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International Journal of Pharmaceutical & Biological Archives2010; 1(1): 44 - 50

ORIGINAL RESEARCH ARTICLE

Statistical Evaluation of Titrimetric Method Using N-Bromosuccinimide (Nbs) and Cyclic Voltammetry for the Assessment of Ascorbic Acid Level in Vegetables and Tuber Products

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ABSTRACT

Titrimetric using N-bromosuccinimide and cyclic voltammetric methods were used to determine the concentration of ascorbic acid in green amaranth (*Amaranth candatus*) and irish potato (*Ipomoea batata*). The results were statistically evaluated using F-test for comparison of precision and t-test for evidence of significant difference. Green amaranth showed comparable precision at 1 % confidence level and no significant difference was found between the means of the two analytical methods at 10 %, 5 % and 1 % confidence levels while irish potato showed comparable precision at both 5 % and 1 % confidence levels, significant difference was found for the means of both methods at all confidence levels. The t-test has shown that cyclic voltammetry produced a better and reliable result for tuber products.

Key Words: Statistical t-test, N-bromosuccinimide, Cyclic voltammetry, Green amaranth.

INTRODUCTION

Vitamin C, known as ascorbic acid, is a water soluble vitamin. It is an electron donor and its biochemical and molecular roles are accounted for by this function [1]. It is a valuable food component and plays a major role as an antioxidant that forms part of defense system against reactive oxygen species and free radicals [2].The requirement to adequately assess vitamin C in food products without underestimating or overestimating its quantity has made researchers to constantly search for methods for its determination.

A wide range of methods have been employed to determine vitamin C content of food products. The quantity of ascorbic acid has been successfully determined in fruits by titrimetric method [2-4]. Fluorimetric and flow injection analysis [5] methods have also found wide application in the determination of ascorbic acid. Some novel spectrophotometric methods have been successfully employed for the determination of vitamin C in simple solution, pharmaceutical preparations and biological samples [6]. Similarly, this vitamin has also been determined voltammetrically using cyclic voltammetry [2], differential pulse polarography [7], chemilumiscence [8] and enzymatically [9].High performance capillary electrophoresis [10] and capillary zone electrophoresis [11] have been found equally suitable for vitamin C determination. High performance liquid chromatography with

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different columns and mobile phases in commercial non-alcoholic beverages [12], tomatoes [13], selected fruit and vegetable products [14] has also been widely used to determine ascorbic acid.

Most of these methods have their shortcoming either they overestimate the level of vitamin C or underestimate it, some are affected by other oxidizable species, some have poor detection limits and some are too expensive to use and maintain. Thus, this article centers on statistical validation of the titrimetric method using N-bromosuccinimide with cyclic voltammetry which is fast, sensitive, selective and gives a linear response at low concentration range [2].

EXPERIMENTAL

Reagents and Materials

Standard ascorbic acid (L-ascorbic acid), N-bromosuccinimide, sodium dihydrogen phosphate (NaH_2PO_4), Oxalic acid were bought from Sigma-Aldrich, Chemie, Steinheim, Germany. Sample and reference solutions were prepared daily, stored in amber bottles and kept away from light in a cupboard to prevent oxidation. All apparatus were acid washed overnight with concentrated trioxonitrate (v) acid, rinsed with deionised water and dried in an oven at 40^{o}C. Deionised water was used to prepare all the solutions. 0.1M phosphate buffer was prepared from NaH_2PO_4 adjusted to pH 2 with phosphoric acid. The pH measurements were made with Metrohm pH meter model 780. All other chemicals were of analytical grade.

Samples analyzed

Two (2) samples, green amaranth and irish potato were bought from Yaba market, Lagos and identified at National Institute of Horticultural Research idi-ishin Ibadan, Nigeria.

Instrumentation, working procedure and analysis of samples by cyclic voltammetry

A BASI-EPSILON potentiostat – galvanostat obtained from bioanalytical system Inc. (West Lafayette, USA) was used in the study. Glass carbon (3 mm) was used as working electrode, a platinum disc (1.6 mm) was used as auxiliary electrode and Ag/AgCl (kept in KCl solution acting as supporting electrolyte to maintain its saturation) was the reference electrode. Before each measurement, glassy carbon working electrode was polished with 0.01 µC and 0.05 µC alumina, cleaned in pyrana and washed with deionised water. Effective reproducible stirring of the solution was done using a magnetic stirrer put into the cell and exclusion of oxygen was achieved by deaerating the solution with nitrogen gas for about 15minutes. For each measurement, the potential was scanned between 200 mV and 1000 mV with a scan rate of 50 mVS^{-1} and sensitivity of 10 µA.

A stock solution of 5 mmolL-1 was prepared by dissolving 0.088g of ascorbic acid in 100 cm^3 0f
0.1 $molL^{-1}$ phosphate buffer dosed with 0.1 $mmolL^{-1}$ EDTA solution. Other standard solutions, 4 $mmolL^{-1}$, 3 $mmolL^{-1}$, 2 $mmolL^{-1}$ and 1 $mmolL^{-1}$ were prepared from stock solution by serial dilution. 15cm^3 of each standard ascorbic acid solution was put into the electrochemical cell and the detailed working procedure was followed as described earlier. All measurements were made at room temperature. The anodic current corresponding to electrochemical oxidation of ascorbic acid appeared at 580mV. Calibration curves were plotted using the peak current values obtained from voltammograms against ascorbic acid concentration.

Each sample was peeled, weighed, minced and blended with deaerated phosphate buffer and filtered through both glass wool and filter paper for clear extract. The working procedure for standard ascorbic acid solution was applied to samples analysis. The ascorbic

acid content was calculated by measuring the peak currents at 580mV using the calibration curve of standard ascorbic acid.

Determination of ascorbic acid by titration with N-bromosuccinimide (NBS)

Stock solution of 0.5 gL^{-1} was prepared by dissolving 0.1 g of L-ascorbic acid in 200 ml of 0.6 $molL^{-1}$ oxalic acid. Other standard solutions, 0.4 gL^{-1}, .0.3 gL^{-1}, 0.2 gL^{-1} and 0.1 gL^{-1} were prepared by serial dilution. 10 ml of each standard ascorbic acid solution was to diluted 100 ml with deionised water; 10 ml of this containing 0.02 $molL^{-1}$ KI, 0.05 $molL^{-1}$ ethanoic acids was titrated against 0.1 gL^{-1} N-bromosuccinimde solution using 50 gL^{-1} starch solution as indicator until a permanent violet color end-point was obtained. All titrations were performed in triplicate and results obtained were used to construct calibration curves.

Each sample was peeled, weighed and blended with 0.6 $molL^{-1}$ oxalic acid solution. The samples were analyzed as described for standard ascorbic acid solution. The content of ascorbic acid in each sample was extrapolated from the calibration curve plotted.

STATISCAL TEST FOR ANALYTICAL METHODS

The test is based on the comparison of the means and the precisions of concentration of ascorbic acid obtained in the samples by the two methods. The test shows how precise the two methods are in determining the concentration of ascorbic acid. The appropriate test to use is t-test at different probabilities but before it can be confidently used, F-test must be used to show that there is no significant difference between the precisions of the two methods.

$$F_{test} = \frac{S_1^2}{S_2^2}$$

$$t_{test} = \frac{\chi_1 - \chi_2}{S_p\sqrt{(1/n_1 + 1/n_2)}}$$

$$S_p = \frac{\sqrt{(n_1-1)(S_1^2) + (n_2-1)(S_2^2)}}{n_1+n_2-2}$$

χ_1 – mean of samples in 1.
χ_2 – mean of samples in 2.
S_p – pooled standard deviation.
S_1^2 and S_2^2 – variances of samples 1 and 2.
n_1 and n_2 – number of observations in samples 1 and 2.

RESULTS AND DISCUSSION

Several voltammograms corresponding to different ascorbic acid concentrations are presented in **Fig. 1**. As it can be seen in **Fig. 1**, no reduction (cathodic) peak was found indicating that electrochemical oxidation of ascorbic acid is an irreversible process [1,2].

The oxidation (anodic) peaks at 580mV were found to vary linearly with ascorbic acid concentrations as shown in **Fig. 1**. This shows that ascorbic acid can be quantitatively measured by cyclic voltammetry.

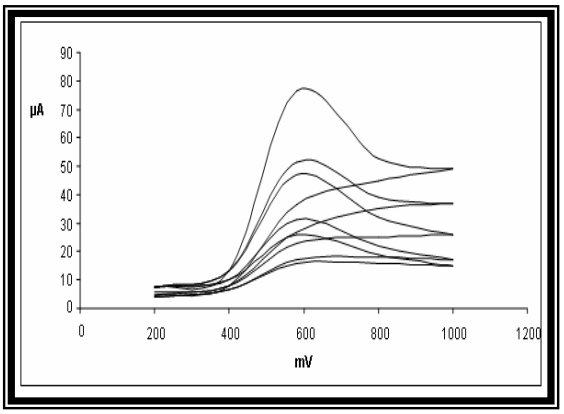

Fig. 1. Voltammograms of [1] 5 mmolL^{-1}, [2] 4 mmolL^{-1}, [3] 3 mmolL^{-1}, [4] 2 mmolL$_{-1}$ and [5] 1 mmolL^{-1} of ascorbic acid concentration in 0.1M phosphate buffer pH 2 dosed with 0.1mM EDTA.

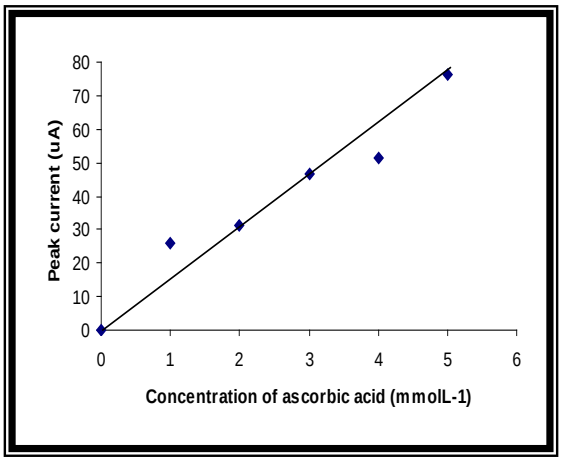

Fig. 2. Variation of peak currents with ascorbic acid concentrations in phosphate buffer pH 2

Table 1 shows the determination of standard ascorbic acid solutions by titrimetric method with NBS and cyclic voltammetric method respectively. The values for titrimetric method represent the average of three concordant determinations whereas values of cyclic voltammetric method represent the average of two values. Calibration curve corresponding to NBS method is shown in **Fig 3**

Table 1: Result obtained for standard ascorbic acid determination by titrimetric method with NBS and cyclic voltammetric method. The values for titrimetric method represent the average of three concordant determinations whereas values of cyclic voltammetric method represent the average of two values.

S/N	Conc. of Ascorbic Acid (G/L^{-1}) By N – Bromosuc Cinimide	Mean Titre Value	Conc. of Ascorbic Acid (Mmoll^{-1}) Cyclic Voltammetry	Peak Current (Ma)
1	50	5.45	5	76.19
2	40	4.20	4	51.32
3	30	3.37	3	46.83
4	20	2.27	2	31.20
5	10	1.37	1	25.79

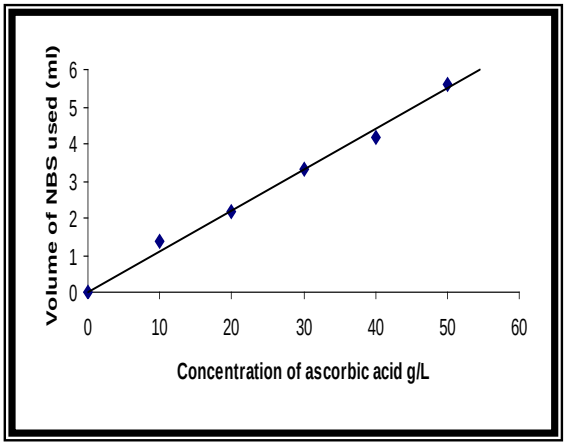

Fig. 3: Calibration curve of standard ascorbic acid with N-bromo succinimide

Table 2 shows NBS determination of ascorbic acid in vegetable sample (green amaranth) and tuber sample (irish potato) obtained from the calibration curve of standard ascorbic acid. Each sample was analyzed five times.

Table 2: Result of Concentration of Ascorbic Acid Determined By Titrimetric Method with Nbs for both Green Amaranth and Irish Potato

Green amaranth				Irish potato		
S/N	**Mass (G)**	**Titre Value (Ml)**	**Concentration of Ascorbic Acid (Mg/100g)**	**Mass (G)**	**Titre Value (Ml)**	**Concentration Of Ascorbic Acid (Mg/100g)**
1	17.44	1.10	56.77	55.50	0.60	9.91
2	17.44	1.20	60.21	55.50	0.60	9.91
3	17.44	1.00	51.61	70.41	0.70	9.83
4	14.14	0.80	55.94	40.00	0.40	9.88
5	14.14	0.90	58.93	40.00	0.40	9.88

Table 3 presents the values obtained from the calibration curve of standard ascorbic acid for both green amaranth and irish potato determined by cyclic voltammetry. Each sample was analyzed four times. The voltammograms corresponding to each food are shown in **Fig. 4** and **5**

Table 3: Level of Ascorbic Acid Determined By Cyclic Voltammetric Method for Both Samples

Green amaranth				Irish potato		
S/N	**Mass (G)**	**Peak Current (Ma)**	**Concentration of Ascorbic Acid Found (Mg/100g)**	**Mass (G)**	**Peak Current (Ma)**	**Concentration Of Ascorbic Acid Found (Mg/100g)**
1	22.27	10.97	60.04	30.34	4.65	17.41
2	22.25	10.95	58.37	30.33	4.50	17.32
3	07.82	03.90	59.27	11.74	1.69	17.29
4	47.79	23.78	58.24	63.40	09.42	17.36

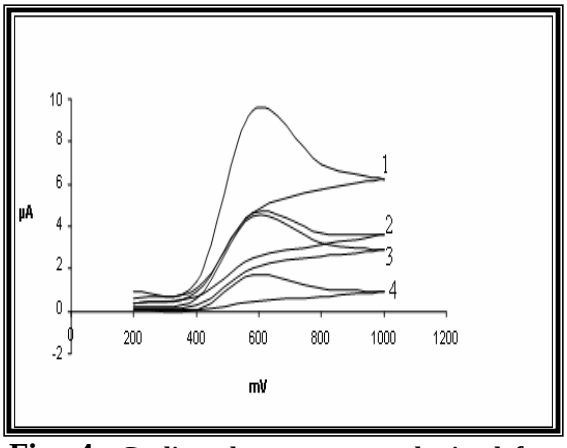

Fig. 4: Cyclic voltammograms obtained for different masses of irish potato. [1] 63.4 g, [2] 30.34 g, [3] 30.33 g and [4] 11.74 g

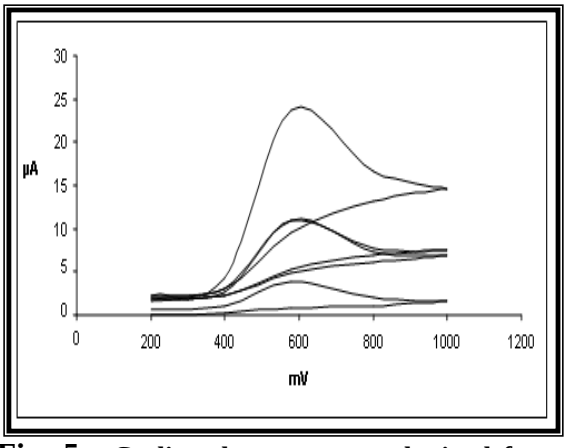

Fig. 5: Cyclic voltammograms obtained for different masses of green amaranth. 22.27 g [1], 22.25 g [2], 7.82 g [3] and 47.79 g [4].

The statistical data obtained are presented in **Tables 4** and **5** for both green amaranth and irish potato using different analytical methods to determine the

concentration of ascorbic acid in them.

Table 4: Statistical Data Obtained for the Determination of Concentration of Ascorbic Acid in Green Amaranth By Both Analytical Methods

	N-bromosuccinimide				Cyclic voltammetry			
S/N	Mass (G)	Conc. of Ascorbic Acid (Mg/100g)	Mean	Variance	Mass (G)	Conc. of Ascorbic Acid (Mg/100g)	Mean	Variance
1	17.44	56.77			22.27	60.02		
2	17.44	60.21			22.25	58.37		
3	17.44	51.61	56.70	10.95	07.82	59.27	58.98	0.69
4	14.14	55.94			47.79	58.24		
5	14.14	58.93						

Table 5: Statistical Data for Assessing the Concentration Of Ascorbic Acid In Irish Potato Determined By The Two Analytical Methods

	N-bromosuccinimide				Cyclic voltammetry			
S/n	Mass (g)	Conof ascorbic acid (mg/100 g)	Mean	Variance	Mass (g)	Conc. of ascorbic acid (mg/100g)	Mean	Variance
1	55.50	9.91			30.34	17.41		
2	55.50	9.91			30.33	17.32		
3	70.41	9.83	9.88	0.0011	11.74	17.29	17.35	0.0082
4	40.00	9.88			63.40	17.36		
5	40.00	9.88						

The results obtained were statistically tested for significant difference using F-test to compare precision and t-test to compare the means of the results at different probabilities of 1 %, 5 % and 10 % confidence levels

For green amaranth

F-test = $\frac{10.95}{0.69}$

$F_{calculated}$ = 15.87

$F_{tabulated}$ = 0.05 0.01
9.12 28.7

$F_{calculated} < F_{tabulated}$ at 1% confidence level. Thus there is comparable precision at the specified probability.

t_{test} =

$\chi_1 - \chi_2 = 2.28$

$S_p = 6.77$

$n_1 = 5$

$n_2 = 4$

Degree of freedom = 5+4-2 = 7

$t = 2.28/6.77\sqrt{0.45}$

$= 2.28/6.77x0.67$

$t_{calculated} = 0.502$

$t_{tabulated}$ = 0.1 0.05 0.01
1.415 1.895 2.998

It was found at different probabilities of 10 %, 5 % and 1 % confidence levels that $t_{calculated} < t_{tabulated}$, thus, there is no significant difference between the means of the methods. Both methods have been found suitable in determining level of ascorbic acid in green amaranth. When the same test was used for

Irish potato, there exists comparable precision at 5 % and 1 % probabilities but there is significant difference at all the confidence levels used because $t_{calculated} > t_{tabulated}$. Cyclic voltammetry presents a better result than N-bromosuccinimide in determining the concentration of ascorbic acid in irish potato because Irish potato contains starch which could have interfered with end-point detection by increasing the quantity of starch in the solution thereby underestimating the concentration of ascorbic acid.

Therefore, to quantitatively and qualitatively determine the level of ascorbic acid in vegetables, both analytical methods are found useful while it is highly recommended that levels of ascorbic acid in tuber and tuber products should be done using cyclic voltammetry to avoid underestimating the concentration of ascorbic acid.

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