

ASCORBIC ACID DETERMINATION IN FRESH AND COMMERCIAL FRUIT JUICES BY DIFFERENTIAL STRIPPING VOLTAMMETRIC TECHNIQUE AT A GLASSY CARBON ELECTRODE

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ABSTRACT. Ascorbic acid, also known as Vitamin C cannot be synthesized by humans. Ascorbic acid is commonly found in a variety of vegetables and fruits such as mangoes, oranges, broccolis and lettuce. Hence, vegetables and fruits become the main sources of ascorbic acid to meet dietary intake. The differential pulse anodic stripping voltammetry (DPASV) technique using glassy carbon electrode (GCE) as a working electrode and phosphate buffer at pH 4.2 as a supporting electrolyte has been proposed for ascorbic acid determination in natural and commercial fruit juices. The optimum instrumental conditions for electroanalytical determination of ascorbic acid by the proposed DPASV technique were initial potential (E_i) = 0 V, end potential (E_f) = 0.8 V, accumulation time (t_{acc}) = 60 s, scan rate (ν) = 0.125 V/s and pulse amplitude = 0.150 V. The anodic peak appeared at 0.3598 V. The curve was linear from 0.028 to 1.703 mM ($R^2=0.9999$) with a detection limit of 0.0114 mM. The precisions in terms of relative standard deviation (RSD) were 1.30%, 0.50% and 0.06%, respectively. The ruggedness of the proposed DPASV technique was tested with statistical F-test. Satisfactory recoveries ranging from 73.65±1.70% to 101.93±1.65% were obtained for three different known concentrations of AA in the fruit juice samples. It can be concluded that the proposed technique is precise, accurate, rugged, low cost, fast and has the potential to be an alternative method for routine analysis of ascorbic acid in natural and commercial fruit juices.

KEYWORDS: Ascorbic Acid, Commercial Fruit Juice, Glassy Carbon Electrode, Voltammetry

INTRODUCTION

Ascorbic acid (AA) was discovered in the twentieth century in 1907 by Holst and Frolich, as a solution for scurvy disease. During that year, the disease had been reported to be 'ship beriberi'. An experiment was carried out on guinea pigs by feeding them with fresh apples, fresh potatoes, fresh cabbage and fresh lemon juices which are high in the AA. The guinea pigs were also fed with a simple diet like oat, barley and wheat. The results indicated that deficiency of AA caused scurvy (Packer & Fuchs, 1997). The AA is important for collagen production, a protein which gives structure to bones, cartilages, muscles and blood vessels as well as an antioxidant and free radical scavenger (Sona *et al.*, 2015; Yilmaz *et al.*, 2008). The AA also helps in iron absorption and maintains capillaries, bones and teeth (Pisoschi *et al.*, 2011). The main natural sources of AA are various fruits and vegetables especially kiwi, mangoes, papayas, lettuce, tomatoes, peppers, strawberries, cantaloupe and broccolis (Sadia *et al.*, 2014). The AA is also known as vitamin C, L-ascorbic and 2,3-didehydro-L-threohexono-1,4-lactone as well as 3-keto-L-gulofuranolactone. It has a chemical formula of $C_6H_8O_6$ and a molecular weight of 176 gmol^{-1} while its chemical structure is shown in Figure 1.

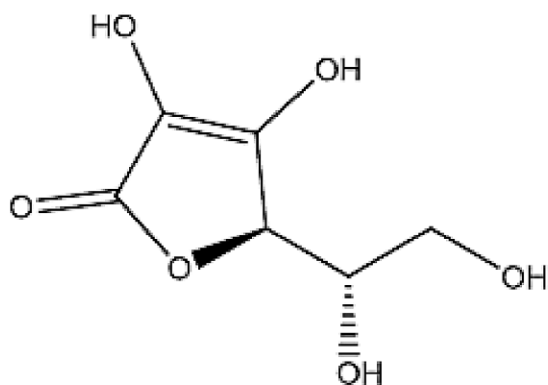


Figure 1: Chemical structure of ascorbic acid

The AA content tends to be lost during processing, storing and packaging since it is highly sensitive to light, heat, temperature and oxygen (Gazdik *et al.*, 2008). Storage of commercial fruit juices in closed containers at ambient temperature for four months showed that the AA loss ranged from 29% to 41% (Morris *et al.*, 2011). Meanwhile, in open containers and in the refrigerator for 31 days, the loss of AA was about 60% to 67%. About 12.5% of AA content has been lost for the commercial fruit juice in open containers which was stored outside the refrigerator for 10 days (Morris *et al.*, 2011). For the same period, about 9% of the AA content has been lost for refrigerated commercial fruit juices (Morris *et al.*, 2011). Hence, it is very important to have a simple, very sensitive, precise, accurate, rugged, low cost and fast method for determining the exact content of AA in the natural and commercial fruit juice in the markets.

Various analytical techniques have been applied for AA determination such as chromatographic method, particularly high-performance liquid chromatography (HPLC) (Klimczak *et al.*, 2015; Tyagi *et al.*, 2014; Valente *et al.*, 2011), titrimetric method (Tareen *et al.*, 2015; Nweze *et al.*, 2015; Dioha *et al.*, 2011), spectrometric method (Liamas *et al.*, 2011). However, HPLC method uses expensive equipment, requires a tedious sample pre-treatment, uses of toxic organic solvent as mobile phase and various harmful reagents (Zhang *et al.*, 2018). In the titrimetric method, difficulties are encountered with titrants and interferences often occur with coloured samples which lead to lack of specificity (Sona *et al.*, 2015). Meanwhile, in direct spectrophotometry, there is a matrix effect in the ultraviolet (UV) region since many organic compounds in samples may also exhibit UV absorbance during measurement (Ogunlesi *et al.*, 2010). In addition, the spectrophotometry is beyond the specific limit and the intensity of absorption is not directly proportional to the concentration (Raghu *et al.*, 2007).

Differential pulse voltammetric (DPV) technique has been used to determine AA in fruit juices and wine based on its oxidation at carbon paste electrodes (Pisoschi *et al.*, 2011). Cyclic and square wave voltammetric techniques were also used for electrochemical behaviour investigation and determination of AA at a glassy carbon electrode in which the methods were tested in some beverages and fresh edible vegetables (Aabraha & Sargawie, 2014). Determination of AA in real samples of tropical fruits was carried out by cyclic voltammetric technique using glassy carbon electrode in phosphate buffer at pH 2 (Okiei *et al.*, 2009).

Differential pulse stripping voltammetric (DPSV) technique has good discrimination against capacitive current which resulting in improved resolution, higher sensitivity, low detection limit and effectiveness to be applied in the analysis of various electrochemically active compounds at their trace amounts at a reasonable cost and through a simple and fast analysis (Skrovanko *et al.*, 2015). The purpose of this study is to optimize and validate a proposed differential pulse anodic stripping voltammetric (DPASV) technique using bare glassy carbon electrode as a working electrode and phosphate buffer solution (pH 4.2) as a supporting electrolyte for quantitative analysis of AA in the several commercial and fresh fruit juices.

METHODOLOGY

Materials

All chemicals used were analytical grade reagents. The AA standard ($MW = 176 \text{ g mol}^{-1}$) with 99% purity was obtained from Sigma Aldrich, UK. All solutions were prepared in deionized water. For the preparation of 2.84 mM AA stock solution, the AA powder was dissolved in a 100 mL volumetric flask. The standard working solution series were prepared by carrying out dilution of the 2.84 mM stock solution. All prepared stock and standard working solutions were protected from light and used within 24 hours to avoid decomposition. Phosphate buffer solution (PBS) was prepared by adding and dissolving 2.70 mL of ortho phosphoric acid, 27.218 g of potassium dihydrogen phosphate, 71.630 g disodium hydrogen phosphate in the 1000 mL of volumetric flask. Sodium hydroxide with a concentration of 0.1 M was used to adjust the pH of the PBS solution to the 4.2 (Pisoschi *et al.*, 2011).

Instrumentation

The voltammetric determination was carried out using Autolab Potentiostat (Metrohm, Switzerland) that consisted of a three-electrodes system. A glassy carbon electrode (GCE) as the working electrode (WE), a platinum wire as the counter electrode (CE) and an Ag/AgCl (3M KCl) as the reference electrode (RE). The GCE was polished with alumina on an alumina pad and then rinsed with deionized water before being used for measurements. The Autolab Potentiostat was connected to a computer, installed with NOVA 1.1 software for information processing. The pH meter (Hanna Instruments, UK) was employed for all pH measurements.

Voltammetric measurement

Validation of proposed DPASV technique

The validation of the proposed DPASV technique was carried out by applying optimum operational instrumental parameters applied were initial potential (E_i) = 0 V, final potential (E_f) = 0.8 V, accumulation time (t_{acc}) = 60 s, scan rate (ν) = 125 mV/s, accumulation potential (E_{acc}) = 0 mV and pulse amplitude = 150 mV, as previously studied by Nur Syamimi *et al.*, (2020).

An appropriate linearity range with acceptable correlation coefficient (R^2), limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and recovery of spiked AA standard into the commercial and fresh fruit juices were analyzed in order to verify the suitability of the proposed DPASV technique for AA determination as proposed by Miranda *et al.*, (2012). The linearity was investigated in the range of 0.028 to 1.703 mM AA standard solution in the electrochemical vessel. The LOD was estimated by additional lower concentration of the AA standard solution until obtaining a response that was significantly different from the response of PBS solution at pH 4.2. The LOQ was calculated by the equations; $LOD = 3 SD/m$ and the value of the LOQ was 3.333 times the value of the obtained LOD.

Three different concentrations (0.028, 0.284 and 0.852 mM) of AA standard solution were applied for intra-day and inter-day precision with three replicate measurements ($n=3$). The precision of the proposed DPASV technique was determined in terms of the relative standard deviation (RSD). The accuracy of the proposed DPASV technique was examined by spiking the three known volumes of AA standard solution which gave a final concentration of 0.028, 0.284 and 0.852 mM in the electrochemical vessel. The actual concentrations of AA standard solution found in the electrochemical cell by the proposed DPASV technique were calculated using the regression equation achieved in linearity range study. The ruggedness of the proposed DPASV technique was investigated with three

replicate (n=3) measurements using the same instrument (Metrohm, Autolab Potentiostat) which operated by two different analysts under the same optimum parameters. Statistical *F*-test was carried out for the ruggedness.

Collection and preservation of fruit juices

Blackcurrant, orange, mango, lychee and guava commercial fruit juices were bought at the nearest mart in Jengka, Pahang. All these commercial fruit juices were centrifuged before being analyzed by the proposed DPASV technique (Pisoschi *et al.*, 2011). Meanwhile, an average-sized pineapple and orange, also bought in Jengka, Pahang was peeled and pressed. The obtained pineapple and orange juice were then being centrifuged and analyzed within one hour after the sample preparation in order to avoid degradation of AA which could contribute to inaccurate voltammetric measurements.

Recovery studies of spiked AA standard in fruit juices

The recovery of AA standard solution in the fruit juices (by pressing) and commercial fruit juices was determined by spiking 1.0 mL of the juices into the measuring cell containing PBS solution at pH 4.2. This study was carried out with three replicates (n=3). Recovered concentrations of the spiked AA standard solution in the samples were calculated using the regression equation from a calibration curve and from the following formula;

$$\text{Recovery (\%)} = (Q_{\text{DET}} - Q_{\text{P}}) / Q_{\text{ADD}} \times 100$$

Where, Q_{DET} represents concentration of AA determined in the juices, Q_{P} represents concentration of AA previously present in the juices and Q_{ADD} represents the concentration of AA added in the juices.

RESULTS AND DISCUSSION

Validation of the proposed DPASV technique

Linearity, limit of detection (LOD) and limit of quantification (LOQ)

The constructed calibration curve was linear from 0.028 mM to 1.703 mM with equation of $I_p \text{ (nA)} = 19337x - 219.4$ at 14 different concentrations of the AA, as represented in Figure 5. The acceptable correlation coefficient (R^2) had been achieved, which was 0.9999. The LOD was 0.0114 mM and the LOQ was 0.0379 mM.

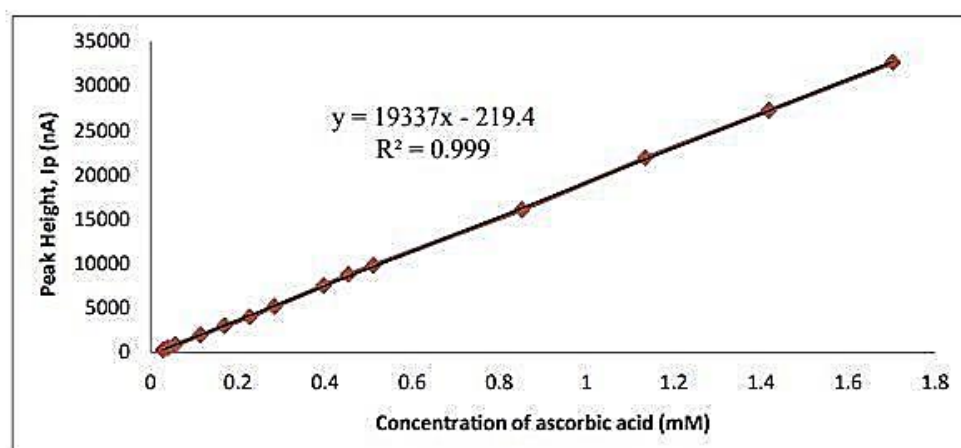


Figure 5: Linear curve of I_p against concentrations of AA in phosphate buffer solution pH 4.2

Precision

The precision of the proposed DPASV technique for AA analysis in terms of RSD was determined using 0.028 mM, 0.284 mM and 0.852 mM AA standard solution in three replicates ($n=3$) measurements. The RSD values obtained were 1.30%, 0.50% and 0.06% for respective concentrations. The proposed DPASV technique was considered precise as the obtained RSD values were less than 2% (Ngai *et al.*, 2013).

Accuracy

The recoveries of 93.58%, 98.54% and 98.78% were respectively achieved for the spiked concentrations of 0.028 mM, 0.284 mM and 0.852 mM AA standard solution, as shown in Figure 6. These results indicate that the proposed DPASV technique was considered accurate since satisfactory recoveries were successfully achieved (Radi *et al.*, 2011).

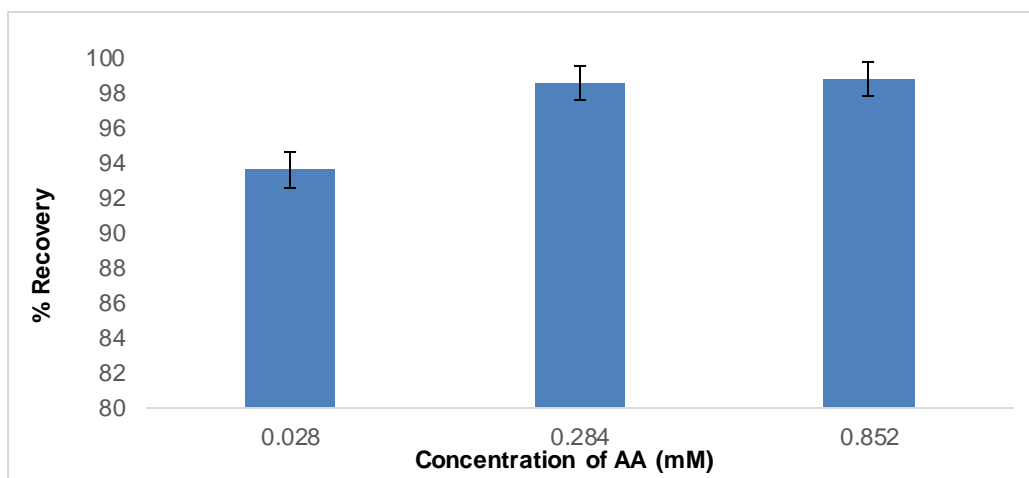


Figure 6: Recoveries (%) for three different known concentrations of AA standard solution

Ruggedness

The RSD values of 1.27%, 2.93% and 0.50%, respectively for 0.028 mM, 0.284 mM and 0.852 mM AA standard solution were achieved for the measurements by the first analyst. Otherwise, 0.91%, 0.07% and 0.07% of RSD achieved for measurements conducted by the second analyst, also for respective AA concentrations (Jain & Rather, 2011). There were no significant differences between the obtained variances for AA when the measurements were conducted by two different analysts with the same instrument (Metrohm, Autolab Potentiostat) at the 5% significance level, as proved by two-tailed *F*-test. The proposed DPASV technique was considered to be rugged.

Recovery and determination of AA in fruit juices

The recoveries achieved for AA content in blackcurrant, orange and mango commercial fruit juices were $80.00 \pm 6.25\%$, $73.65 \pm 1.70\%$ and $97.48 \pm 16.90\%$. Meanwhile, the recovery of $101.93 \pm 1.65\%$ was obtained for fresh pineapple juices, as shown in Table 1. According to *t*-test, there were no significant differences between recovery and spiked value at the 95% confidence level since all the calculated *t* values are lower than the theoretical *t* value, which was 4.303 (Bergamini *et al.*, 2010). These satisfactory recoveries also indicated that the matrix did not affect the measurement of the AA in the fruit juices by the proposed DPASV technique.

No AA was detected in both lychee and guava commercial fruit juices. Tables 2 and 3 show the content of AA in the blackcurrant, orange and mango commercial fruit juices were 2.0213 mM, 1.8286 mM and 2.9798 mM, respectively. On the other hand, the content of AA in the fresh orange and pineapple juices were 0.8000 mM and 0.698 mM. The AA content in the commercial fruit juices much higher compared to the fresh fruit juices might be due to the AA enrichment during the commercial fruit juices processing.

Table 1: Recovery for spiked AA standard solution in the fruit juices (n=3)

Samples	Spiked Concentration of AA Standard Solution (mM)	Found Concentration of AA Standard Solution (mM)	Recovery (%)	Average Recovery \pm SD (RSD)
Blackcurrant (Commercial Fruit Juice)	0.0852	0.0760 0.0653 0.0709	89.19 76.69 83.24	80.00 \pm 6.25 (7.8 %)
Orange (Commercial Fruit Juice)	0.0852	0.0611 0.0638 0.0634	71.71 74.86 74.38	73.65 \pm 1.70 (2.31 %)
Mango (Commercial Fruit Juice)	0.0852	0.0799 0.0987 0.0705	93.86 115.90 82.69	97.48 \pm 16.90 (17.34 %)
Pineapple (Fresh Fruit Juice)	0.0390	0.0402 0.0400 0.0390	102.95 102.85 100.03	101.93 \pm 1.65 (1.62 %)

Table 2: AA content in the commercial and fresh fruit juices

Samples	AA Content (mM)	AA Content (mg/100 cm³)
Blackcurrant (Commercial Fruit Juice)	2.0213	35.59
Orange (Commercial Fruit Juice)	1.8286	32.24
Mango (Commercial Fruit Juice)	2.9798	52.52
Lychee (Commercial Fruit Juice)	Not detected	Not detected
Guava (Commercial Fruit Juice)	Not detected	Not detected
Orange (Fresh Fruit Juice)	0.800	14.10
Pineapple (Fresh Fruit Juice)	0.698	12.30

CONCLUSION

The proposed DPASV technique had been successfully applied to determine the AA in fruit juices. The present method had an advantage that a very simple sample pre-treatment was required. It was also found to be practically rapid, convenient, sensitive, accurate, precise, rugged and inexpensive. Therefore, it could be an excellent alternative method for the routine determination of AA in fruit juices in future.

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