH adjustment.

The volume the analyzed sample was 15 ml and all measurements were performed at home room temperature, using a 0.1 M KCl solution as supporting electrolyte. For cyclic voltammetry measurements the potential was scanned within the range -100 to 1000 mV, with a 50 mV/s scan rate, in operational parameter influence study scan rate varies from 25-150 mV/s for both electrodes. For differential pulse was scanned voltammetry measurements the potential was scanned within the range -100 to 1000 mV, potential scan rate of 50 mV/s, pulse amplitude of 50 mV, pulse width of 25 ms and

pulse period of 125 ms. For the investigation of the influence of the operational parameters on the electroanalytical signal, the pulse amplitude varied between 25 and 150 mV, potential scan rate of 50 mV/s and the pulse period ranged between 75 and 200 ms for both electrodes.

Choice of solvent

Since the purpose of this paper is to analyze the level of ascorbic acid in selected biological samples with electrochemical method, it is important to select the most suitable medium which dissolve samples. For electrochemical experiments, the solvent should meet the requirements as followed:

Miscibility with samples in order to get homogenous mixture.

Good dielectric constant of the solvent and solubility of supporting electrolyte in order to obtain a conductive mixture contained biological sample.

Lower toxicity for safe analytical application[68].

For ascorbic acid analysis in different samples different solvents are employed, aqueous solvent[2,8,10], Phosphate buffer[3].

Calibration graph for voltammetric determination

For both electrodes under cyclic and differential pulse voltammetry in optimum conditions a linear calibration curve for CV and DPV analysis was constructed in ascorbic acid concentrations range 2 mM-12 mM, pH = 1 to pH = 5/6 and scan rate 50-150 mV/s (CV). The purpose of calibration was to optimize the parameters.

RESULTS AND DISCUSSION

Voltammetric studies of a standard ascorbic acid at a glassy carbon and platinum working electrodes.

Cyclic voltammetric behavior of standard ascorbic acid at glassy carbon and platinum electrodes

In order to understand the electrochemical oxidation process of ascorbic acid occurring on platinum and glassy carbon electrodes; it was studied using cyclic voltammetry. Experimental results in pH optimization show that the maximum peak current was obtained in the acidic media (0.1 M KCl buffer at GCE and Pt electrode) which indicates participation of a proton transfer in the electrode process. Also, the results showed that the shape and intensity of the curves were better in O.1 M KCl buffer pH = 1 for Pt electrode and pH = 2 for GCE. Therefore, the KCl buffer at pH = 1 for Pt and pH = 2 for GCE was chosen for the analytical determination.

The voltammograms of 0.1 M KCl solution and 12 mM ascorbic acid solution at for glassy carbon (pH = 2) and platinum (pH = 1) working electrode are shown in figure 3.

In both cases no peak current were observed in the voltammograms of the supporting electrolyte in the potential range of -100 to 1000 mV but were observed for 12 mM ascorbic acid solution in the supporting electrolyte in figure 3 for both electrodes at 270 mV at GCE and 490 mV at Pt electrode. No reduction peak current was observed indicating an irreversible heterogeneous charge transfer in this system[60]. These indicate that ascorbic acid concentration can be measured quantitatively by cyclic voltammetry.

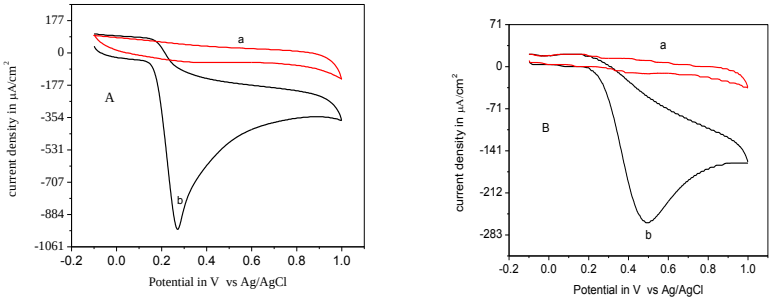


Figure 3. Cyclic voltammograms of (a) 0.1 M KCl solution and (b) 12 mM ascorbic acid for GCE (A) under optimized pH=2 and Pt working electrode (B) at optimized pH = 1.

The peak current was directly proportional the rate of electrolysis at the electrode surface. The voltammetric peaks is better defined at the GCE than at the Pt electrode. The peak potential shifts towards more positive values when electrode radius or sphericity of the electrode increases.[60]

Effect of concentration

The effect of concentration was studied using cyclic voltammetry on both working electrodes. As can be seen from Figure 4, when the concentration of ascorbic acid increases the peak current also increases successively on both glassy carbon and platinum electrodes.

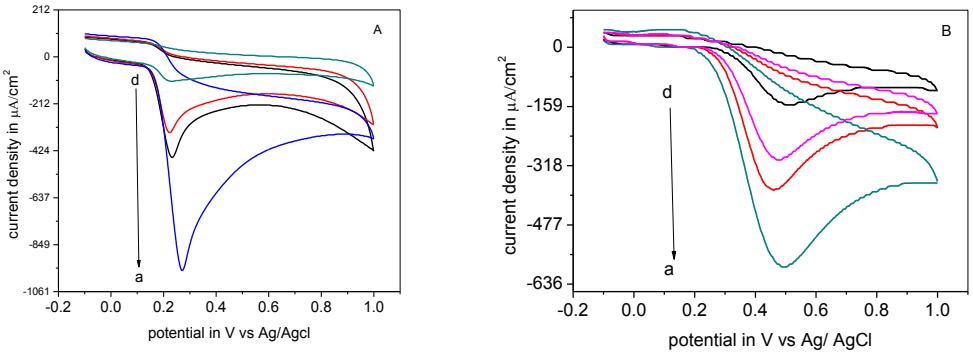


Figure 4. Cyclic voltammograms obtained from different concentrations of ascorbic acid expressed as mM: (a) 12 (b) 10 (c) 8 (d) 6, at (A) GCE (pH = 2) and (B) Pt working electrode (pH=1) in 0.1 M KCl buffer and a potential scan rate of 50 mV/s

The ratio of peak current versus concentration decreases with an increase in concentration of vitamin C which indicates the process in KCl is complicated by adsorption phenomena.[59]

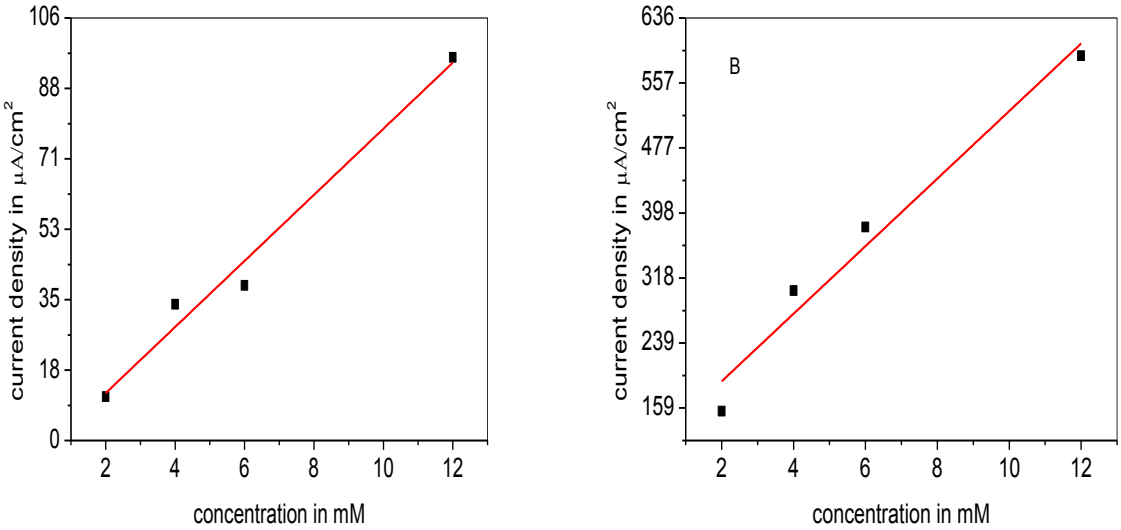


Figure 5. Calibration graph obtained from ascorbic acid determination by cyclic voltammetry at different concentrations expressed as mM using GCE (A) and Pt (B) working electrodes.

The relation between ascorbic acid concentration and cyclic voltammetry peak current is linear and their linear dependence was depicted from calibration curve shown in figure 5 with linear regression equations y=23.4611X + 13.2489 and y=5.19714X + 13.7071 for GCE and Pt respectively and correlation coefficients (R^2=0.97241 for GCE and R^2=0.95579 for Pt electrode).

The value calculated for the relative standard deviation R.S.D. were 2.76%, for GCE and 4.42% for Pt. The values obtained for the limit of detection and the limit of quantification were 0.0035294 mM and 0.0176 mM for GCE and 0.025519 mM and 0.085066 mM for Pt electrode respectively. This is calculated using equation 9 and 10 below.

$$LOD = 3 \cdot \frac{S.D}{m} \quad \text{.....1}$$

$$LOQ = 10 \cdot \frac{S.D}{m} \quad \text{.....2}$$

Where, S.D= standard deviation
m= slope

Effect of scan rate

Typical cyclic voltammograms of 12mM ascorbic acid at various scan rates at GCE and Pt electrode in 0.1M KCl buffer (pH = 2 for GCE and pH =1 for Pt electrode) is given in figure 6.

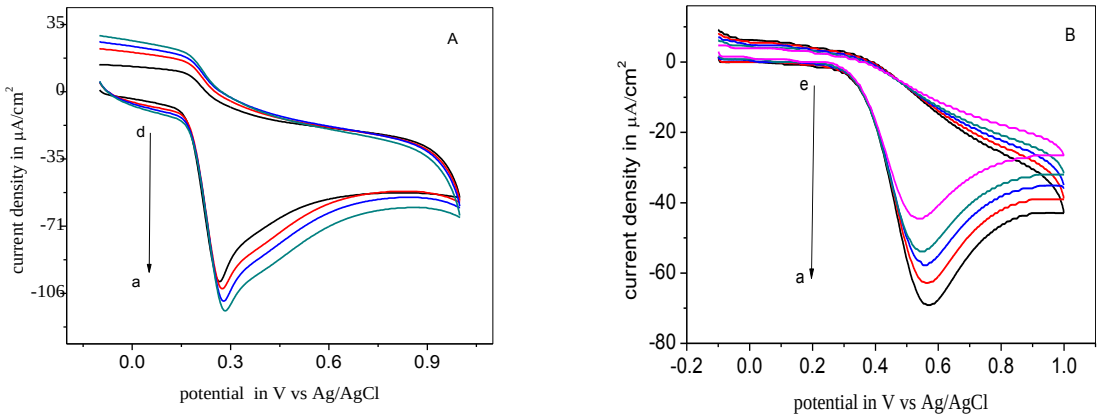


Figure 6. Cyclic voltammograms of 12 mM ascorbic acid in 0.1 M KCl buffer solution at various scan rates as: (a) 125, (b) 100, (c) 75, (d) 50, (e) 25 mVs-1 using GCE (A) and Pt (B) electrodes

The peak potential shifted to more positive values as the scan rate increased as shown in Figure 7, in agreement with the irreversible electrochemical behavior observed for ascorbic acid oxidation in diffusion controlled process.[60] Scan rates are chosen because at this values; Sensitivity was relatively high and the voltammetric curves were well shaped with relatively narrow peak widths.[58]

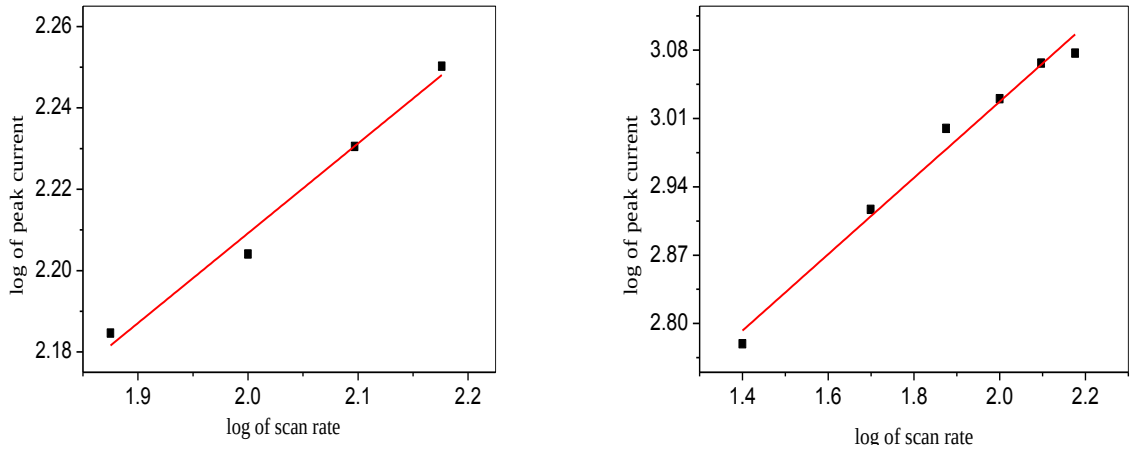


Figure 7. Calibration graph for logarithm of peak current versus logarithm of scan rate for GCE (A) and Pt (B) electrodes

Plot of the logarithm of peak current versus the logarithm of the scan rate gave a straight line with correlation coefficient (R^2=0.97571 for GCE and R^2=0.97903 for Pt) and a slope of 0.22074 for GCE and 0.39085 for Pt electrode, very close to the theoretical value of 0.3 and 0.4 respectively, which is expressed for an ideal reaction of the diffusion-controlled electrode process[8, 10].

Differential pulse voltammetric behavior of standard ascorbic acid using glassy carbon and platinum electrodes

The electrochemical oxidation process of ascorbic acid occurring on platinum and glassy carbon electrodes was studied by differential pulse voltammetry technique. The pH was optimized for DPV method in the electrochemical oxidation behavior of ascorbic acid in a pH range between pH = 1 up to 5 at GCE and Pt electrode in aqueous media. Maximum peak current was obtained in the acidic media (0.1 M KCl buffer at GCE and Pt electrode) that indicates participation of a proton transfer in the electrode process. Also, the experimental results showed that the shape and intensity of the curves were better in O.1 M KCl buffer pH = 1 for GCE and Pt electrode. Therefore, the KCl buffer at pH = 1 for GCE and Pt electrode was chosen for the analytical determination in DPV technique. DPV response was strongly pH dependent.

The voltammograms of 0.1 M KCl buffer solution and 12 mM ascorbic acid solution at pH = 1 for glassy carbon and platinum working electrode are shown in figure 5.6 below. In both cases no peak current were observed in the voltammograms of the supporting electrolyte in the potential range of -100 to 1000 mV but were observed for 12 mM ascorbic acid solution in the supporting electrolyte. No reduction peak current was found indicating an irreversible heterogeneous charge transfer in this system[60]. These indicate that ascorbic acid concentration can be measured quantitatively by differential pulse voltammetry.

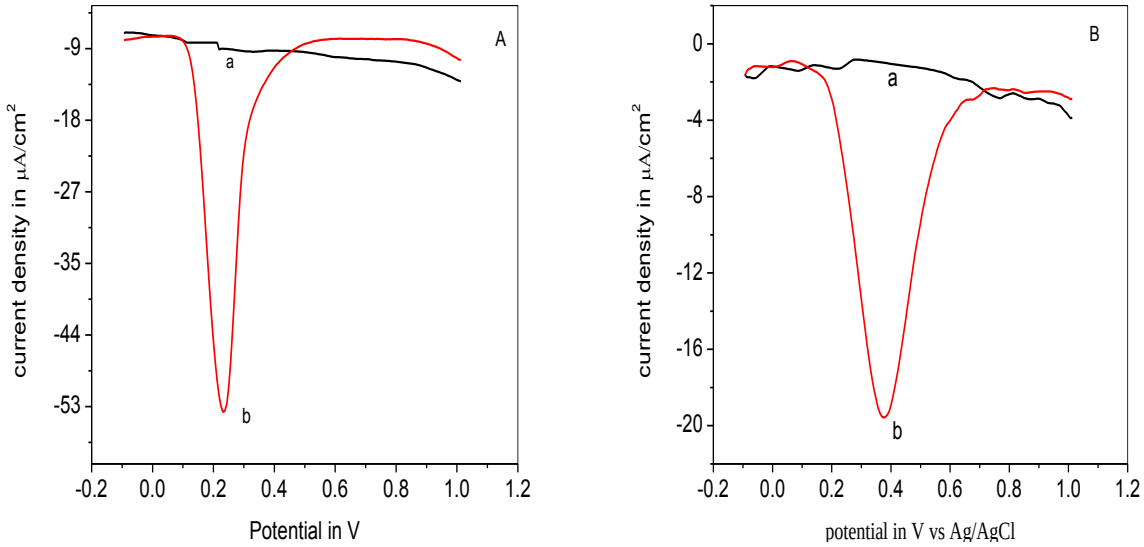


Figure 8. Differential pulse voltammograms of 0.1 M KCl buffer solution and 12 mM ascorbic acid at optimized pH = 1 for GCE (A) and Pt (B) electrode

Differential pulse voltammetry was used for quantification of vitamin C since it gives voltammograms in which the peaks are sharper and better defined than those obtained by cyclic voltammetry.

Effect of concentration

In figure 9, several differential pulse voltammograms, obtained at GCE and Pt working electrode, for different ascorbic acid concentrations at pH = 1 are presented. The peak corresponding to ascorbic acid oxidation appeared at 235 mV and 375 mV (versus Ag/AgCl reference electrode) for GCE and Pt respectively. The calibration graph (Figure 4.8) shows a linear range obtained between 2 and 12 mM ascorbic acid and linear regression of y = 1.201X + 0.530393 for GCE and y = 0.0521393X + 0.506857 for Pt electrode, (R^2 = 0.99609 for GCE and R^2 = 0.95032 for Pt).

The value calculated for the relative standard deviation R.S.D. were 0.391%, for GCE and 4.969% for Pt. The values obtained for the limit of detection and the limit of quantification were 0.12412 mM and 0.4137 mM for GCE and 0.22497 mM and 0.74 mM for Pt electrode respectively.

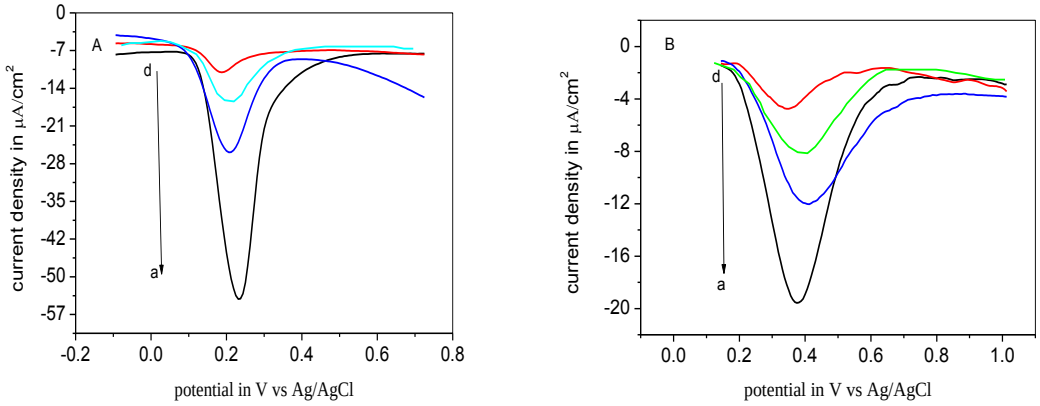


Figure 9. Differential pulse voltammograms obtained with GCE (A) and Pt (B) electrodes for different ascorbic acid concentrations, expressed as mM: (a)12 (b) 10 (c) 8 and (d) 6; experimental conditions : pulse amplitude 50 mV, pulse period 125 ms, potential scan rate 50mV/s.

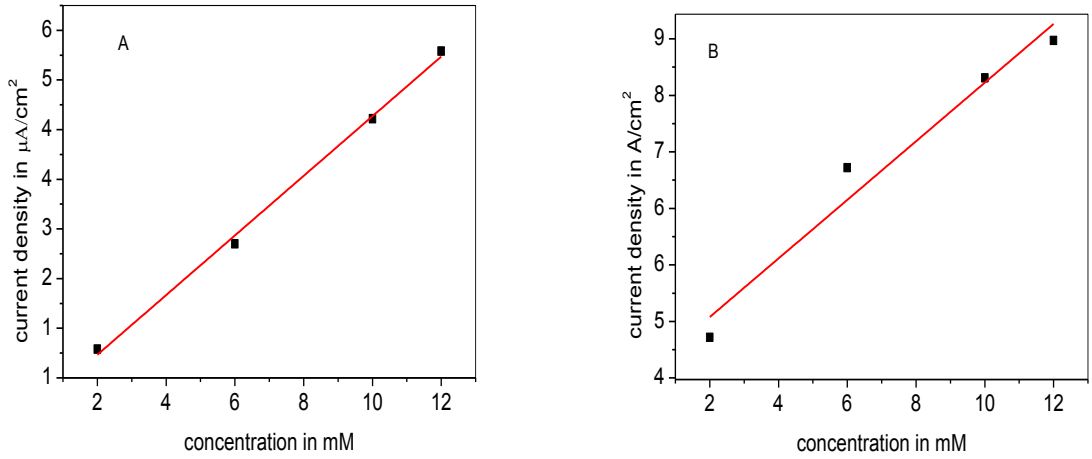


Figure 10. Calibration graph obtained from ascorbic acid determination by DPV at different concentration values using GCE (A) and Pt (B) working electrodes

Effect of pulse amplitude

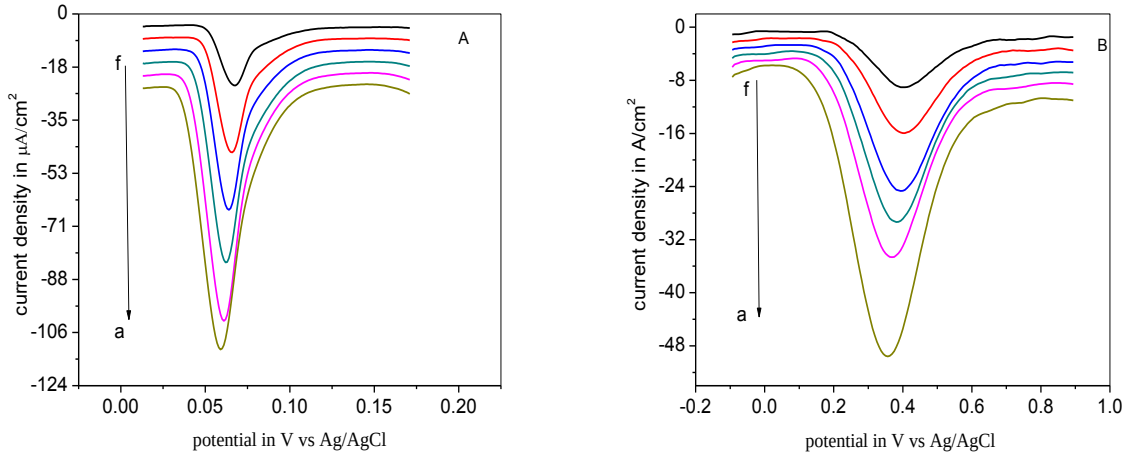


Figure 11. Influence of pulse amplitude of DPV on ascorbic acid determination, using GCE (A) and Pt (B) electrodes; (a) 25 mV (b) 50 mV (c) 75 mV (d) 100 mV (e) 125 mV and (f)150 mV; experimental conditions: pulse period 125ms, potential scan rate 50 mV/s

For the investigation of the influence of the pulse amplitude for GCE and Pt electrodes (figure 11), the parameter was varied between 25 to 150 mV, at 125 ms pulse period and 50 mV/s potential scan rate. By analyzing the results presented in figure 4.9, it can be noticed that the value of the measured current intensity increases with the applied pulse amplitude. The peak height increases with the decrease of the pulse amplitude. An optimum value of 50 mV was chosen for further studies and in real sample analysis at both electrodes because above this value the peak becomes distorted and below this values the peak width becomes large which results peak interpretation difficult.

Influence of pulse period

For the investigation of the influence of the pulse period (Figure 12) we varied this parameter between 75 and 200 ms, at 50 mV pulse amplitude and 50 mV/s potential scan rate. The peak height and width increases with the decrease of the pulse period. The optimum value chosen for further studies was 125 ms. Smaller values of the pulse amplitude were not used, in order to diminish the influence of noise at values of the pulse period below 100 ms, larger values of pulse amplitude result with poor peak resolution.

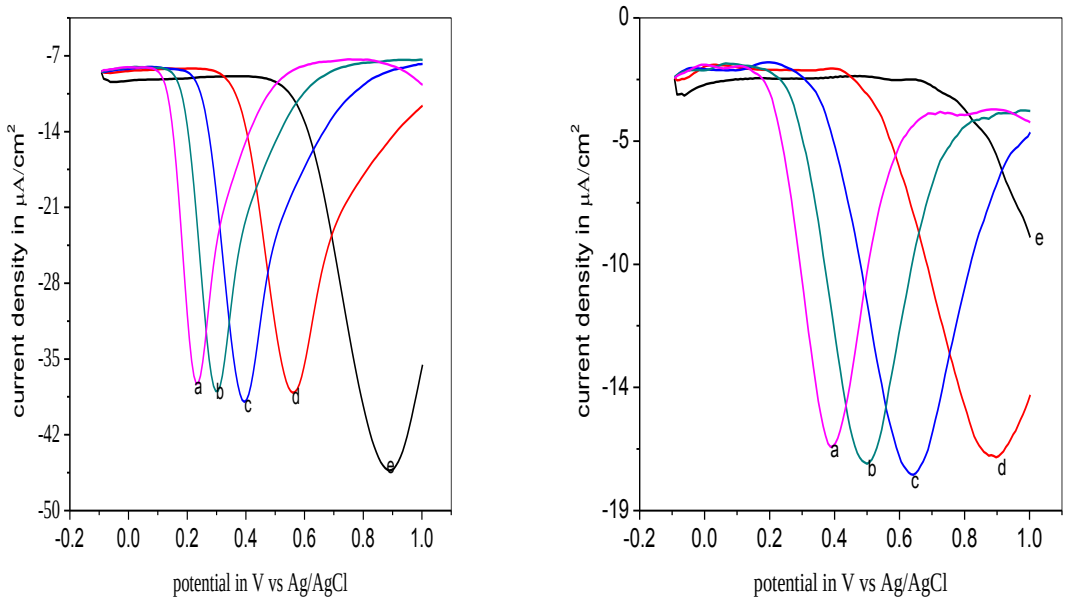


Figure 12. Influence of the pulse period on the analytical response at ascorbic acid determination by differential pulse voltammetry, using GCE (A) and Pt (B) electrodes; (a) 75 ms (b) 100 ms (c) 125 ms (d) 150 ms (e) 200 ms; experimental conditions: pulse amplitude 50 mV, potential scan rate 50mV/s

Real sample analysis

Biological samples of plant and animal sources were investigated. These include Ox Liver and Tomato Sauce. In the case of liver, the sample was washed with water and milled and minced using mortar and pistil. 5 g of the homogenized sample was weighted and dissolved in to 25 ml of 0.1 M KCl by shaking it for 15 minutes. The dissolved sample is then allowed to settle and filtered in order to get a clear solution for analysis using suction filter paper and introduced in to the cell for run. But for tomato 5g of the sauce was weighted and the weighted amount is directly dissolved in 0.1 M KCl solution by shaking it and ready for run. The working procedure employed for standard ascorbic acid solutions was also applied to biological sample analysis by cyclic and differential pulse voltammetry.

The voltammogram of the supporting electrolyte was recorded then a known volume (V_u) of unknown concentration (C_u) of the investigated sample was added and the resulting cyclic and differential pulse voltammetry was recorded and Ip1 was measured then a known volume (V_s) of known concentration (C_s) of standard was added and the cyclic and differential pulse voltammetry was recorded and I_{p2} was measured. The C_u can be calculated using the following equation:

$$C_u = \frac{I_{p1} C_s . V_a}{(I_{p2}(V_u + V_s)) - I_{p1} V_u} \quad \text{.....3}$$

Cyclic voltammetric determination of Vitamin C in liver

From figure 13, Vitamin C content of liver can be determined using cyclic voltammetry at GCE (A) and Pt (B) working electrodes. From the voltammograms the amount of Vitamin C in GCE was determined to be 20mg/100g and in Pt electrode 16mg/100g of liver.

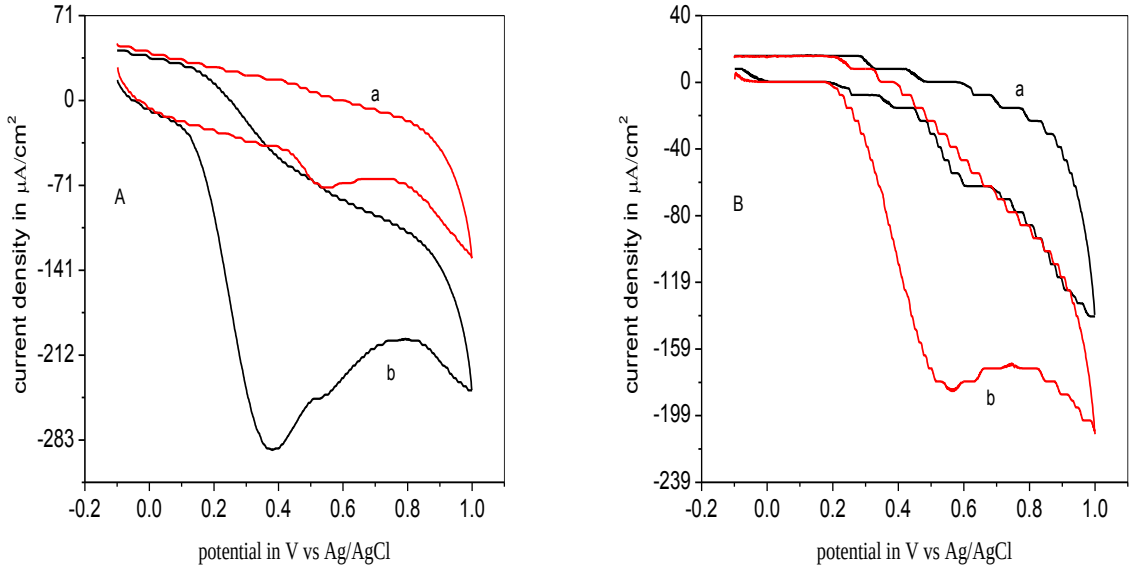


Figure 13. Cyclic voltammogram of liver on GCE (A) and Pt (B) electrode

Cyclic voltammetric determination of Vitamin C in Tomato

Cyclic voltammograms of tomato at GCE (A) and Pt (B) electrodes show 25.4 mg/100g and 20.6mg/100g respectively.

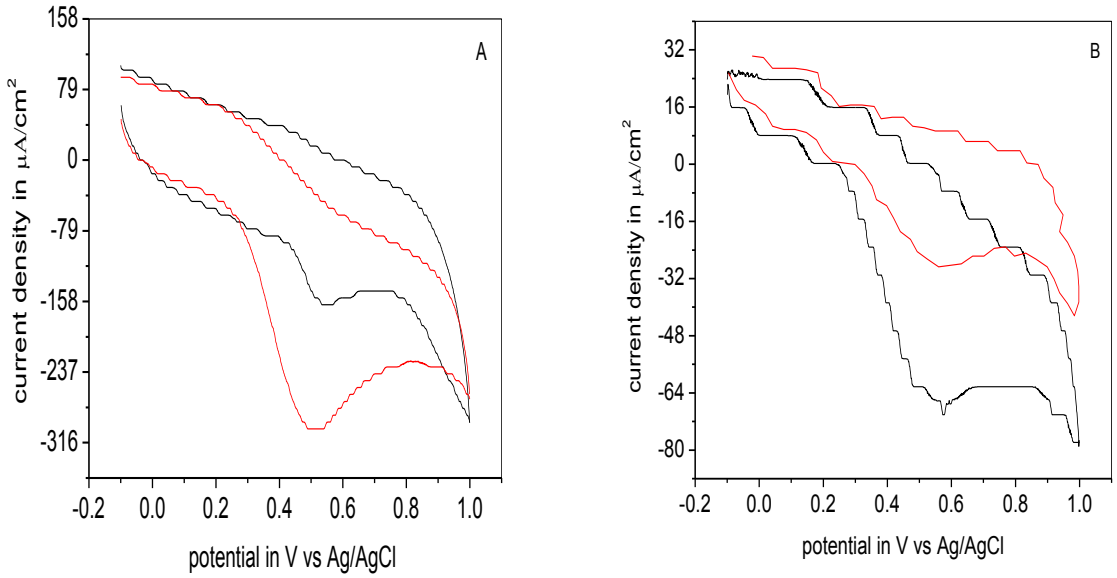


Figure 14. Cyclic voltammograms of ascorbic acid in tomato using GCE (A) and Pt (B) working electrodes

Differential pulse voltammetric determination of ascorbic acid in liver

Differential pulse voltammograms for liver in GCE (A) and Pt (B) electrodes are shown in figure 15 and the amounts of ascorbic acid in liver sample was calculated as 21.9 mg/100g and 19.3 mg/100g respectively.

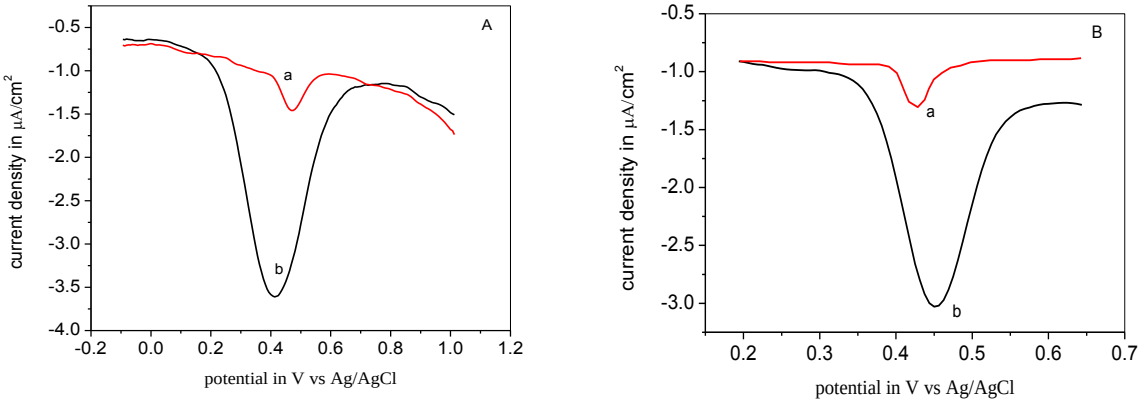


Figure 15. Differential pulse voltammogram for liver using GCE (A) and Pt (B) electrodes under experimental conditions set

Differential pulse voltammetric determination of ascorbic acid in tomato

Differential pulse voltammogram of tomato under experimental conditions was depicted as in figure 16 and the content of ascorbic acid in tomato by DPV was 26.4 and 21.1 mg/100g of tomato sauce.

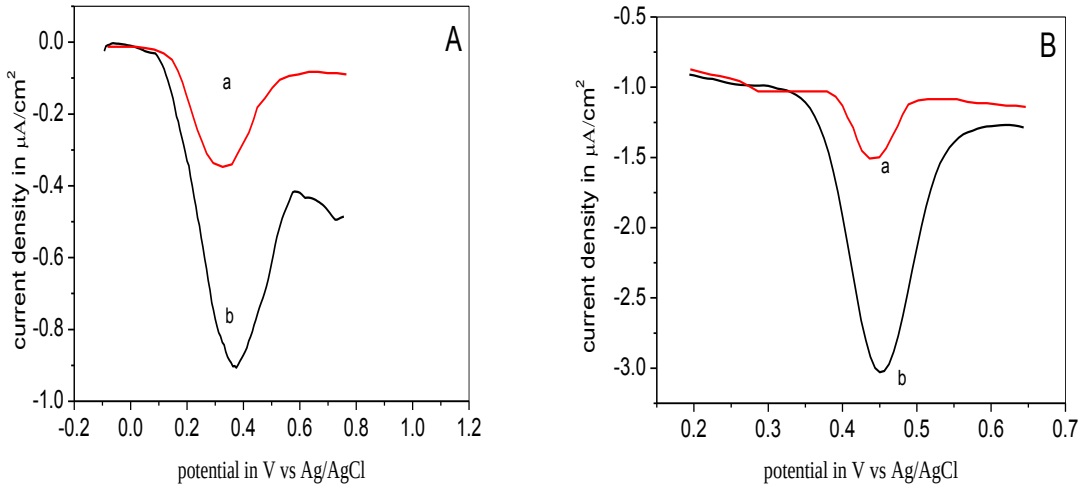


Figure 16. Differential pulse voltammogram for tomato using GCE (A) and Pt (B) under experimental conditions set

CONCLUSION AND RECOMMENDATION

The electrochemical determination of ascorbic acid can be determined either by cyclic voltammetry or differential pulse voltammetry at glassy carbon and platinum electrodes. The electrochemical oxidation ascorbic acid was irreversible and pH dependent.

The oxidation peak potential of ascorbic acid were 270 mV and 235 mV for GCE in CV and DPV respectively but for Pt electrode 490 mV for CV and 370 mV for DPV (versus Ag/AgCl reference electrode). Based on the electrochemical oxidation, quantitative determination of ascorbic acid in liver and tomato was studied using cyclic and differential pulse voltammetry techniques.

The result in the quantitative measurements of vitamin C concentration by CV and DPV gives higher vitamin C content in tomato for both electrodes and those for liver is lower. Also, my results show there is a significant difference in the values of vitamin C quantified between electrode types and method type; glassy carbon gives better result than Pt electrode in cyclic voltammetry and in differential pulse voltammetry, the difference in the result is due to the sphericity or radius of the electrode.

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