

Determination of Ascorbic Acid in *Citrus Sinensis* and *Ananas Comosus* Using Poly (3, 4-Ethylenedioxythiophene) Modified Glassy Carbon Electrode

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Abstract: This study describes the preparation and application of a poly (3, 4-Ethylenedioxythiophene) (PEDOT) modified glassy carbon electrode for ascorbic acid determination in fruit samples by square wave voltammetric method. The PEDOT modified electrode was prepared by electrochemical polymerizing of 3, 4-Ethylenedioxythiophene (EDOT) on a glassy carbon electrode in a non-aqueous medium (0.1 M Tetrabutylammoniumhexafluorophosphate and 0.1 M EDOT in acetonitrile). The best performance of the PEDOT modified electrode in 0.1 M phosphate buffer was obtained at pH 5.0. In addition to this other parameters like electrode thickness, square wave amplitude, and square wave frequency were optimized. Under these conditions, an oxidation potential of ascorbic acid was observed at 395.24 mV versus Ag/AgCl/ reference electrode. The square wave voltammetric study indicated that the PEDOT modified electrode showed a very good electrocatalytic activity by reducing the overpotential by 113.69 mV for ascorbic acid oxidation. The modified electrode also showed a linear response range between 50 and 90 μ M. The proposed method was checked by determining the recovery of ascorbic acid in two commercially available tablets and provided an excellent recovery (i.e. 99.43 and 93.80%). Finally the method was applied in Orange and Pineapple fruits and 68.11 \pm 0.012 and 45.46 \pm 0.023 mg ascorbic acid per 100 g of the sample was found in each fruits respectively.

Keywords: Ascorbic Acid, Glassy Carbon Electrode, PEDOT, Square Wave Voltammetry

1. Introduction

Vegetables and fruits are part of man's most important food categories being necessary for the maintenance of life and health. Especially they are a precious source of vitamins, mineral substances and other substances that are necessary for food completion (some proteins with indispensable amino-acids etc.) [1]. Fruits play an important role as protective foods, with therapeutic effects appreciated since antiquity [2]. Today it is known that populations that have a predominantly vegetarian diet show a lower frequency of cardiovascular diseases, cancer, obesity, kidney and liver disease. In traditional medicine, largely confirmed by rigorous clinical research, a large number of fruits are recommended for the treatment of gastrointestinal, cardiovascular, nutrition, metabolism, kidney, respiratory, endocrine, central nervous system and dermatological

diseases [3].

Vitamins are organic nutrients that are required in small quantities for a variety of biochemical functions. They generally cannot be synthesized by the human body and must be supplied in diet. Vitamin C (ascorbic acid) is a water soluble antioxidant which helps protect against cancer, heart diseases and stress and which is found in variable quantities in fruits and vegetables [4]. Antioxidants protect cells from damage caused by unstable molecules known as free radicals [5]. Abubakar *et al* [6] also stated that interest has increased considerably in finding naturally occurring antioxidants from plants because of their potential in health promotion and disease prevention, and their high safety and consumer acceptability. In addition vitamin C is required for the production of collagen in the connective tissue, teeth and bones [4]. There is a wide variety of food containing vitamin C. The public generally known best sources of vitamin C are citrus fruits such as Orange and their juices. A wide variety of

other foods also contain sufficient quantities of vitamin C, such as Pineapples and Sweet peppers [7].

Therefore, measuring the quality, safety, nutrition and stability of food products is the primary concern now a day's and the importance and awareness of nutrition in the public health issues have resulted in increased demands for knowledge of the nutrient content of the foods. Accordingly, there has been a growing tendency to introduce more accurate, reproducible and objective procedures for the evaluation of quality in foods in place of the old fashion, slow and relative inadequate methods in food analysis [8]. Therefore, 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 instability 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 later reaction is irreversible [9].

Traditional methods for ascorbic acid assessment involve titration with an oxidant solution; Dichlorophenol indophenols (DCPIP), Potassium iodate or Bromate. Chromatographic methods, particularly High performance liquid chromatography (HPLC) with electrochemical detection, have turned out to be a selective and sensitive method for ascorbic acid assessment in foodstuffs and biological fluids. Fluorimetric methods and Ultra violet visible (UV-VIS) absorbance-based determinations were also used for ascorbic acid estimation. Now a day's electroanalytical methods such as cyclic voltammetry, differential pulse voltammetry, square wave voltammetry, etc have been raised towards determination of ascorbic acid. But these methods need high voltage and this high voltage can be reduced using chemically modified electrodes which give simple, stable, and selective, with a short response time and ahigh sensitivity. Therefore; this study was aimed at using modified glassy carbon electrode which is poly (3, 4-Ethylenedioxythiophene) (PEDOT) and applying on the determination of ascorbic acid in *Citrus Sinensis* and *Ananas Comosus* by square wave voltammetry.

2. Materials and Methods

2.1. Chemicals and Reagents

The reagents and chemicals used were ascorbic acid (GPR, England), disodium hydrogen phosphate (Lamberg chemicals, India), sodium dihydrogen phosphate anhydrous (Nice, India), phosphoric acid (Nice, India), sodium hydroxide (CDH, India), acetonitrile (Sigma-Aldrich), Tetrabutylammonium hexafluoro phosphate (TBAHFP) (Sigma-Aldrich chemical company), 3, 4-ethylenedioxythiophene (Aldrich chemical company), polishing alumina powder (BAS 0.05micron, USA) and tablets: Epharm (500 mg, Ethiopia) and Chewette (100 mg, Malasiya) were used in the experiment. All chemicals were used as received. Distilled water was used for the preparation of all solutions. For all of the experiments a mixture of 0.1 M

Na_2HPO_4 and 0.1 M NaH_2PO_4 buffer solution was used.

2.2. Apparatus

The experiments were carried out using Bioanalytical system (BASCV-50W, USA) electrochemical voltammetric analyzer coupled with personal dell computer with conventional three electrodes consisting of poly (3, 4-Ethylenedioxythiophene) (PEDOT) modified glassy carbon electrode as working electrode, silver-silver chloride electrode and Quasi-reversible (Silver wire) as reference electrode and platinum electrode as counter electrode. Digital pH meter (Milwaukee model pH 600 meter) was used to measure the pH of the buffer solution. Stainless steel knife was used for peeling the fruits. Juice squeezer (Nima, NM 343, Japan) was used for squeezing the juice from fruits. Centrifuge (model 80-2) was used for centrifuging the solution in order to make clear solution.

2.3. Preparation of Working Electrode

To prepare poly (3,4-Ethylenedioxythiophene), 0.1M Tetrabutylammoniumhexafluorophosphate (TBAHFP) was prepared by taking 1.935 gram in 50 ml acetonitrile used as a solvent. Then 0.01M 3, 4-Ethylenedioxythiophene (EDOT) solution was prepared in 0.1M TBAHFP in acetonitrile. Before modification, the glassy carbon electrode (GCE) was polished with polishing alumina (0.05micron) powder and then rinsed with distilled water. The EDOT was electropolymerized on the dried GCE by running cyclic voltammetry from 0 to 1300 mV for ten cycles. Then the modified electrode was cycled again for ten cycles in 0.1M TBAHFP/acetonitrile solution for stabilization. After electrochemical polymerization the PEDOT modified GCE was carefully washed with acetonitrile.

2.4. Preparation of a Standard Solution

1.0 mM ascorbic acid solution was prepared by dissolving 0.0088 g of ascorbic acid in 50 ml of 0.1M buffer solution for the entire experiment and 100 μM fresh stock solution of ascorbic acid was prepared by dissolving 0.00176g of ascorbic acid in 100 ml of 0.1M buffer solution at pH=5 for real sample analysis. From stock solution, serial dilutions were made in 0.1M phosphate buffer solution by taking appropriate amounts of standard ascorbic acid solutions to obtain 100 μM , 90 μM , 80 μM , 70 μM , 60 μM , 50 μM , 40 μM , 30 μM , 20 μM and 10 μM ascorbic acid solutions. These solutions were used for calibration curve. The potential of each measurement were scanned between 0 to 600 mV underscan rate of 100 mV/s. The anodic peak currents for the electrochemical oxidation of ascorbic acid were recorded.

2.5. Fruit Sample Collection and Sample Preparation

For real sample measurements, some Pineapple and Orange average sized fruits were collected from Hawassa Etfuit markets. Fruits of these samples were washed with tap water followed by distilled water in order to remove dust particles on the surface of the fruits. The juice from fruits

was extracted using juice squeezer and the extracted juice was transferred into a beaker. The contents of the flask were centrifuged with a centrifuge (centrifuge model 80-2) and finally filtered with filter paper (Whatman No.541) until a clear solution was obtained. The obtained clear sample solution was transferred into volumetric flasks. From each juices 3ml of the clear solution was immediately added to 100 ml of 0.1 M phosphate buffer solution (pH = 5). The potential of all solutions were scanned as described in the standard ascorbic acid solutions. The ascorbic acid content of the fruit samples were determined by measuring the peak current from the calibration curves and expressed in milligrams per 100 g of sample.

3. Results and Discussion

3.1. Electrochemical Polymerization of EDOT to PEDOT

Electrochemical polymerization of EDOT to PEDOT on this research has been done in an organic solution of Tetra butyl ammonium hexafluorophosphate in acetonitrile. The electrochemical polymerization was done by applying a potential of -1900 to 2300 mV and the respective cyclic voltammogram of the electrochemical polymerization of the monomer was depicted in [Figure 1] in which the inset graph showed the characteristic of monomer oxidation.

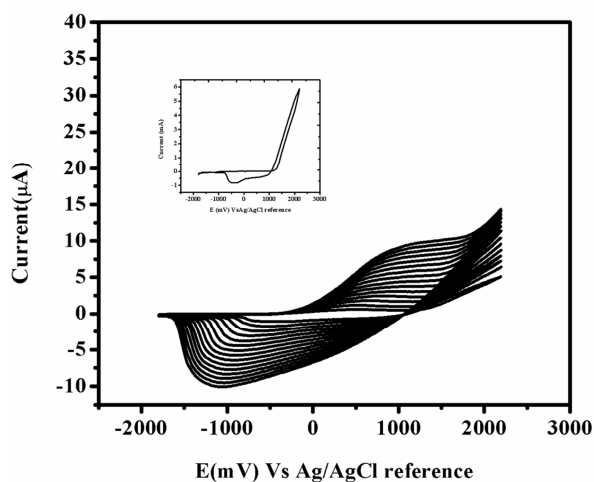


Figure 1. Electrochemical polymerization of EDOT to PEDOT.

3.2. Comparison of the Modified Glassy Carbon Electrode with Bare Glassy Carbon Electrode

The PEDOT modified glassy carbon electrode was also compared with bare glassy carbon electrode and the result is shown in [Figure 2]. [Figure 2] curve (a); showed the cyclic voltammogram of PEDOT modified glassy carbon electrode in 0.1M phosphate buffer solution at pH=5 in the absence of ascorbic acid at a scan rate of 100 mV/s. The result indicated that there is no anodic peak at the blank solution (solution without ascorbic acid), this also indicated that there was no peak interference of the PEDOT at the oxidation peak current of ascorbic acid. In the same figure curve (b); showed the

cyclic voltammogram of 1.0 mM of ascorbic acid in phosphate buffer solution at bare glassy carbon electrode in which the electrochemical oxidation of ascorbic acid was observed with anodic peak current at 0.073 mA and high anodic potential at 508.93 mV and this was a large potential as compared to the potential obtained from modified electrode. Whereas curve (c) showed the catalytic oxidation of ascorbic acid at the glassy carbon electrode modified with PEDOT. And the result showed that the anodic peak current was markedly enhanced to 0.089 mA and the anodic peak potential was shifted negatively to 395.24 mV. But no cathodic peak current was found, which indicated that there is an irreversible heterogeneous charge transfer reaction in the system. So the possible explanation for the negative shift observed in the oxidation peak potential of ascorbic acid could be due to the prevention of the electrode surface fouling by the oxidation product. The remarkable enhancement in the anodic peak current and lowering of anodic potential provided clear evidence of the catalytic effect of PEDOT modified glassy carbon electrode towards the detection of ascorbic acid.

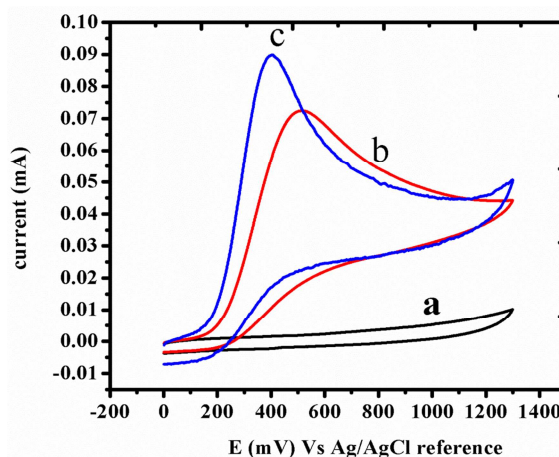


Figure 2. Cyclic voltammogram of 0.1 M phosphate buffer solution at PEDOT modified glassy carbon electrode (a), 1.0 mM ascorbic acid at bare glassy carbon electrode (b) 1.0 mM ascorbic acid at PEDOT modified glassy carbon electrode and (c) at pH of 5 and scan rate of 100 mV/s.

3.3. Effect of PEDOT Film Thickness

In order to find an excellent thickness of the modifier, optimization was done using 3, 5, 7, 9, 10, 15, 17 and 20 number of cycles of polymerization. The optimal thickness of the modifier which produced the best electrochemical signal in terms of anodic peak signal was obtained at 10 cycles. For cycles higher than 10 the anodic peak current became decreased. This might be due to the increase of thickness of the polymer (PEDOT) beyond the optimum in the electrode and the consequent reduction of the electrical conductivity of the electrode. Therefore in the present study the best thickness of the modifier for the electrode was found to be the one produced with 10 cycles of electropolymerization [Figure 3].

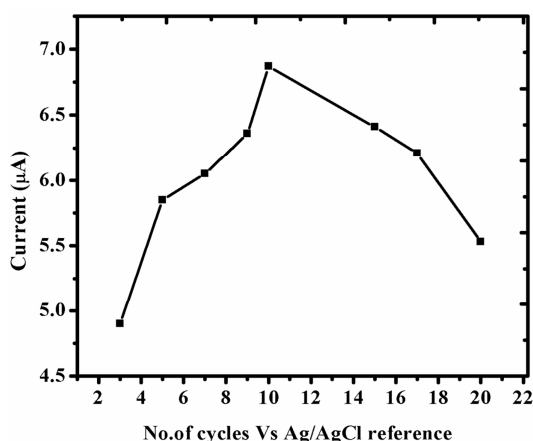


Figure 3. The effect of PEDOT film thickness at PEDOT modified glassy carbon electrode on the cyclic voltammetric peak current of 1.0 mM ascorbic acid in 0.1M phosphate buffer solution of pH = 5 at a scan rate of 100 mV/s.

3.4. Effect of Scan Rate

For an irreversible system, the peak current and peak potential are affected by the scan rate and it will be dependent on potential scan rate. Therefore; in the present study the effect of scan rate on the peak current of ascorbic acid at PEDOT modified glassy carbon electrode was studied between scan rates of 20 - 500 mV/s while maintaining other parameters constant. And the result showed that the peak current increased with increasing scan rate [Figure 4]. Mehmet *et al* [10] also stated that linear increase of the anodic peak current with the increase of scan rate indicated that transference charge between the species within the polymeric film takes place more rapidly.

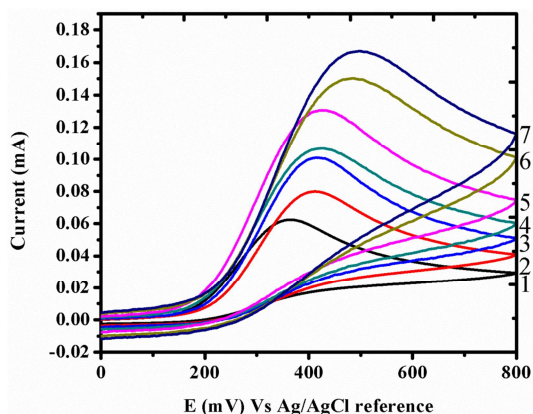


Figure 4. Cyclic voltammogram of 1.0 mM ascorbic acid in 0.1M phosphate buffer solution at different scan rates; (1) 20 mV/s, (2) 40 mV/s, (3) 60 mV/s, (4) 100 mV/s, (5) 150 mV/s, (6) 300 mV/s (7) 500 mV/s at pH = 5.

3.5. Optimization of Square Wave Voltammetric Techniques

Optimization of Square wave voltammetric was done in order to deal the response of PEDOT modified glassy carbon electrode for ascorbic acid oxidation using pH, scan rate, Square wave amplitude and Square wave frequency and the optimized results were presented in [Table 1].

Table 1. Optimum experimental parameters for the determination of ascorbic acid by Square wave voltammetry at PEDOT modified glassy carbon electrode.

Parameters	Optimum value
Modified electrode film thickness	10 cycle
pH	5
Scan rate	100 mV/s
Square wave amplitude	25 mV
Square wave frequency	12 Hz

The influence of pH on the catalytic oxidation of ascorbic acid was investigated in the pH range of 3-7. And the result indicated that the highest sensitivity was observed at pH 5 [Figure 5].

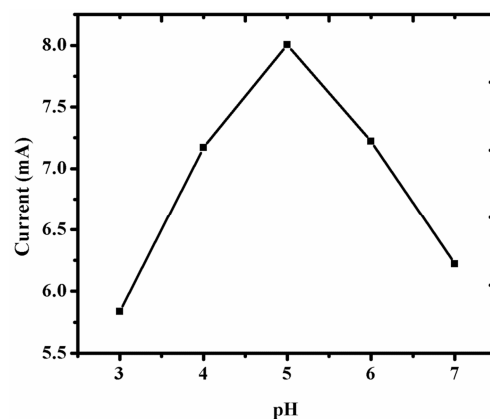


Figure 5. Effect of pH on the anodic peak current of 1.0 mM ascorbic acid solution at PEDOT modified glassy carbon electrode in 0.1 M phosphate buffer solution at a scan rate of 100 mV/s.

The effect of square wave amplitude was also optimized by taking 5, 10, 15, 20, 25, 30 and 40 mV and the result showed that the current was continuously increased starting from 5 mV to 25 mV but decreased from 30 mV onwards [Figure 6]. As a result 25 mV was considered as the optimal amplitude. Other parameters which needs optimization condition was optimized at this optimized square wave amplitude.

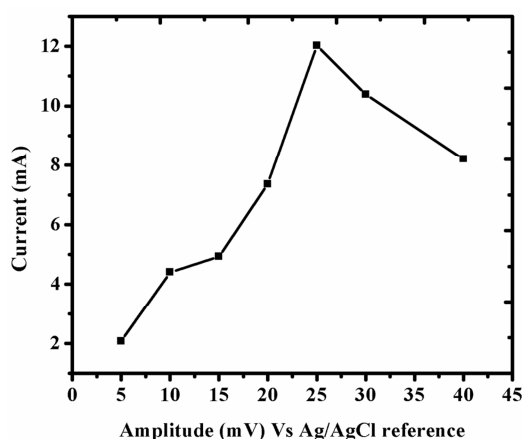


Figure 6. The effect of square wave amplitude of the solution on the anodic peak current of 1.0 mM ascorbic acid solution at PEDOT modified glassy carbon electrode in 0.1 M phosphate buffer solution of pH = 5 at a scan rate of 100 mV/s.

The effect of square wave frequency was also studied and optimized by taking frequency of 2, 4, 6, 8, 10, 12 and 14 Hz and keeping other parameters constant.

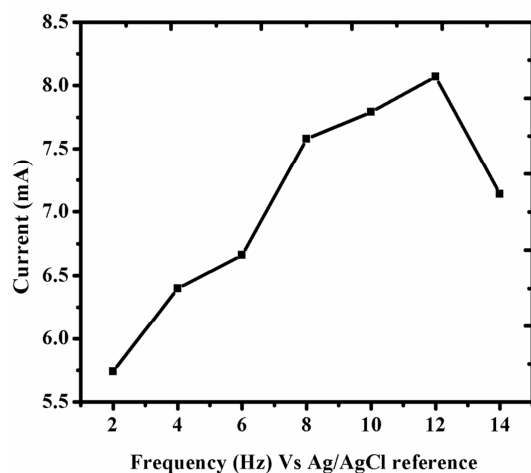


Figure 7. The effect of Square wave frequency of the solution on the anodic peak current of 1.0 mM ascorbic acid solution at PEDOT modified glassy carbon electrode in 0.1 M phosphate buffer solution of pH = 5 at a scan rate of 100 mV/s.

As can be seen from [Figure 7], the result showed that the magnitude of the peak current increased with increasing the square wave frequency; however, the slope diverted to a lower value after frequency of 12 Hz. This indicated that the dependence of the peak current on frequency is being distorted due to an accompanying peak broadening. In addition, it was observed that the peak current was unstable at higher frequencies and this can also affects the reproducibility of the measurement. As a result, 12 Hz was chosen as the optimum square wave frequency for the subsequent experiments.

On this study the effect of ascorbic acid concentration in the PEDOT modified glassy carbon electrode was also studied. It was performed at different concentrations as shown in [Figure 8]. The result revealed that the Square wave voltammogram of the anodic peak current increased rapidly and linearly with increasing concentration of ascorbic acid from 50 to 90 μ M. This indicated that the oxidation peak current of ascorbic acid was linearly dependent on the ascorbic acid concentration. Thus the increased of anodic peak current as concentration of ascorbic acid increased showed the sensitivity and stability nature of the modified electrode in the presence of ascorbic acid and this enhanced the suitability of the electrode for the determination of ascorbic acid.

Table 3. Comparison of the analytical data obtained by some modified and standard electrodes proposed for the determination of ascorbic acid.

Electrode material	Modifier	Method	Linear range	LOD	References
CPE		DPV	0.31 – 20 mM	88 μ M	[11]
GCE		DPV	6 \times 10 ⁻⁶ - 8 \times 10 ⁻⁴ M	51.7 μ M	[9]
CPE	EBTMCPE	DPV	0 - 9 \times 10 ⁻⁵ M	27 μ M	[12]
GCE	SWCNT/WO ₃	CV	20 – 100 μ M	80 μ M	[13]
GCE	PEDOT	SWV	50 – 90 μ M	23.3 μ M	Present work

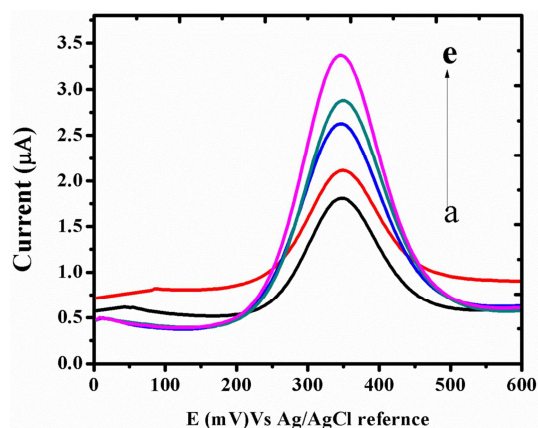


Figure 8. Square wave voltammogram obtained for different ascorbic acid concentrations expressed as μ M: (a) 50 (b) 60 (c) 70 (d) 80 (e) 90 μ M in 0.1 M phosphate buffer solution of pH = 5 at scan rate of 100 mV/s.

3.6. Method Validation

Since there was no reference material in the laboratory the modified electrode was validated by determination of the degree of recovery of ascorbic acids purchased from pharmaceutical drug shops. These tablets were Epharm (500 mg) from Ethiopia and Chewette (100 mg) from Malaysia. The detected amount of ascorbic acid in the tablets using SWV gave an excellent recovery as shown in [Table 2].

Table 2. Amount of ascorbic acid detected and calculated recovery using SWV.

Pharmaceutical Formulations	Labeled amount of AA in mg	AA Obtained in mg	% Recovery
Epharm	500 mg	469.04 \pm 0.065	93.80
Chewette	100 mg	99.43 \pm 0.045	99.43

As shown in [Table 2], the degrees of recovery of ascorbic acid (AA) in the two tablets were 93.80% and 99.43%, which indicated the modified electrode is very suitable for the determination of the amount of ascorbic acid.

In addition to this the linear range and limit of detection (LOD) of ascorbic acid at PEDOT modified glassy carbon using SWV of this work was compared with other standard voltammetric methods. As it can be seen from [Table 3] the electrochemical sensor of square wave voltammetry based on PEDOT modified glassy carbon electrode gave a comparable and in some case better analytical performance and detection limit as compared to results obtained from the other standard methods.

3.7. Determination of Ascorbic Acid in Orange and Pineapple at PEDOT Modified Glassy Carbon Electrode

The modified electrode was applied to the determination of ascorbic acid concentration in freshly squeezed Orange and Pineapple fruits. The experimental result showed that the amount of ascorbic acid in both Orange and Pineapple were 0.06811 ± 0.00 and 0.04546 ± 0.02 g/100g solution respectively [Table 3]. The absence of cathodic peak current also indicated the irreversible oxidation character of Orange and Pineapple. The obtained shape and peak position of the fruits were similar to the standard ascorbic acid solution. It has been also found that there was a linear dependence of peak current (I_p) on ascorbic acid concentration. Generally the amounts of ascorbic acid in the samples were successfully determined by on the modified electrode using square wave voltammetry technique.

Table 4. Amount of ascorbic acid obtained by SWV from Orange and Pineapple fruits.

S.No	Sample		mg/ 100 gram of AA
	Common name	Botanical name	
1	Orange	<i>Citrus Sinensis</i>	68 ± 0.012 mg
2	Pineapple	<i>Ananas Comosus</i>	45 ± 0.023 mg

Two sided t-tests were performed by using Microsoft Excel for statistically significant difference in the mean value of ascorbic acid concentrations between the two samples. At 95% confidence interval difference in mean values were accepted as they were being statistically significantly different at ($P = 0.05$). Thus it can be concluded that Orange has more ascorbic acid content than Pineapple.

The obtained values were also compared with other literatures done by other methods. As shown from [Table 4] the present results were somewhat very good results as compared to others values from the literature. This indicated that the PEDOT modified glassy carbon is an excellent modifier in the determination of ascorbic acid in orange and pineapple fruits.

Table 5. Comparisons of the obtained amount of AA with other literatures.

Types of fruit	Method used for determination	Obtained amount of AA in mg/100 g	References
Orange	CV	64.0 mg	[14]
Orange	DPV	40.68 mg	[15]
Orange	DCPIP	30.48 mg	[16]
Orange	SWV	68.11 ± 0.012 mg	Present work
Pineapple	Titration by 1% Sodium Metabisulphite	32.5 ± 1.00 mg	[17]
Pineapple	CV	35.2 mg	[14]
Pineapple	SWV	45.46 ± 0.023 mg	Present work

4. Conclusions

The electrochemical oxidation of ascorbic acid was successfully studied by SWV with PEDOT modified glassy carbon electrode. The linear working range was found to be $50 - 90 \mu\text{M}$ and the detection limit was greatly improved to allow a sensitive detection of ascorbic acid. The very low concentration detection limit and its high sensitivity suggested that the modified glassy carbon electrode can act as a useful electrode material for the development of electrochemical sensor for ascorbic acid. The effect of film thickness of modified electrode, pH, concentration of ascorbic acid and scan rate was observed on voltammetric responses. The optimum conditions for the oxidation of ascorbic acid were determined. These after optimization of all these parameters validation of the modified electrode was done in terms of percentage recovery. And the values were to be 93.80 and 99.43% which is very good recovery. Finally the PEDOT modified glassy carbon electrode was applied in the determination of ascorbic acid in Orange and Pineapple fruit juices using SWV and was found 68.11 ± 0.012 and 45.46 ± 0.023 mg of ascorbic acid per 100 gram of fruit juice respectively. Finally depending on the output of the experimental data the PEDOT modified glassy carbon electrode was found preferable for the determination of ascorbic acid in fruits.

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