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Research Article

Determination of Ascorbic Acid Content of Wine & Soft Drinks by Voltammetric Techniques at Glassy Carbon Electrode

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- Glassy carbon electrode
- Wine
- Soft drinks

Abstract

Ascorbic acid is a white crystalline powder with a molecular formula of C6H8O6 and a formula weight of 176.12 g/mol. It is a water-soluble, antioxidant vitamin, important in forming collagen, cartilages, muscles, and blood vessels. Therefore, this research aims to develop method for quantitative determination of ascorbic acid in different wine and soft drinks by using cyclic and square wave voltammetey. The oxidation peak for Ascorbic acid occurs at about 596 mV (versus Ag/AgCl) on a glassy carbon working electrode by using cyclic voltammetry and about 450 mV by using square wave voltammetry. For square wave voltammetry method, the influence of pulse amplitude, step potential, frequency, PH dependence and concentration dependence was investigated. For cyclic voltammetry method, the influence of scan rate, concentration and PH was also investigated. The obtained calibration graph shows a linear dependence between the peak height and Ascorbic acid concentration within the range 1.0 mM - 8.0 mM with a glassy carbon working electrode for both cyclic and square wave voltammetry methods. The Ascorbic acid content determined ranged between 2.76 mg/100 mL for sprite and 14.71mg/100 mL for kemila white wine by using cyclic voltammetry and 4.13 mg/100 mL for sprite and 16.34 mg/100 mL for kemila white wine by using square wave voltammetry method. Different Ascorbic acid concentrations (from standard solution) were added to analyzed samples. The degree of recovery being comprised between 97.45 and 100.72 %.

INTRODUCTION

Wine

The term wine describes an alcoholic beverage that contains products of fermentation of the Juice from the grapes, pears, apples, berries, and even flowers such as dandelions [1]. Wine naturally contains about 85–89% water, 10–14% alcohol, less than 1% fruit acids, and hundreds of aroma and flavor components in very small amounts. Wine character, its taste and smell, is derived from many factors including the grapes it is made from, where they were grown, and the production techniques applied by the wine maker [2]. Wines are consumed in all over the world. Wine is also widely produced and consumed in Ethiopia. Awash Winery is the first commercial winery in Ethiopia, established in 1943 E.C. The winery produces a range of well made wines, including Gouder Red, Dukem Red, Awash Crystal White, Axumite Red and Kemila White [3].

Soft drinks

Soft drinks are non-alcoholic water-based flavored drinks that are optionally sweetened, acidulated, carbonated and which may contain fruit juice and salts. Their flavor may derive from vegetable extracts or other aromatic substances [4].

Non-alcoholic soft drink beverage can be divided into fruit drinks and soft drinks. The top soft drink brands are coca-cola and Pepsi. The other popular soft drink brands include Fanta, Miranda, 7Up, Sprite etc.

Soft drinks available in glass bottles, aluminum cans, PET bottles or disposable containers can be divided into carbonated and non-carbonated drinks. Cola, lemon and oranges are carbonated drinks and fruit juices and squashes are non-carbonated drinks. The major ingredient of soft drinks is water and it accounts for 86%-90% of the soft drink composition. Aromatic substances are added to soft drinks to give a pleasant taste and better stability to the taste.

The most common acids used in soft drinks are citric acid, phosphoric acid and malic acid. The function of acidity in the drink is to balance the sweetness, make the drink fresh and thirst-quenching. Color is added to soft drinks to make them presentable and appetizing. Preservatives like natrium benzoate, potassium sorbate and sulphur dioxide are added to increase the life of the product.

Antioxidants are substances, which prevent reactions that destroy aromatic substances in soft drinks. The most common antioxidant used is ascorbic acid.

Vitamin C (Ascorbic acid)

Vitamin C is a white crystalline powder with a molecular formula of $C_6H_8O_6$ and a formula weight of 176.12 g/mol. It is a water-soluble, antioxidant vitamin, important in forming collagen, cartilages, muscles and blood vessels. It prevents tissue damage & used in treatment of certain diseases such as scurvy, anemia, diabetes, common cold, hemorrhagic disorders, wound healing, cough, influenza, sores, gingivitis, skin diseases, diarrhea, malaria, bacterial infections, plug poisoning, liver disease, allergic reactions, arteriosclerosis as well as infertility in males. Vitamin C also aids in the absorption of iron, immune response activation and helps maintain capillaries, bones, and teeth. Ascorbic acid is known for its reductive properties and it is used on a large scale as antioxidant in the pharmaceutical, chemical, cosmetic and food industry [5].

Adequate intake of vitamin C from foods and supplements is vital for normal functioning of the human body. Recommended Dietary Allowances of 75 mg/day and 90 mg/day have been established for adult women and men, respectively, and 45 mg/day for children 9–12 years old. For smokers, the recommended values are increased by an additional 35 mg [6].

An ascorbic acid excess can lead to gastric irritation, and the metabolic product of vitamin C and causes renal problems [7]. In some cases, excessive quantities of ascorbic acid may result in the inhibition of natural processes occurring in food and can contribute to taste deterioration [8]. Its oxidation can be accelerated by excessive heat, light, and heavy metal cations. Ascorbic acid content of foodstuffs and beverages represents a relevant indicator of quality which has to be carefully monitored, regarding its variation during manufacturing and storage [9]. Humans do not produce Vitamin C due to a mutation in the gulonolactone oxidase gene, which results in the inability to synthesize the protein [10]. Therefore humans depend on exogenous sources of the vitamin which include citrus fruit, leafy vegetables, tomatoes, green and red peppers, grapes, soft drinks, wines or food supplements and pharmaceutical preparations [11].

There is a need to find an accurate, reliable, rapid and easy to implement method 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 is due to its oxidation to dehydroascorbic acid, which is a reversible reaction and subsequently to 2, 3-diketo-L-gulonic acid. The latter reaction is irreversible which is shown at scheme I [12], (Figure 1). The instable ascorbic acid oxidation to dehydroascorbic acid & subsequently to 2, 3- diketo L-gulonic acid [12].

The determination of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical and food application. Traditional methods for ascorbic acid assessment involve titration with potassium iodate [13]. Chromatographic methods, particularly HPLC with electrochemical detection [14], have turned out to be a selective and sensitive method for ascorbic acid assessment in foodstuffs and biological fluids.

A recently developed voltammetric methods allow rapid, simple, selective and sensitive determination of low molecular weight antioxidants and vitamins and drugs, without the necessity of time consuming separation [5].

Square-wave voltammetry was used to determine ascorbic acid, based on its oxidation at a zeolite modified carbon paste electrode [15]. Cyclic and differential pulse voltammetry were used for electrocatalytic ascorbic acid determination, at a carbon paste electrode, modified with 2,7-bis (ferrocenylethynyl) fluoren-9-one [16]. Cyclic voltammetry at a bare Pt electrode was applied to ascorbic acid content estimation in citrus juices and soft drinks [17].

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-disulphonicacid [18]. Differential pulse voltammetry was used for the assessment of ascorbic acid, at poly (3,4-ethylendioxythiophene) modified electrodes [19]. Simultaneous determination of vitamins C and B6 in pharmaceuticals formulations was performed using differential pulse voltammetry at a glassy carbon electrode [20].

Dopamine was determined in the presence of ascorbic and uric acids by differential pulse voltammetry at a bare glassy carbon electrode [21]. 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 1×10^{-6}M - 5×10^{-4}M, the achieved detection limits for ascorbic acid and uric acid were 2×10^{-7}M & 1×10^{-7}M, respectively [22].

A multi-walled carbon nanotube-tetradecyltrim ethylammonium bromide film coated graphite electrode was used to study the electro oxidation of ascorbic acid in differential pulse, cyclic and square-wave voltammetry [23].

The electro catalytic oxidation of ascorbic acid (cyclic and

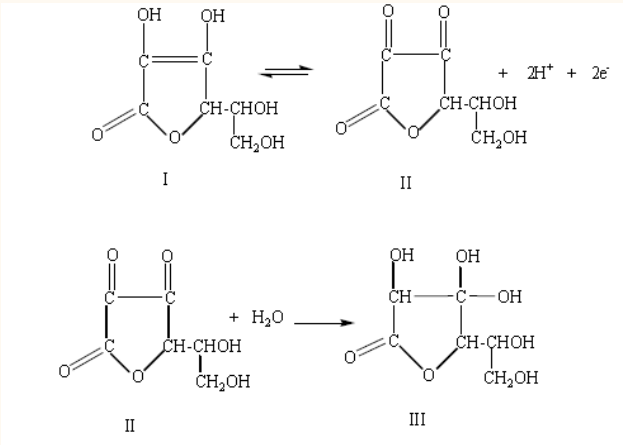

Figure 1 The instable ascorbic acid oxidation to dehydroascorbic acid & subsequently to 2, 3- diketo L-gulonic acid.

differential pulse voltammetry) was investigated with a carbon nanotube paste electrode modified with 2,2'-[1,2-ethanediylbis (nitriloethylidine)]-bis-hydroquinone. 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 [24]. Differential pulse voltammetry with a poly (sulfonazo III) modified glassy carbon electrode enables the highly selective determination of ascorbic acid, dopamine and uric acid [25].

Cyclic voltammetry and differential pulse voltammetry at a binuclear copper complex modified glassy carbon electrode were also applied to determine ascorbic acid and dopamine. 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 1.4×10^{-6} M for dopamine and 2.8×10^{-6} M for ascorbic acid. The modified electrode was used for ascorbic acid and dopamine determination in medicine and foodstuffs [26]. Differential pulse voltammetry at a glassy carbon electrode was applied to quantitative determination of ascorbic acid in tablet dosage form and in some fruit juices [27].

This study aims at investigating the ascorbic acid determination by square wave voltammetry and cyclic voltammetry at glassy carbon working electrode. The developed method is applied to ascorbic acid content assessment in wine & soft drinks. The results obtained by cyclic voltammetry and square wave voltammetry performed at the working electrode is compared. More over the content of ascorbic acid in different wine samples & different soft drinks produced in Ethiopia can be compared and the sample containing appropriate ascorbic acid concentration can be recommended for someone having deficiency in ascorbic acid.

EXPERIMENTAL PART

Chemicals and reagents

All chemicals and reagents are of analytical grade. Chemicals and reagents used for this work include:-

- 99% L-ascorbic acid ($C_6H_8O_6$) (Blulux)
- 98% Sodium phosphate monobasic ($NaH_2PO_4.2H_2O$) (Blulux)
- 85% Phosphoric acid (H_3PO_4) (Blulux)
- 98% sodium hydroxide (NaOH) (Blulux)
- 98% $Na_2EDTA.2H_2O$ (Blulux)
- Alumina powder (Al_2O_3) *(*Pharmacos *LTD*)
- Distilled water

Samples selected for analysis

a) Wines: Gouder red wine, Axumite red wine and Kemila white wine

b) Soft drinks: Mirinda**,** Fanta orange**,** Pepsi**,** Coca cola and Sprite

Apparatus

The experiments were performed using the, electrochemical analyzer BAS 100B [bioanalytical systems,USA], which controlled from a magnetic stirrer with a hot plate was used for stirring in PH adjustments. The PH of the buffer is measured using a digital pH meter (3305) [28].

Electrochemical cell

Glassy carbon electrode as working electrodes, Ag/AgCl as reference electrode and coiled Pt as counter electrode was used. The electrode was polished using alumina powder and rinsed with distilled water before each measurement [28]. The cell stand is shown in Figure (2).

Procedure

Preparation of supporting electrolyte: 0.1 M phosphate buffer solution composition was prepared daily with 0.1 mM $Na_2EDTA.2H_2O$, and 0.1 M sodium phosphate monobasic and pH values was adjusted from 1- 5 with phosphoric acid and NaOH.

Standard preparation: A stock solution of 8 mM was prepared by dissolving 0. 141 g of ascorbic acid in 100 mL of 0.1M phosphate buffer dosed with 0.1mM $Na_2EDTA.2H_2O$ solution. Other standard solutions, 8.0 mM, 6.0 mM, 4.0 mM, 2.0 mM and 1.0 mM were prepared from stock solution by serial dilution with phosphate buffer to the final volume of 50 mL. 15 mL of each standard ascorbic acid solution was used in the electrochemical cell.

For cyclic voltammetry measurements, the potential was scanned in the range 0 - 1V, at scan rate of 100 mV/s. The effect of the scan rate was studied in the range 25 mV/s - 125 mV/s. For the square wave voltammetry measurements the potential was scanned in the range 0 -1V. For the investigation of the influence of the operational parameters on the analytical signal, the pulse amplitude was varied between 25 and 125 mV.

Sample preparation and analysis: The commercial wine and soft drink samples were analyzed without any pretreatment. Aliquots of 10 mL mixed with 5 mL of 0.1M phosphate buffer (PH = 3 for CV method and PH = 2 for OSWV) was transferred to the electrochemical cell. The voltammograms were registered and peak heights (hx) were measured. After adding a single aliquot (Va = 8 mL) of standard solution having concentration of Cst, new voltammograms were registered and new peak heights (ha) were also measured. Samples concentrations (Cx) were obtained using equation 2.1 [29].

$$Cx = \frac{hx}{(ha - hx)} \frac{CstVa}{Vx} \tag{2.1}$$

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies via standard addition method and percentage difference. All electrochemical measurements were carried out at room temperature (~ 25 °C).

RESULT AND DISCUSSION

Cyclic voltammetric behavior of AA

Figure (3.1) shows (a) the cyclic voltammograms for phosphate buffer and (b) the oxidation of 8 mM ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at pH = 3. The buffer solution gave no response while anodic peak current was observed at 596 mV in the voltammogram of the ascorbic

Figure 2 Electrochemical cells stand.

acid solution. No cathodic peak current was found indicating an irreversible heterogeneous charge transfer due to the absence of electro activity on the reverse scan during cyclic voltammetry [30].

Optimization of the solution pH: The electrochemical behavior of ascorbic acid in buffered solutions has been studied at different pH values from 1 - 4 in cyclic voltammetry technique. The voltammogram is shown in Figure (3.2a) and the plot of Ip vs PH is shown in Figure (3.2b).

Variation in the electrolyte pH results variations in the formal potential of ascorbic acid. The results indicated that the peak potential, Ep, shifted to more negative values with increasing pH. Such a behavior suggests that the participation of protons in the electrode process and the acidic dissociation of ascorbic acid occur at or before the rate determining step [16]. The peak current shows a maximum at PH = 3 as shown in Figure (3.2). Owing to its efficiency of oxidation, pH = 3.0 was chosen as optimal pH.

Effect of varying scan rate: The effect of scan rate (ν) on the cyclic voltammograms of ascorbic acid in phosphate buffer supporting electrolyte was studied (Figure 3.3a). The peak current was found to be linearly proportional to the square root of scan rate as shown in Figure (3.3b) it can be given by equation 3.1 with its correlation coefficient of 0.998.

$$Ip\ (\mu A) = 26.85\nu^{1/2} + 2.353 \quad (3.1)$$

The result illustrates that the process of ascorbic acid oxidation is diffusion controlled process [31].

The peak potential shifted to more positive values as the scan rate increased as shown in (Figure 3.3c); in agreement with the irreversible electrochemical behavior observed for ascorbic acid oxidation [32]. In order to obtain information on the rate-determining step a Tafel slope, b, was determined using Equ.3.2 for a totally irreversible diffusion controlled process [33].

$$Ep = (b/2)\log\nu + \text{constant} \quad (3.2)$$

The slope of Ep *vs* log ν plot was found to be 155.89 mV, with correlation coefficient 0.997 and its linear regression equation as expressed by equation 3.3:

$$Ep\ (mV) = 155.89\log\nu + 326.03 \quad (3.3)$$

Thus b = 2×155.89 = 311.78 mV. This slope value indicates a one-electron transfer to be rate limiting step [33].

Effect of varying ascorbic acid concentrations: The cyclic voltammograms show that peak current increases linearly with increasing concentration of ascorbic acid from 1.0 to 8.0 mM as shown in Figure (3.4). The calibration graph of various ascorbic acid concentrations immersed in 0.1 M phosphate buffer (pH = 3.0) was determined as shown in Figure (3.4b). The linear regression equation is given in equation 3.4

$$Ip\ (\mu A) = 23.19\ C_{AA} + 36.81 \quad (3.4)$$

Where Ip represents the value of the current intensity, from which the background value was subtracted and C_{AA} is the ascorbic acid concentration and the correlation coefficient is 0.997. This shows that ascorbic acid can be quantitatively measured by cyclic voltammetry.

Application of the electrochemical method to ascorbic acid determination

Validation of the proposed procedure: The repeatability of the measurement was calculated from five independent runs of ascorbic acid solution as shown in equatio.3.4.Validation of the procedure for the quantitative determination of the ascorbic acid was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), and repeatability. LOD and LOQ were calculated from the peak current using the following equations [34].

$$LOD = 3\ s/m, \quad (3.5)$$

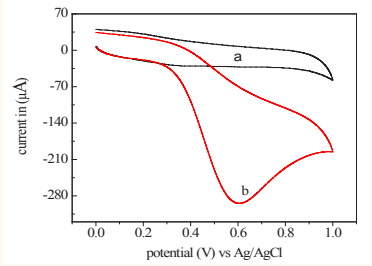

Figure 3.1 Cyclic voltammograms for of 8 mM ascorbic acid in 0.1 M phosphate buffer (pH= 3) at a scan rate of 100 mV/s.

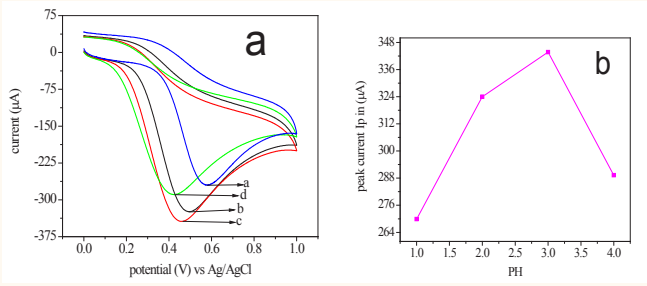

Figure 3.2 a) Cyclic voltammogram of 8 mM ascorbic acid in 0.1 M phosphate buffer containing 0.1 mM Na2EDTA.2H2O for pH values of (a) 1, (b) 2, (c) 3, and (d) 4 at a scan rate of 100 mV s^{-1}.
b) Plot of peak current vs pH.

$$LOQ = 10\,s/m \quad (3.6)$$

Where s is the standard deviation of the peak currents (n = 5) and m is the slope of the calibration curve (Figure 3.4). LOD and LOQ were obtained as 0.073 mM and 0.243 mM, respectively.

Application to real sample analysis: In order to analyze the accuracy of the method for ascorbic acid determination in wines and soft drinks, the standard addition method were applied to Kemila, Axumite, Gouder, Mirinda, Pepsi, sprite, coca cola and Fanta orange samples.

The following figures shows cyclic voltammograms of samples of Kemila, Axumite, Gouder, Mirinda, pepsi, sprite, coca cola, fanta orange and samples containing acid standard solutions respectively. The shapes and positions of peak potentials and the increase in peak currents are similar to the voltammograms of the standard ascorbic acid. But there is a shift in the oxidation potential because of the matrix effect.

The onset potentials, peak potential, offset potentials and the increase in peak current are similar to the voltammograms of standard ascorbic acid for each sample.

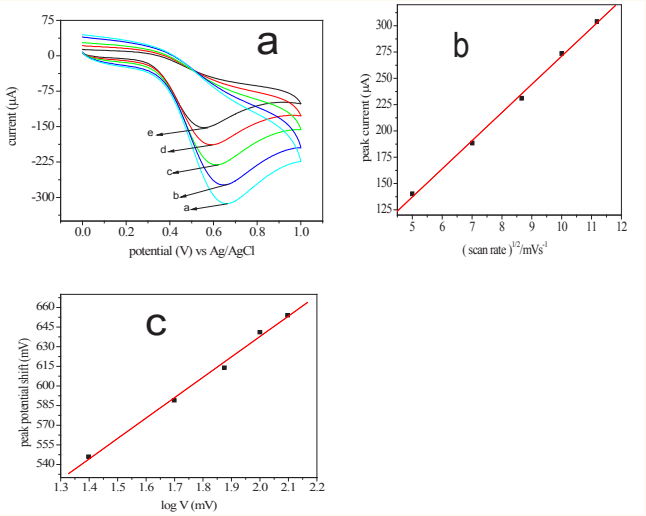

Figure 3.3 a) Cyclic voltammograms of 8 mM ascorbic acid in 0.1 M phosphate buffer solution (pH = 3.0) at various scan rates: (a) 125, (b) 100, (c) 75, (d) 50, and (e) 25 mVs^{-1}. (b) A plot of peak current *vs* square root of scan rate ($v^{1/2}$). c) Ep vs log v.

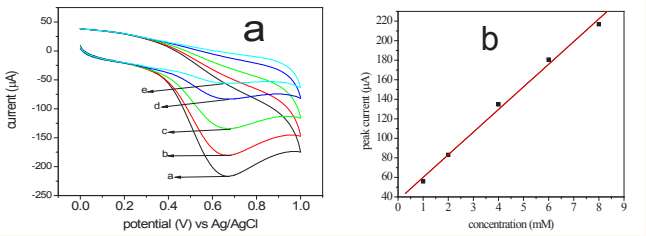

Figure 3.4 a) Cyclic voltammograms of ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at pH = 3.0 for concentrations of 8.0 (a), 6.0 (b), 4.0 (c), 2.0 (d), 1.0 mM (e) at a scan rate of 100 mVs-1. b) Calibration graph for ascorbic acid at various concentrations.

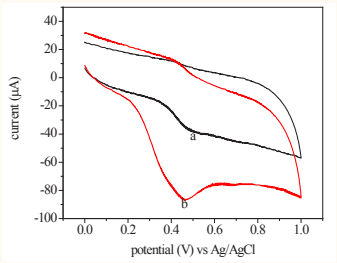

Figure 3.5 Cyclic voltammogram of (a) sample of Kemila and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-.

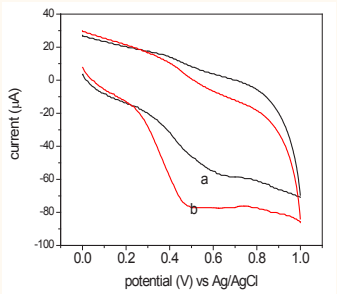

Figure 3.6 Cyclic voltammogram of (a) sample of Gouder and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

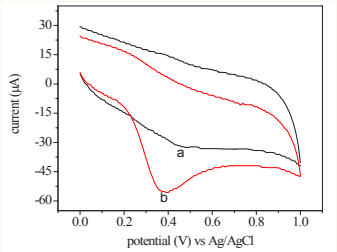

Figure 3.7 Cyclic voltammogram of (a) sample of sprite and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

Determination of degree of recovery for each soft drink and red wine: 10 mL of each sample was mixed with 5 mL of phosphate buffer (pH = 3). 15mL of the homogenized samples were transferred to the electrochemical cell. Their voltammograms were registered and their peak heights (hx) were measured. After adding a single aliquot (Va = 8 mL) of standard ascorbic acid solution having concentration of Cst, a new voltammograms were registered and new peak heights (ha) were also measured as shown in the above figures. The degree of recovery were determined by Equ.3.7 using a standard addition or spike procedure

Figure (3.5) Cyclic voltammogram of (a) sample of Kemila and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1. Fig.3.6 Cyclic voltammogram of (a) sample of Gouder and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

$$R_A\% = \frac{Q_A(O+S) - Q_A(O)}{Q_A(S)} \times 100 \quad (3.7)$$

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

The degrees of recovery for each sample were determined from (Figure 3.5 - 3.12), respectively. They are very close to the ideal value 100% as shown in Table (1).

Square wave voltammetric behavior of ascorbic acid

Figure (5.13) shows the square wave voltammograms of the phosphate buffer (a) and the oxidation of ascorbic acid (b) in 0.1 M phosphate buffer supporting electrolyte at pH = 2.0. The buffer solution gave no response while anodic peak current was observed at 450 mV in the voltammogram of ascorbic acid solution as shown in (a) and (b) of Figure (3.13), respectively.

Optimization of the solution pH: The influence of pH on the electrochemical oxidation of ascorbic acid was investigated in the range 1- 4 as shown in Figure (3.14). In all cases the concentration of the phosphate buffer was maintained at 0.1 M and voltammograms were obtained using 8.0 mM ascorbic acid. The peak current for the electrochemical oxidation of ascorbic acid was strongly affected by the solution pH as shown in Figure (3.14). Maximum peak current was observed at PH = 2 (Figure 3.14 b). Therefore, all subsequent measurements were performed at pH = 2. This pH is also suitable for better stability of ascorbic acid.

The results indicated that the peak potential, Ep, shifted to less positive values with increasing pH. Such a behavior suggests that the participation of protons in the electrode process and the acidic dissociation of ascorbic acid occur before the rate determining step [35].

Effect of square wave voltammetric parameters: The electroanalytical method for the determination of ascorbic acid was studied using square wave voltammetry, which is an effective and well-established pulse voltammetric technique suitable for determination of organic compounds [33]. The response obtained by square-wave voltammetry was dependent on parameters such as frequency (f), pulse height (ΔEp) and step potential (ΔEs), which have a combined influence on the peak current. Hence, they were analyzed in order to optimize the experimental parameters for ascorbic acid determination. The square wave parameter optimization was carried out in solutions of 8 mM ascorbic acid in 0.1 M phosphate buffer of pH =2 containing 0.1 mM Na2EDTA.2H2O.

Effect of pulse amplitude: For the investigation of the influence of the pulse amplitude on the analytical signal the parameter was varied between 25 and 150 mV, at 15mV step potential and 60 Hz frequency. The value of the measured current intensity increased with the applied pulse amplitude. An optimum value of 100 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.

Effect of step potential: The influence of step potential, which determines the amount of potential changes between two data points in the experiment, was investigated between 5 and 20 mV at fixed f and Δ Ep. The peak height increased up to 15 mV because the effective scan rate was increased, but at higher values of step potential, the peak heights decreased. Accordingly step potential of 15 mV was chosen for further study. The potential shift is due to the increase in scan rate as a result of increasing step potential.

Effect of Frequency: The effect of square wave frequency on peak current and peak potential of ascorbic acid was studied in the range of 15 - 90 Hz at constant Δ Ep and ΔEs as shown in Figure (3.16). The peak current was found to increase linearly with square wave frequency as shown in Figure (3.16b) and the relation between Ip and f can be represented by the equation 3.8

$$Ip\ (\mu A) = 1.93429\, f + 425.633 \quad (3.8)$$

The correlation coefficient for the expression was 0.994. The peak current increased with the frequency due to the increase in the effective scan rate but the peak shape and baseline were distorted at frequencies higher than 60 Hz and therefore 60 Hz was selected as optimum frequency for further study. This was attributed to the greater contribution of the capacitive current at higher frequencies [36].

The peak potential also shifted to more positive potential with increase in square wave frequency and the plot of Ep *vs* log f was linear and its correlation coefficient was 0.997 as shown in (Figure 3.18). The variation of Ep with log f obeys equation 3.9:

$$Ep\ (mV) = 116.78 \log f + 208.73 \quad (3.9)$$

These observations are in agreement with the properties

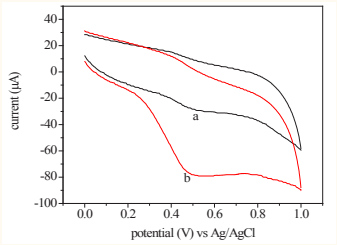

Figure 3.8 Cyclic voltammogram of (a) sample of Axumite and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

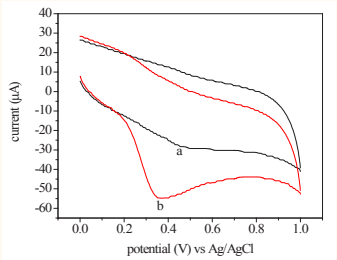

Figure 3.9 Cyclic voltammogram of (a) sample of coca cola and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

of irreversible electrochemical processes which are diffusion controlled [36]. These results support the inferences obtained from cyclic voltammetry studies.

Effect of varying ascorbic acid concentrations: Under the optimum parameters (pH =2, ƒ = 60 Hz, ΔEp = 100 mV, ΔEs = 15 mV), the calibration graph for determination of ascorbic acid was obtained in the concentration range of 1.0-8.0 mM. (Voltammograms for different concentrations of ascorbic acid are shown in Figure (3.18). The regression equation is represented by equation 4.10 with a correlation coefficient of 0.997 as shown in Figure (3.18 b).

$$Ip\,(\mu A) = 51.8457\,CAA + 240.108 \qquad (3.10)$$

b) Calibration plot of peak current *vs* concentration under the optimized parameters.

The peak current increased with increasing concentration indicating diffusion controlled irreversible oxidation process. Accordingly ascorbic acid can be determined using square wave voltammetry.

Application of the SWV method for ascorbic acid determination

Validation of the proposed procedure: The repeatability of the measurement was calculated from five independent runs of ascorbic acid solution. LOD and LOQ were obtained as 0.0815 mM and 0.385 mM, respectively from the calibration graph as shown in Figure (3.18 b).

Application to real sample analysis: In order to analyze the accuracy of the method for ascorbic acid determination in wines and soft drinks, the standard addition method were applied.

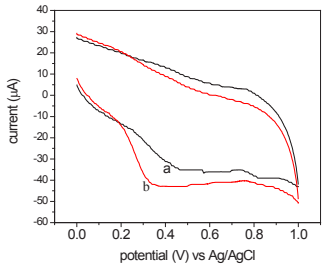

Figure 3.10 Cyclic voltammogram of (a) sample of Pepsi and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

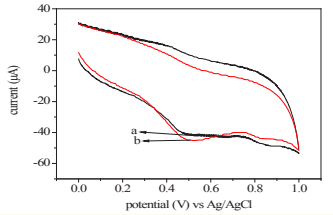

Figure 3.11 Cyclic voltammogram of (a) sample of Mirinda and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

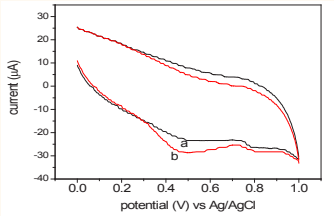

Figure 3.12 Cyclic voltammogram of (a) sample of Fanta orange and (b) a + 8 mL of 8 mM ascorbic acid standard at pH = 3 and scan rate of 100 mVs-1.

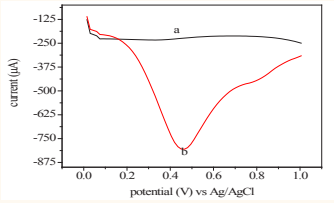

Figure 3.13 Square wave voltammograms for (a) 0.1 M phosphate buffer supporting electrolyte (b) a + 8.0 mM ascorbic acid at PH = 2.0. Experimental conditions: f = 60 Hz, ΔEp = 100 mV, ΔEs = 15 mV.

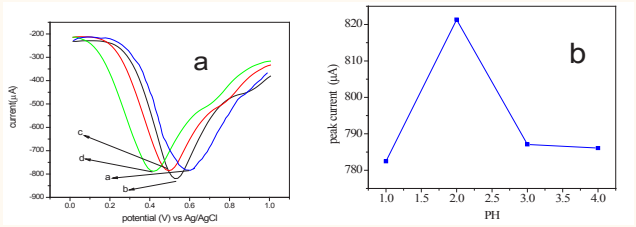

Figure 3.14 Square wave voltammogram of 8 mM ascorbic acid in 0.1M phosphate buffer containing 0.1 mM Na2EDTA.2H2O for pH values of (a) 1, (b) 2, (c) 3, (d) 4. b) Plot of peak current vs pH. Experimental parameters: f = 60 Hz, ΔEp = 100 mV, ΔEs = 15 mV.

The following figures show square wave voltammograms of samples of Kemila, Axumite, Gouder, Mirinda, Pepsi, sprite, coca cola andante orange (a) and a + 8 mL of 8 mM standard ascorbic acid solution (b) under the optimized parameters.

The shapes, baselines and positions of the peak potentials and the increase in peak current are similar to the voltammogram of the standard ascorbic acid.

OSWV determination of degree of recovery for each sample: The degree of recovery for each samples were determined from the above Figures (3.19-3.26), as shown in Table (2) with an excellent recovery value of 100 %. The concentration of ascorbic acid determined in different samples using CV and SWV are compared in Table (3).

The results obtained by square wave voltammetry were greater than the results obtained by cyclic voltammetry for all samples. They are also in a good agreement with the data reported in literature regarding the ascorbic acid content of red wine. The ascorbic acid content of wine obtained by cyclic and differential voltammetry is 15.05 and 16.22 mg/100 mL sample [37].

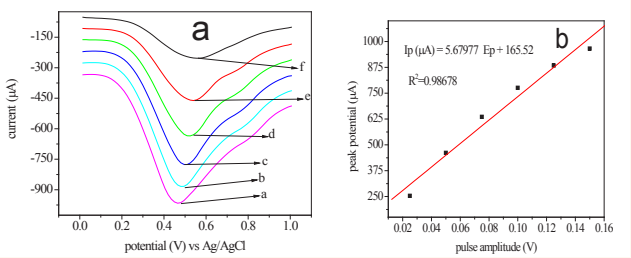

Figure 3.15 Influence of the pulse amplitude on the analytical response at ascorbic acid determination by squarewave voltammetry ; (a) 150 mV, (b)125 mV, (c) 100 mV , (d) 75 mV, (e) 50 mV and (f) 25 mV ; experimental conditions: ΔEs = 15 mV, f= 60 Hz. b) peak current vs pulse amplitude.

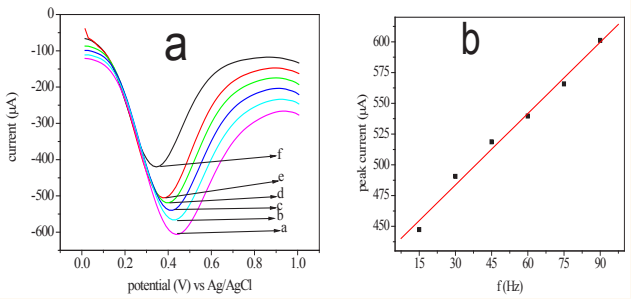

Figure 3.16 Square-wave voltammogram of 8 mM ascorbic acid in 0.1 M phosphate buffer solution (pH = 3) at various frequencies: (a) 90, (b) 75, (c) 60, (d) 45, (e) 30, and (f) 15 Hz. b) plot of peak current vs frequency. Experimental parameters: ΔEp = 100 mV, ΔEs = 15 mV.

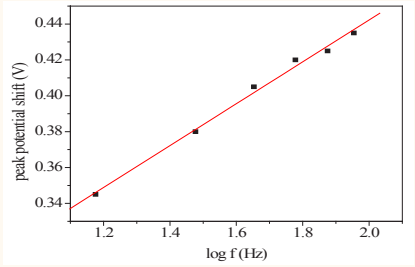

Figure 3.17 Effect of square wave frequency on the peak potential shift of 8 mM ascorbic acid at pH= 2. Experimental parameters: ΔEp = 100 mV, ΔEs = 15 mV.

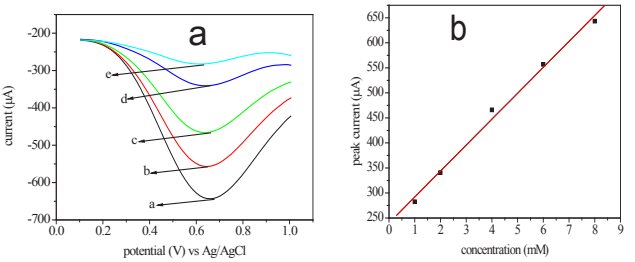

Figure 3.18 Square wave voltammograms of ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at PH = 2.0 for various concentrations: (a) 8.0, (b) 6.0, (c) 4.0, (d) 2.0, and (e) 1.0 mM.

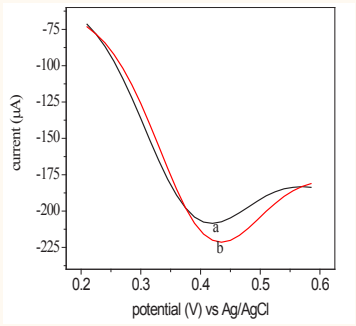

Figure 3.19 Square voltammogram of (a) sample of Kemila and (b) a + 8 mM AA standard at pH = 2. Experimental conditions: ƒ= 60 Hz; ΔEs= 15 mV; ΔEp =100 mV.

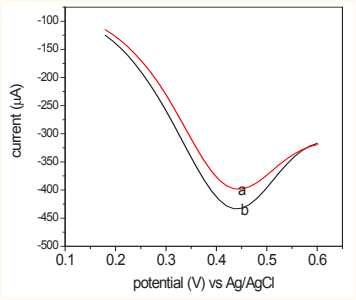

Figure 3.20 Square voltammogram of (a) sample of Axumite and (b) a + 8 mM AA standard at pH = 2. Experimental conditions: ƒ= 60 Hz; ΔEs =15 mV; ΔEp = 100 mV.

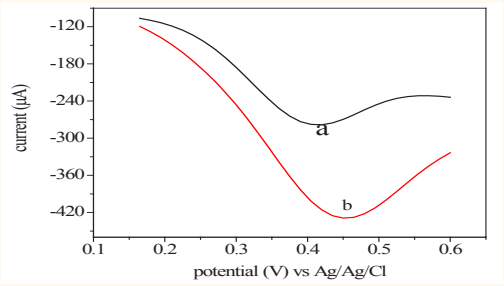

Figure 3.21 Square voltammogram of (a) sample of Gouder and (b) a + 8 mM AA standard at pH 2. Experimental conditions: ƒ= 60 Hz; ΔEs =15 mV; ΔEp= 100 mV.

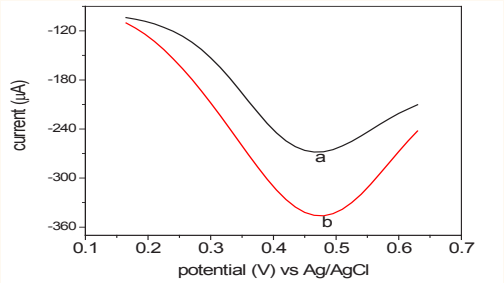

Figure 3.22 Square voltammogram of (a) sample of Mirinda and (b) a + 8 mM AA standard at PH= 2. Experimental conditions: ƒ= 60 Hz; ΔEs =15 mV; ΔEp= 100 mV.

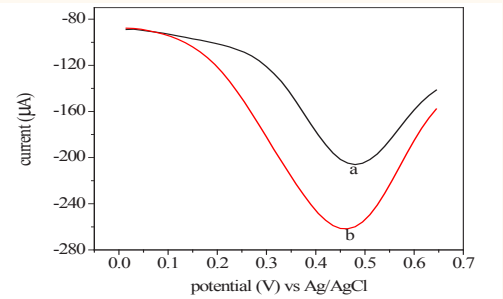

Figure 3.23 Square voltammogram of (a) sample of Pepsi and (b) a + 8 mM AA standard at pH= 2. Experimental conditions: ƒ= 60 Hz; ΔEs= 15 mV; ΔEp= 100 mV.

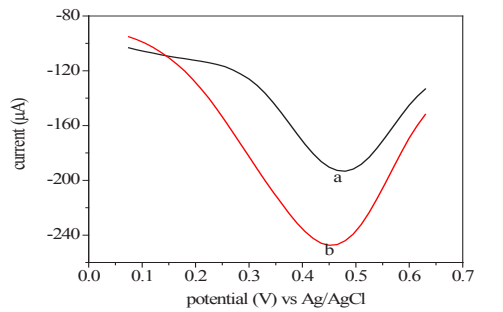

Figure 3.24 Square voltammogram of (a) sample of sprite and (b) a + 8 mM AA standard at pH = 2. Experimental conditions: ƒ= 60 Hz; ΔEs =15 mV; ΔEp= 100 mV.

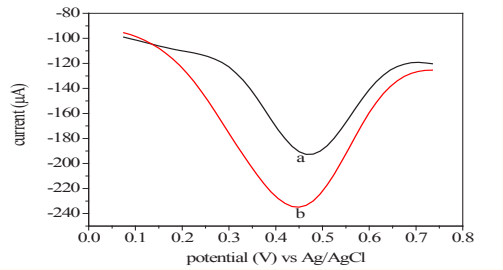

Figure 3.25 Square voltammogram of (a) sample of coca cola and (b) a + 8 mM AA standard at pH = 2. Experimental conditions: ƒ= 60 Hz; ΔEs= 15 mV; ΔEp =100 mV.

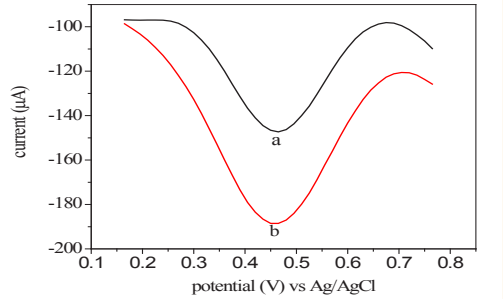

Figure 3.26 Square voltammogram of (a) sample of Fanta orange and (b) a + 8 mM AA standard at pH = 2. Experimental conditions: ƒ= 60 Hz; ΔEs= 15 mV; ΔEp =100 mV.

Table 1: Determination of degree of recovery for wine and soft drink samples.

Nº.	Sample	Concentration before addition of standard to sample (mg/100 mL)	Concentration after addition of 141 mg AA to sample (mg/100 mL)	% recovery
1	Kemila	14.71	152.12	97.45
2	Gouder	13.14	151.98	98.47
3	Sprite	2.76	141.65	98.50
4	Axumite	11.86	152.82	99.97
5	Coca-Cola	3.43	144.00	99.69
6	Pepsi	9.76	149.76	99.29
7	Mirinda	8.98	150.05	100.05
8	Fanta orange	4.04	144.96	99.94

Table 2: Determination of degree of recovery for each sample.

No.	Samples	Concentration before addition of standard solution (gm/100 mL)	Concentration after addition of 141 mg AA to sample (gm/100 mL)	% recovery
1	Kemila	16.34	158.36	100.72
2	Axumite	13.23	153.43	99.43
3	Gouder	14.98	156.00	100.01
4	Mirinda	9.02	148.65	99.03
5	Pepsi	10.27	150.82	99.68
6	Sprite	4.13	143.79	99.05
7	Coca cola	5.03	146.05	100.01
8	Fanta orange	5.88	146.88	100.00

CONCLUSION

The studied voltammetric methods (square wave voltammetry and cyclic voltammetry) for ascorbic acid determination are characterized by sensitivity, rapidity and reproducibility. The degree of accuracy of the investigated voltammetric methods is confirmed by the values obtained for the degree of recovery, which ranged between 97.45 and 100.72%.

The level of vitamin C determined from each wine and soft drinks was in the range of 2.76mg/100 mL for sprite to 14.71 mg/100 mL for Kemila white wine for cyclic voltammetry and 4.13 mg/100 mL for sprite and 16.34 mg/100 mL for Kemila white wine for square wave voltammetry technique. The results obtained by square wave voltammetry were better than the results obtained by cyclic voltammetry when compared with literature value.

RECOMMENDATION

Wines and soft drinks are good nutritional sources of vitamin C. Hence people may drink soft drinks and wines such as Mirinda, Pepsi, coca cola, Fanta, Gouder, Kemila and Axumite

Table 3: Ascorbic acid concentrations in some wines and soft drinks as determined by cyclic voltammetry and square wave voltammetry.

№	Samples	Concentration of AA from CV (mg/100 mL)	Concentration of AA from SWV (mg/100 mL)	% Difference
1	Kemila	14.71	16.34	11.08
2	Axumite	11.86	13.23	11.55
3	Gouder	13.14	14.98	14.00
4	Mirinda	8.98	9.02	0.45
5	Pepsi	9.76	10.27	5.23
6	Sprite	2.76	4.13	49.64
7	Coca cola	3.43	5.03	46.65
8	Fanta orange	4.04	5.88	45.54

to prevent deficiency of vitamin C. Square wave voltammetry is more preferable than cyclic voltammetry for quantitative determination of vitamin C from different types of soft drinks and wines.

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