POLAROGRAPHIC DETERMINATION OF ASCORBIC ACID IN FRUIT JUICES USING STANDARD ADDITION CALIBRATION

Safa Abd Alwahhab Emhmed Albakkoush[1] and Aliyu M. Hamisu[2*]

[1]Department of Chemistry, College of Education Zaltan, Sabratha University, 250, Sabratha, Libya.
[2]Department of Polymer Technology, College of Science, Hussaini Adamu Federal Polytechnic Kazaure, 5004, Kazaure, Nigeria.

الملخص:

تم تطبيق طريقة الاستقطاب لتحديد عدد كبير من المواد العضوية وغير العضوية. يُعزى ذلك إلى البساطة ، والحساسية ، والسرعة ، وخصوصًا عند اقترانها بمعايرة إضافة قياسية ، ليست هناك حاجة لإعداد عينة معقدة وتتطلب وقتًا حيث يمكن الاهتمام بتأثير المصفوفة. على سبيل المثال ، يعطي حمض الأسكوربيك (فيتامين ج) أكسدة محددة جيدًا في عازلة بريتون روبنسون والتي تعتبر مثالية لتحديد الكميات على نطاق تركيز واسع. هنا ، نجحنا في تحديد تركيز حمض الأسكوربيك في عصائر البرتقال والجوافة الوردية والمانجو الاستوائية في محلول بريتون روبنسون باستخدام طريقة معايرة الإضافة القياسية. تم اكتشاف أن قيم تيار الذروة تزداد مع زيادة التركيز المختلف لحمض الأسكوربيك وتم الحصول على منحنيات معايرة الخط المستقيم. تتوافق القيمة المستنبطة من تحديد المعايرة المعيارية الداخلية القطبية بشكل جيد مع القيم المقتبسة. كانت التركيزات المحددة 154 و 165 و 143 جزء في المليون للجوافة البرتقالية والوردية والمانجو الاستوائية على التوالي مقارنة بالقيمة المبلغ عنها وهي 150 جزء في المليون على ملصق كل عصير فاكهة. أظهرت نسبة حمض الأسكوربيك التي تم العثور عليها لقيم الملصق المُبلغ عنها أن طريقة الاستقطاب هذه موثوقة ومناسبة الغرض للتحليل المقصود.

الكلمات المفتاحية: الاستقطاب. إضافة قياسية حمض الاسكوربيك؛ التحليل

Abstract

Polarographic method of determination has been applied for large number of substances, both organic and inorganic. This is attributed to the simplicity, sensitivity, fast and especially when coupled with standard addition calibration, there is no need for complex and time demanding sample preparation as matrix effect can be taken care of. For example, ascorbic acid (Vitamin C) gives a well-defined oxidation in Britton-Robinson buffer which is ideal for the quantities determination over a wide concentration range. Herein, we successfully determined the concentration of ascorbic acid in orange, pink guava and tropical mango juices in Britton-Robinson buffer using standard addition calibration method. It was discovered that, the peak current values increases with increase in different concentration of ascorbic acid and straight line calibration curves were obtained. The extrapolated value from the polarographic internal standard calibration determination is in good agreement with the quoted values. The determined concentrations were 154, 165 and 143 ppm for orange, pink guava and tropical mango respectively compared to the reported value of 150 ppm on the label of each fruit juice. Ratio of ascorbic acid found to reported label values showed this polarographic method is reliable and fit-for-purpose technique for the intended analysis.

Keywords: Polarography; Standard Addition; Ascorbic Acid; Analysis.

1. INTRODUCTION

Vitamin C is a water-soluble compound that is essential for life, it is often used as an antioxidant and antimicrobial agent if food products. Primarily, ascorbic acid and the oxidized form of ascorbic acid (dehydroascorbic acid) exhibits the biological activity of vitamin C. several structural functional groups contributes to their biological activity, including lactones, two enolic hydroxyl group, and a both primary and secondary groups. Enediol functional group which can easily get oxidized to diketones endows it with the antioxidant activity. (Njus et al., 2020) Vitamin C is a vital supplement in our daily dietary intake to improve cardiac health and prevent degenerative diseases and has been an important material applied for protection against high blood pressure, endothelial dysfunction and the blood vessel changes that precede heart disease, but usually taken for granted. (Arrigoni and De Tullio, 2002)

The analytical determination of the ascorbic acid content, involved the conversion of the reduced form to the oxidized form. The most frequently used method for the determination of ascorbic acid is High-Performance Liquid Chromatography (HPLC), which offers high selectivity and sensitivity compared to spectrophotometric, enzymatic or titration methods (Nováková et al., 2008). Ascorbic acid is a typical example of an unsaturated hydroxylactone that is electrochemically active and therefore get oxidized on dropping mercury electrode and give a cathodic wave. The ascorbic acid oxidation wave is inferred based on the mechanism involving a reversible

electrode reaction followed by the irreversible conversion of an unstable intermediate to stable dehydroascorbic acid. (Gupta, 2015)

Polarographic study of the oxidation mechanism of ascorbic acid was carried out in an acid medium and the process involves two electrons and the polarographic wave shows that the limiting current is governed by diffusion. (Ruiz et al., 1977) The oxidation process of the two electron is composed of two separate but consecutive one electron transfers, the second electron being the rate determining step. In pharmaceutical settings, polarographic method for the estimation of ascorbic acid in formulations was developed, parameters like pH, concentration of supporting electrolyte, mercury flow rate and drop time and maximum suppressor were fully optimized. As such, a well-defined polarographic response with diffusion current proportional to the amounts of ascorbic acid is observed under the optimum conditions. (Ruiz et al., 1977, Sahbaz and Somer, 1992)

On the other hand, standard additions calibration is used in instrumental analysis to determine the concentration of an unknown sample by spiking the sample with known amount of analyte to create a series of different standard were the sample is present within each of the standard. Unlike standard series calibration, where calibration is made using series of known amounts of analyte and then measure the unknown analyte separately. Standard additions calibration approach is particularly useful for analysing complex samples and eliminating the matrix effects. Within any sample in analytical science we have the analyte which is we are interested in analysis or quantifying and by

definition anything else is the matrix. The matrix components typically affects the instrument response to the analyte, however standard addition calibration method can helps to overcome the matrix effects. (Gupta, 2015, Lau et al., 1985, Jeyaseelan and Joshi, 2002, Lento et al., 1963, Owen and Smyth, 1975, Burns and Walker, 2019, Patil et al., 2019, Kalaycı et al., 2020, Mazurek et al., 2020)

For the analysis ascorbic acid using polarographic method, the peak height is a function of pH. For instance, a pH of 4.5 was used by Sontag and coworkers (Sontag and Kainz, 1978) to obtain a maximum peak height achieved by using Britton–Robinson buffer with addition of sodium hydroxide solution to adjust the pH to 4.5. The supporting electrolyte used was an equivalued 2M acetic acid and sodium acetate plus 2% oxalic acid solutions. However, Lau and coworkers observed that, Britton–Robinson buffer with the above composition is not suitable for the determination of ascorbic acid in vegetables and fruits due to large background signal overlapping with the peak of interest, hence they use 2M acetate buffer as both supporting electrolyte as well as buffer owing to its low background signal. As such, small modification on the pH of the Britton-Robinson buffer to 3.5 will be desired for the determination of absolute ascorbic acid in fruits and vegetables. Other type of buffers has also been used for the determination of ascorbic acids in fruits as the supporting electrolyte. (Sahbaz and Somer, 1992, Kozar et al., 1988) High buffer capacity is desirable for any buffer to be suitable as a supporting electrolyte for increased measurement precision.

Herein, we report a simple, fast, and effective differential pulse polarographic technique for the quantitative determination of ascorbic acid in Peel Fresh orange, pink guava and tropical mango juices using standard addition method and modified Britton-Robinson buffer pH to 3.5 as the pH of the supporting electrolyte. Our results shows that, the determined concentrations of the ascorbic acids in these fruit juices using our polarographic method agrees well with the amount of the ascorbic acid on the label of the fruit juice. This denotes the method as not only fit for purpose for the determination of ascorbic acids in fruits juices, but also straightforward, cost-effective, fast and requiring no sample preparation, hence no concern on matrix effects.

2. METHODOLOGY

All chemicals were of analytical reagent (A.R.) grade. Ascorbic acid - $C_6H_8O_6$, Acetic acid - CH_3COOH glacial, Phosphoric acid - H_3PO_4, Boric acid - H_3BO_3, Sodium hydroxide – NaOH, all chemicals were obtained from Sigma Aldrich. The Peel-Fresh orange, pink guava and tropical mango juices were obtained from a grocery shop located at college twelve, the University of Malaya, Kuala Lumpur Malaysia. Metrohm 797 VA Computrace Polarograph (Dropping Mercury Electrode, DME) was used for the analysis. The equipment works with three electrodes comprising the working electrode (mercury multimode), reference electrode (Ag/AgCl-KCl) and auxiliary platinum electrode. The sample is manually injected and quantification was done by standard addition method. Ascorbic acid was determined from fruit

juices without sample preparation at the dropping mercury electrode. Section 2.4 provides the instrument setup for the analysis.

2.1. Preparation Supporting Electrolyte (Britton Robinson pH 3.2)

A Briton-Robinson buffer was used as the supporting electrolyte and prepared by mixing equal amount (100 mL each) of 0.04 M acetic acid, 0.04 M phosphoric acid and 0.04 M boric acid into 500 mL glass beaker. (Britton and Robinson, 1931) The pH of Britton-Robinson buffer was adjusted to precisely a pH of 3.2 by addition of 0.2 M sodium hydroxide solution and tested by a pH meter.

2.2. Preparation Ascorbic Acid, Stock Standard Solution (300 µg/mL)

A standard solution of ascorbic acid was prepared by dissolving accurately weighed 30 mg ascorbic acid in 100 mL of the pH-adjusted supporting electrolyte. determination. This standard solution adjusted to pH3.0 using pH meter. A calibration curve was prepared using the standard solution. About 20µl of aliquots of ascorbic acid was successively added to 10.0ml of de-aerated Britton–Robinson buffer in the polarographic instrument. The differential pulse polarograms yielded peaks at +0.08V which respectively belong to the ascorbic acid.

2.3. Sample preparation

10 mL of the fruit juice sample was filtered by a filter paper to separate any solid suspension present in the solution. This was done in a controlled environment and as fast as possible to the analysis step in order to reduce the risks of oxidation of ascorbic acid with oxygen in the air.

2.4. Instrument setup

Below experimental setup was used for the analysis of all the samples in Britton–Robinson buffer supporting electrolyte using standard addition method.

Electrode	DME
Display direction	Negative (-)
Initial potential	-0.1V
Drop time	1 sec
Current range	1-2µA
Scan rate	2 mV/sec
Scan direction	Positive (+)
Modulation Amplitude	25 mV
Low pass filter	Off
Mode	Differential pulse

2.5. Sample analysis using Metrohm 797 VA Computrace Polarography

The cell of Metrohm 797 VA Computrace polarograph and the electrodes were rinsed with distilled water before analysing the sample. This step was done carefully in order to handle the mercury waste appropriately.

10 mL of the prepared supporting electrolyte was measured by a graduated pipette and dispensed into the polarographic cell and purged for 5 minutes with nitrogen gas to ensure the exclusion of oxygen gas from the solution.

An aliquot of filtered juice (60 µL) sample was transferred into the polarographic cell by micropipette. The solution was then scanned from 0.0 to +0.18 volts versus a saturated calomel electrode. Differential pulse mode was used and hence a peak for ascorbic acid occurred approximately at +0.08 volts. Subsequently, 10 µL standard solution of ascorbic acid (300 µg/mL) was added into the polarographic cell, Purged with nitrogen gas and the peak current was recorded. This step was done consecutively 3 times for different additions.

The above procedures were repeated for different types of fruit juice and all the polarograms obtained were recorded and analysed. Finally, the graph of peak current, I (A) versus ascorbic acid concentration in ppm were obtained. The extrapolated line intersecting the response axis indicates the concentration of ascorbic acid in the sample.

3. RESULT AND DISCUSSION

Differential pulse polarography using standard addition method and Britton-Robinson buffer as a supporting electrolyte is a very convenient technique for the determination of ascorbic acid in fruits. A Britton-Robinson buffer was used as supporting electrolyte, prepared by mixing equal amount (100 mL each) of 0.04 M acetic acid, 0.04 M phosphoric acid and 0.04 M boric acid. (Britton and Robinson, 1931) The pH of Britton-Robinson buffer was adjusted to a pH range of 3.2 by adding 0.2 M sodium hydroxide solution and tested by a pH meter. The final pH of the Britton-Robinson buffer used for this experiment was exactly pH 3.2.

A Britton-Robinson buffer has demonstrated usefulness as supporting electrolyte used in the analysis of the ascorbic acid. At higher pH values, ascorbic acid is unstable and a strong reducing agent that can potentially reduce atmospheric oxygen. Therefore, low pH value is preferred and pH 3.2 was selected because maximum sensitivity was achieved. At pH lower than 3.2, the peak current becomes very steep interfering with the oxidation wave of mercury making it difficult to measure the anodic current with high accuracy. Purging with nitrogen gas ensures the solution was de-aerated and all trace of oxygen gas removed to enable good anodic waves to be recorded. In this work, orange, pink guava and tropical mango fruits juices obtained from the market were tested for their ascorbic acid contents. No extensive sample preparation involved as the samples were simply filtered using filter papers to remove any suspended solids which could interfere with the analyte signal. The determination was done directly on the sample aliquots with differential pulse polarography mode using standard addition calibration method. The anodic wave that corresponds to the oxidation of the enediol compound that occurs at the surface of the dropping mercury electrode was measured and recorded.

Table 1 summarizes the ascorbic acid concentrations in orange, pink guava and tropical mango versus the quoted values based on the label using our method.

Table 1: Polarographic estimation of ascorbic acid in Peel-Fresh samples

Peel Fresh Sample	Amounts of Ascorbic acid (ppm)		
	Sample	Found	Ratio of Ascorbic Acid Found to Label Value
Orange	150	154 (2)	1.03
Pink Guava	150	165 (3)	1.1
Tropical Mango	150	143 (2)	0.95

The straight line is obtained by plotting the peak current against the concentration of ascorbic acid determined in Britton-Robinson buffer pH 3.2 for all the samples, shows that the peak current I(A) is directly proportional to the concentration of the ascorbic acid standard solution. The precision of the measurement was ascertained by duplicate measurement Based on the label on the Peel-Fresh processed fruits, the concentration of ascorbic acid in orange, pink guava and tropical mango juice samples is 15.0 mg/0.1 L (150 ppm) each. The obtained values in this work 154(2), 165(3) and 143(2) ppm for orange, pink guava and tropical mango juice samples respectively agrees well with the quoted values.

For orange juice, the linear response against the concentration of internal standard, Figure 1 and Table 2, indicates a linear relationship between the peak current I(A) and the concentration of ascorbic acid additions.

Table 2: Internal standard addition, concentration of ascorbic acid standard and the corresponding peak current, I(A) in orange sample in 60 µL aliquot.

No. of addition	Concentration of standard ascorbic acid added, ppm	Peak current, I(A)
0	0	42.21
1	300	55.18
2	600	68.18
3	900	83.08

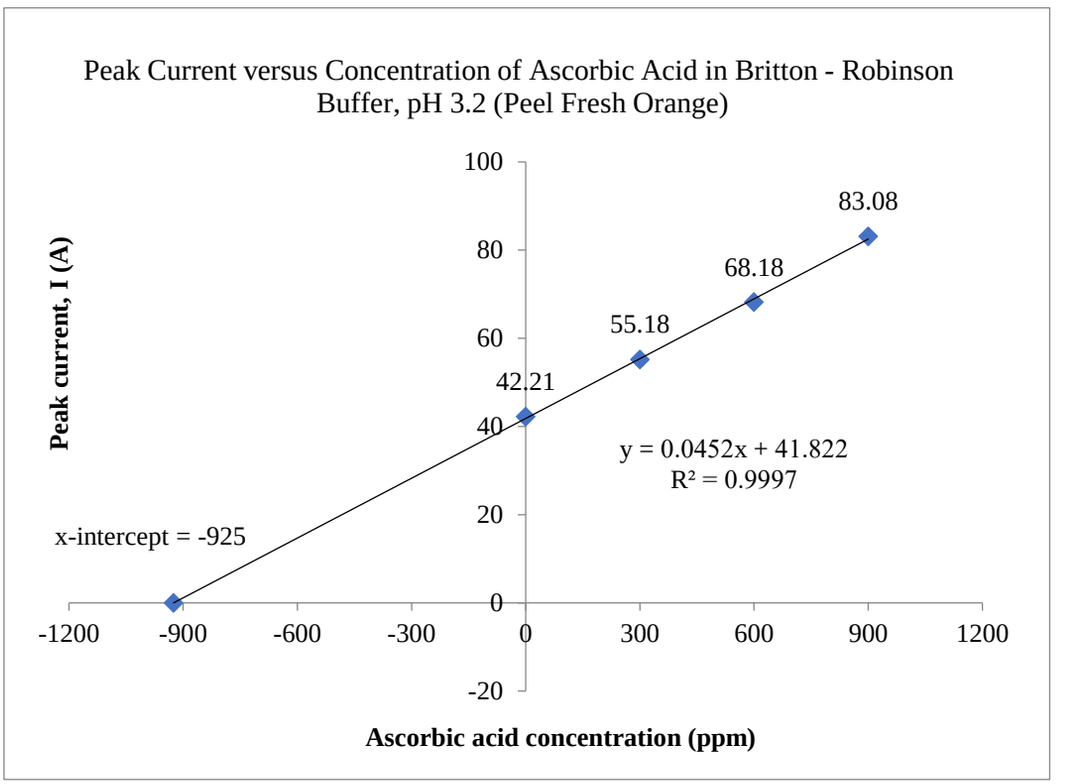

Figure 1: Peak current versus concentration of ascorbic acid in orange juice sample

Figure 1, the *x-axis* intercept is equals to -925 when y equals zero and based on the linear regression equation, $y = 0.0452x + 41.822$ which has a very good fit (R^2 = 0.9997), when $y = 0$, $x = -925$. Therefore, concentration of ascorbic acid in 60 µL aliquot Peel-Fresh orange juice sample is 154 ppm. The extrapolated value of the ascorbic acid agrees well with the quoted value of 150 ppm on the label.

Similarly, for pink guava Table 3 and Figure 2 while that of tropical mango, Table4 and Figure 3 also shows a very linear relationship between the peak current I(A) and the concentration of the ascorbic acid in these samples.

Table 3: Internal standard addition, concentration of ascorbic acid standard and the corresponding peak current, I(A) in pink guava sample in 60 µL aliquot.

No. of addition	Amount of standard ascorbic acid added, ppm	Peak current, I(A)
0	0	43.08
1	300	58.05
2	600	71.11
3	900	83.02

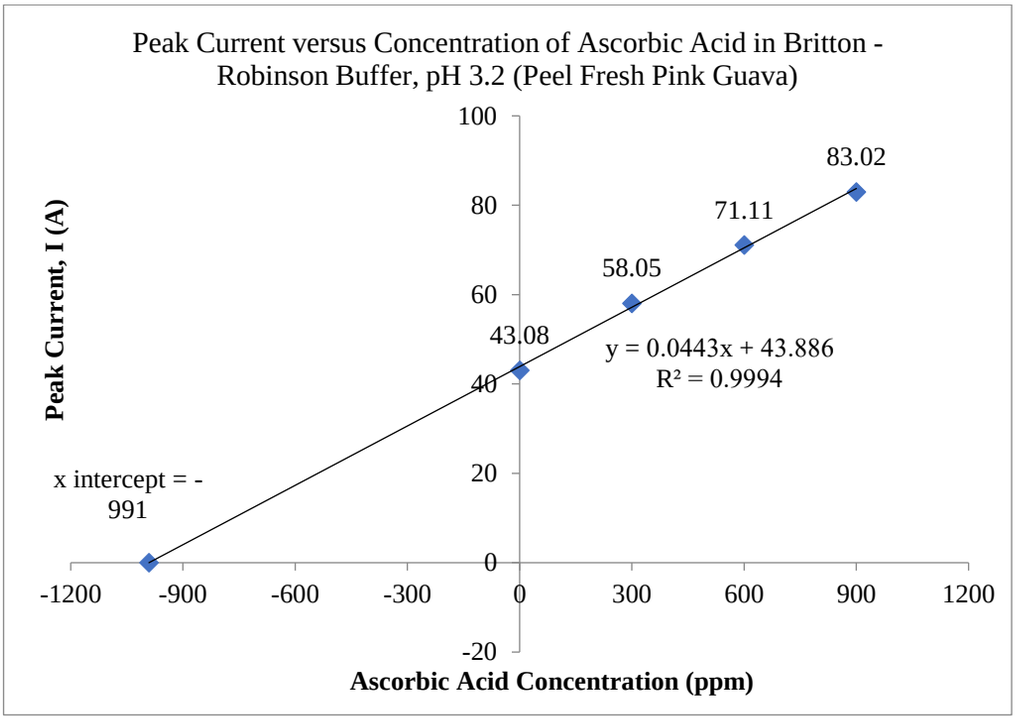

Figure 2: Peak current versus concentration of ascorbic acid in pink guava juice sample

Based on the linear relationship between the peak current and the concentration of the ascorbic acids in pink guava sample, the linear regression equation $y = 0.0443x + 43.886$ has been derived. When $y = 0$, x equals -991 in 60 µL aliquot which is equivalent to 165 ppm ascorbic acid in the pink guava sample. The obtained value of the ascorbic acid concentration agrees to some extent with the quoted value of 150 ppm on the pink guava label.

Table 4: Internal standard addition, concentration of ascorbic acid standard and the corresponding peak current, I(A) in tropical mango sample in 60 µL aliquot.

No. of addition	Amount of standard ascorbic acid added, ppm	Peak current, I(A)
0	0	41.52
1	300	54.58
2	600	69.55
3	900	84.45

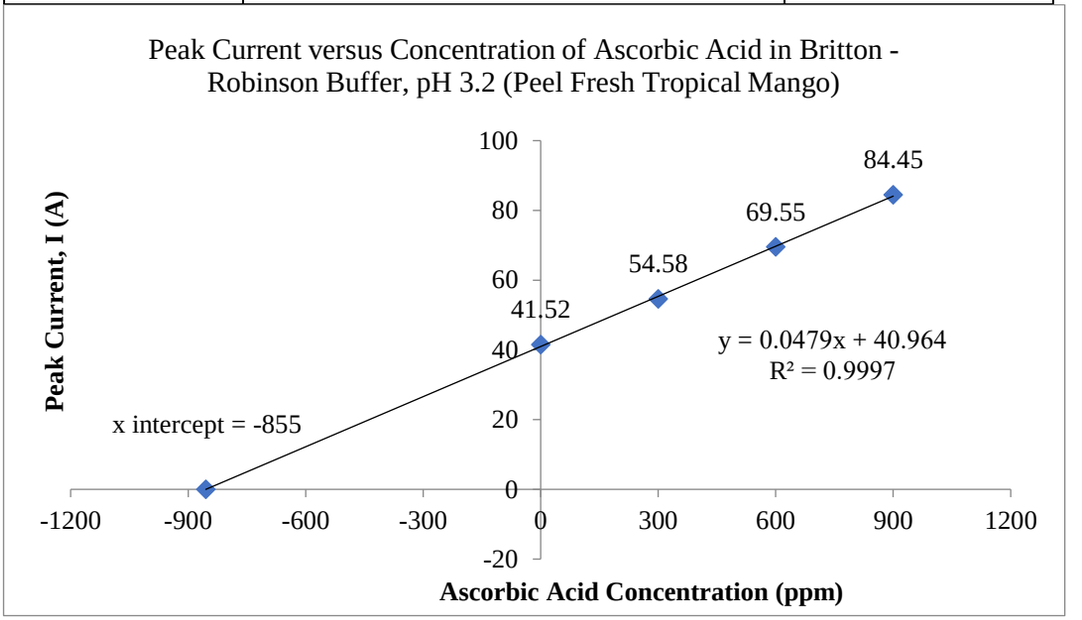

Figure 3: Peak current versus concentration of ascorbic acid in tropical mango juice sample

Tropical mango sample, also the response is proportional to the ascorbic acid concentration leading to a straight line, Table 4 and Figure 3. The extrapolated value at *x* intercept equals -855 when *y = 0* in 60 µL aliquot

which corresponds to 143 ppm in tropical mango juice. The quantified value in tropical mango juice sample also agrees to the quoted value 150 ppm on the label.

The quantified values for the Peel-Fresh orange, pink guava and tropical mango juices samples Table 1, are in good agreement with the quoted values on the label. This substantiates the methods as fast and sensitive with good accuracy. The internal standard calibration graph in each case was found to be linear and the quantitative determination of ascorbic acid citrus fruit showed that the concentration of ascorbic acid found in pink guava juices is the highest with the mean value of 165 ppm followed by in orange and tropical mango with the mean concentration of 154 ppm and 143 ppm respectively. The recoveries for the same order above were 110, 102.7 and 95% respectively. Most manufactures add considerably more vitamin C to their product than the labelled value to compensate for any loss due to degradation.

There are several factors that may contribute to the slight decrease in concentration of ascorbic acid content found in tropical mango in this experiment. The first factor may be attributed to the fluctuation of nitrogen gas pressure supply during the analysis. The essence of using nitrogen gas to purge the solution in the polarographic cell is to minimize oxidation by oxygen in the air. Another factor might be due to appreciable air oxidation occurred during the filtration since it contains a dense amount of solid suspension and contribute to a longer filtration time.

4. Conclusion

The concentration of ascorbic acid in Peel-Fresh juices (Orange, Pink Guava and Tropical Mango) was determined quantitatively by differential pulse polarography technique using a standard addition method. It was discovered that, the peak current values increases with increase in concentration of the ascorbic acid. The straight lines obtained by plotting peak current versus different concentration of ascorbic acids are presented in Figure 1, 2 and 3. The extrapolated value from the polarographic internal standard calibration determination is in good agreement with the quoted values. Ratio of ascorbic acid found to label values showed polarographic method is reliable and fit-for-purpose technique for the intended analysis. This polarographic method is straightforward, requiring no sample extraction, hence time saving. It is also rapid, cost effective, simple instrumental operation with less sample and reagent consumption. In addition to this, the advantage of using standard addition as a means of calibration and quantification is the matrix effects present in the sample are taken into account. Thus, these features make it suitable for analysis of vitamin C in fruits and juices.

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