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Titrimetric determination of vitamin C in natural and commerical fruit juice samples using a locally extracted dye

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ABSTRACT

An alternative method has been developed for accurate determination of vitamin C in various juice samples using aqueous extract of fresh exoderm of fluted pumpkin seeds. This locally extracted dye (LED) gives a better percentage recovery by titration method when compared to the conventional titrimetric method using 2,6 - Dichlorophenol-indophenol (DCPIP). Student's t-test statistical analysis for correlated paired data showed that there is no significant difference ($\alpha = 0.01$, $N = 21$) in precision and reproducibility in the determination by both the new LED and conventional DCPIP methods. Vitamin C content of 21 samples including natural fruits, commercial fruit juice and vitamin C tablets have been accurately determined using this method. The method developed has also been successfully adapted to spectrophotometric titration for determination of trace amount (<0.064mg/ml) of vitamin C in fruit juice samples.

Key words: Vitamin C, titrimetric method, local dye extract (LED).

INTRODUCTION

Vitamin C also known as ascorbic acid is one of the critically needed vitamins for the maintenance of human health. It is water soluble and readily available in most fruits and other plant food items. Unfortunately, this essential vitamin cannot be manufactured or stored in the human system. They must therefore be taken by man from outside source like fruits, vitamin C supplements etc. [1,2].

Other mammals apart from primates and Guinea Pigs in addition to man can synthesize and store vitamin C in their systems. There are many reports on the functions of vitamin C as antioxidant, primary ingredient of collagen, disease defender, cardio metabolism support and wound healer[1]. Other biochemical functions include the biosynthesis of the amino acids, carnitine and the catecholamine that regulate the nervous system. It also helps the body to absorb iron and to breakdown histamine, the inflammatory component of many allergic reactions[2]. Disease conditions ameliorated by vitamin C include cataract development[3], Hemolytic and sickle cell anaemia, periodontal disease[2,4] bone disorders, diabetes[4] and cancer. Pathologic disease conditions due to vitamin C deficiency in man are more severe than in other living species. Man lacks the liver enzyme 'gulonolactone oxidase' required for converting L-gulono-lactone from D-glucose to L-ascorbic acid (vitamin C)[5].

Several methods are described in literature[6,7] for the determination of ascorbic acid in biological samples and blood serum. In all of them hazardous chemicals and complicated procedures are involved[8]. The main thrust of this work therefore, is to develop a simple, cheap but very sensitive alternative for the determination of vitamin C in natural fruit samples, commercial tablets and juices. The use of a locally extracted dye in titrimetric determination of vitamin C would replace the relatively costly indophenol and other reagents currently in use.

MATERIAL AND METHODS

Material and reagents

Laboratory materials included L-ascorbic acid tablets (vitamin C - 500mg/ tab) from Philip Harris, 2,6-Dichlorophenol-indophenol (DCPIP) from Philip Harris, 100% Acetic acid (AnalaR grade) from BDH, grape fruit, orange, tangerine, lime, pineapple, fresh palm wine and fresh fluted pumpkin seeds. Others were commercial fruit juice, vitamin C tablets obtained randomly within Uyo capital city, Nigeria.

Extraction and preparation of standard solutions

(i) Standard solution of locally extracted dye (LED) was prepared by peeling the soft outer covering (blue-black in colour) of ten fresh fluted pumpkin seeds each approximately 5.0g in size. This was dissolved in 1dm^3 of distilled water and filtered through fluted filter papers. The filtrate was used for analysis.

(ii) Standard 2,6-Dichlorophenol-indophenol (DCPIP) was prepared by dissolving 1.0g of DCPIP in 100ml of distilled water.

(iii) For standard vitamin C solution, a tablet of L-ascorbic acid (containing 500mg of vitamin C) was completely dissolved in 500ml of distilled water (1ml/1.0mg vitamin C).

Standardization of LED and DCPIP solution

Standardization of LED was done by titrating 0.5ml standard vitamin C solution above with the LED. The end-point was noted when the blue-black colour of the solution turned to reddish brown. Average volume of LED needed (end-point) = 0.587ml. This was equivalent to 0.5mg of vitamin C. Hence 1ml/ 0.825mg of vitamin C (titre value for LED).

DCPIP solution prepared in (ii) above was titrated with 0.5ml of vitamin C. The endpoint was noted when DCPIP was completely decolorized. The average endpoint of DCPIP was 0.817ml. This was equivalent to 0.5mg of vitamin C. 1ml DCPIP/ 0.612mg of vitamin C (titre value for DCPIP).

Recovery Experiment

The entire analysis by both the new LED and conventional DCPIP was preceded by a recovery experiment. This experiment was conducted by purposely dissolving a known amount of vitamin C in distilled water and determining this amount titrimetrically using each of the two procedures. This was to ascertain whether the new method was capable of detecting vitamin C present before adopting it for analysis of experimental samples.

The recovery experiment was also intended to show how sensitive and precise each method can recover the total vitamin C content in samples. This was done by finding the percentage recovery % recovery

$$= \frac{\text{amount of vitamin C determined}}{\text{amount of vitamin C dissolved in } H_2O} \times 100$$

The amount of vitamin C determined was obtained by multiplying the endpoint by the titre value.

Extraction and Determination of Vitamin C in Samples

Natural fruit samples (viz: lime, orange, pineapple, grape and tangerine) were peeled and the juice squeezed out into a conical flask. 0.5ml of the juice was taken for titrimetric analysis of vitamin C. A solution of guava was prepared by adding 10ml distilled water to an average size (1.5g) guava which had been thoroughly grinded. This was filtered through Watman No.11 filter paper and 0.5ml of the filtrate taken for analysis. One tablet each of commercial vitamin C tablet was completely dissolved in 10ml distilled water contained in two different beakers. 0.5ml of each solution was also taken for analysis. For all commercial fruits juice (viz: chivita (CA), lucomalt (LU), 5Alive (5A), Lucozade boost (LB)) and palm wine, 0.5ml was taken directly for analysis. All these samples were titrated to endpoint with standard LED and DCPIP in turn. The results are presented in tables 2 and 3.

Spectrophotometric determination of vitamin C using standard LED

This analysis was preceded by the determination of the wavelength of maximum absorption (λ_{max}) of standard LED using Winelight Uv spectrometer with 10cm silica cell. 1.0ml standard LED solution was diluted to 10ml with distilled water. This dilution was necessary so as to

match with the sensitivity range of the instrument. Figure 1 shows the variation of absorbance with wavelength for standard LED. This plot shows that standard LED has a λ_{max} at 210nm. The Uv spectrometer was adjusted to this wavelength throughout the experimental process.

For the determination of vitamin C by spectrophotometric method using standard LED, 5Alive juice, grape fruit and Emzor vitamin C tablet representing samples in commercial fruit juices, natural fruits and commercial vitamin C tablets respectively were used. In each case, 1.0ml in 20ml of distilled water was used for standard LED. The absorbance of each sample was first noted without adding standard LED. This was taken as blank absorbance with 0.0ml standard LED. 10ml of each sample was accurately transferred into a conical flask. 0.5ml standard LED was added, swirled and about 1.0ml pipetted into the sample cell of the spectrometer. The absorbance was read and recorded, the solution in sample cell was returned to the stock solution while both pipette and sample cell rinsed with distilled water. The experiment was repeated by adding up to 5.0ml of standard LED, 0.5ml in each succession and the corresponding absorbance taken. The variation of absorbance with volume of standard LED added and the corresponding first derivative plots for 5Alive, grape fruit and Emzor vitamin C tablet are shown in figures 2,3, and 4.

RESULTS AND DISCUSSIONS

Tables 1a and b give the results of recovery experiment in the determination of vitamin C with standardized locally extracted dye (LED) and 2,6-Dichlorophenol-indophenol (DCPIP) titrimetric methods of analysis. Values obtained with standardized LED method were on the average higher than those with standardized DCPIP. This showed that the newly developed method is capable of determining vitamin C present in samples even better than the conventional DCPIP method. However, at concentration of 0.2mg/ml, determination by LED (72.4%) showed a poor percentage recovery as compared to DCPIP (91.8%) This implies that at that concentration or less, the amount of vitamin C determined by LED might not be reliable. DCPIP serves as a better substitute at such low concentrations. At concentrations of 1.0 -7mg/ml, LED gave a better percentage recovery. This showed that within this concentration range, the new LED method is preferred to DCPIP as it is more sensitive, accurate and precise for vitamin C determination.

However, at concentrations of 1.8 -2.0mg/ml, the percentage recovery decreased though not as low as that obtained with DCPIP. This implied that at the working concentration of LED, the amount of vitamin C determined by LED becomes less accurate. With such high concentrations, there is a need to dilute the solution of samples before determination using LED. The result of the determination of vitamin C in 21 samples (commercial and natural fruit juices) using the newly developed method and the conventional indophenol methods are tabulated in tables 2and 3 respectively. A casual comparison of the two tables revealed a consistent measurement of vitamin C for each sample by both methods. Statistical analysis of result data was done. The result showed that there was no significant difference in precision and reproducibility between the two methods (t_{cal} = 0.32, t_{crit} = 2.704, α=0.01, df = 40).

In other words, the two methods measured with the same precision and reproducibility even at α = 0.05, where t_{crit} = 2.021. From this observation, it can be seen that any of the two methods can therefore be used in the determination of vitamin C in any sample without sacrificing accuracy and precision.

Student's t-test for comparing difference of two means for small data (N<30) [9] was also applied in comparing the means obtained by the two methods. The test statistic further revealed that there was no significant difference between the means of data obtained by the two methods, (t_{cal} =0.1350, t_{crit} =2.704, α =0.01, df =40) and at α =0.05, df =40, t_{crit} = 2.021. This indicates that the new LED titrimetric methods developed in this research measured with no significant difference in precision and accuracy with the conventional DCPIP method at 99% and 95% levels of significance.

Table 1(a): Recovery experiment with standard LED

Vol. of Vit. C solution (mL)	Theoretical amount of Vit. C /mg (y_1)	Average end point (mL)	*Amount of Vit. C determined / mg (y_2)	% Recovery ($^{y_2}/_{y1} \times {}^{100}/_{1}$)
0.2	0.2	0.17	0.1448	72.4
0.3	0.3	0.29	0.2471	82.4
0.4	0.4	0.41	0.3493	87.3
0.5	0.5	0.50	0.4260	85.2
0.6	0.6	0.60	0.5112	85.2
0.7	0.7	0.70	0.5964	85.2
0.8	0.8	0.80	0.6816	85.2
0.9	0.9	0.90	0.7668	85.2
1.0	1.0	1.10	0.9372	93.7
1.1	1.1	1.20	1.0224	92.9
1.2	1.2	1.30	1.1076	92.3
1.3	1.3	1.40	1.1928	91.8
1.4	1.4	1.50	1.2780	91.3
1.5	1.5	1.60	1.3632	90.9
1.6	1.6	1.70	1.4484	90.5
1.7	1.7	1.90	1.6188	95.2
1.8	1.8	1.90	1.6188	89.9
1.9	1.9	2.00	1.7040	89.7
2.0	2.0	2.10	1.7892	89.7

* Mean of 3 determinations

Table 1(b): Recovery experiment with standard DCPIP

Vol. of Vit. C solution/ml	Theoretical amount of Vit. C (y_1)	Average end point/ml	*Amount of Vit. C determined / mg (y_2)	% Recovery ($^{y_2}/_{y1} \times {}^{100}/_{1}$)
0.2	0.2	0.30	0.1836	91.8
0.3	0.3	0.43	0.2632	87.7
0.4	0.4	0.56	0.3427	85.7
0.5	0.5	0.70	0.4284	85.7
0.6	0.6	0.80	0.4896	81.6
0.7	0.7	1.00	0.6120	87.4
0.8	0.8	1.08	0.6609	82.6
0.9	0.9	1.30	0.7956	88.4
1.0	1.0	1.40	0.8568	85.7
1.1	1.1	1.60	0.9792	89.0
1.2	1.2	1.70	1.0404	86.7
1.3	1.3	180	1.1016	84.7
1.4	1.4	2.00	1.2240	87.4
1.5	1.5	2.20	1.3464	89.8
1.6	1.6	2.30	1.4076	87.9
1.7	1.7	2.40	1.4688	86.4
1.8	1.8	2.50	1.5300	85.0
1.9	1.9	2.60	1.5912	83.7
2.0	2.0	2.80	1.7136	85.7

* mean of 3 determinations

A further attempt was made by using the student's t-test to measure any significant difference existing between the vitamin C content obtained for each of the samples determined by the two methods. In tables 2 and 3, items number 1,13,14,15 and 21 showed no significant difference (α =0.05, df =4, t_{crit} = 2.776) while items number 5,7 and 9 showed a significant difference at α =0.01, t_{crit} =4.604 between the amount of vitamin C determined by the two methods. The remaining items showed no significant difference at all. Significant difference in determination by the two methods as observed in the above listed items are suspected to have been due to contributory effects of coloured samples and other reducing agents present in the samples. It is suggested that bleaching of coloured samples and masking of other reducing agents may be a sure way of improving determination of vitamin C in such samples.

Generally, the LED method in the determination of vitamin C was very sensitive, accurate and precise: hence it is recommended as a better alternative to a relatively costly, scarce and health hazardous DCPIP method. Worthy of note to support the above assertion is that the approximate composition of vitamin C as indicated by manufacturers in most of the commercial juices considered and data in USRDA [10, 11] were on the average close to those determined by the new LED method without any significant difference ($p \leq 0.05$, n =25).

A plot of result of spectrophotometric titration of standard LED with vitamin C in three different samples as shown in figures 2, 3 and 4 revealed that the new LED method for determining vitamin C in samples can also be monitored using sophisticated instruments (spectrometers).

Table 2: Titration of 0.5mL samples with standard LED

S. No.	Samples endpoint (mL)	Mean of triplicate present (mg/mL) X_1	Amount of Vit. C
Commercial fruit juice			
1	CA	0.38	0.32
2	CA + Acetic acid	0.40	0.34
3	LU	0.43	0.37
4	LU + Acetic acid	0.42	0.36
5	LU + Animal Charcoal	0.43	0.37
6	5A fruit juice	0.49	0.42
7	5A + Acetic acid	0.49	0.42
8	LB+ Acetic acid	0.43	0.37
9	LB juice	0.41	0.35
10	LB + Animal Charcoal	0.51	0.44
Natural fruit samples			
11	Lime juice	0.53	0.45
12	Orange juice	0.53	0.45
13	Orange juice + Acetic Acid	0.51	0.44
14	Lime juice + Acetic Acid	0.50	0.43
15	Pineapple	0.49	0.42
16	Grape fruit	0.43	0.37
17	Tangerine fruit	0.45	0.38
18	Fresh palm wine	0.70	0.60
19	Emzor vitamin C tablet	1.12	0.95
20	Ulticare vitamin C tablet	1.09	0.93
21	Guava	1.35	1.15

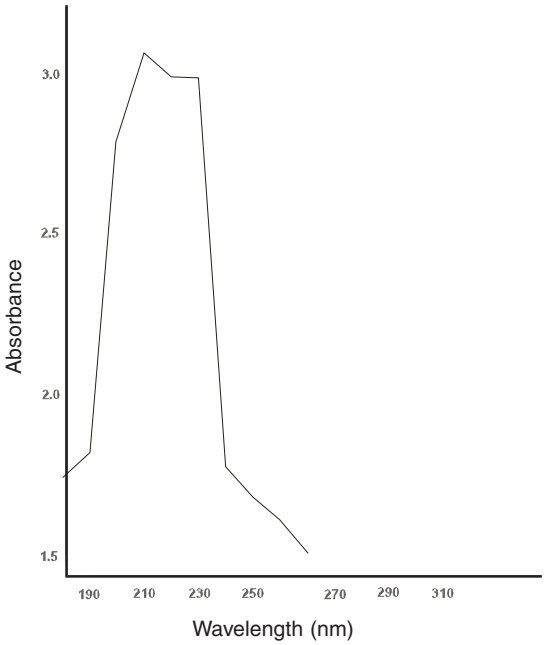

Fig. 1: Variation of absorbance with wavel length for standard LED.

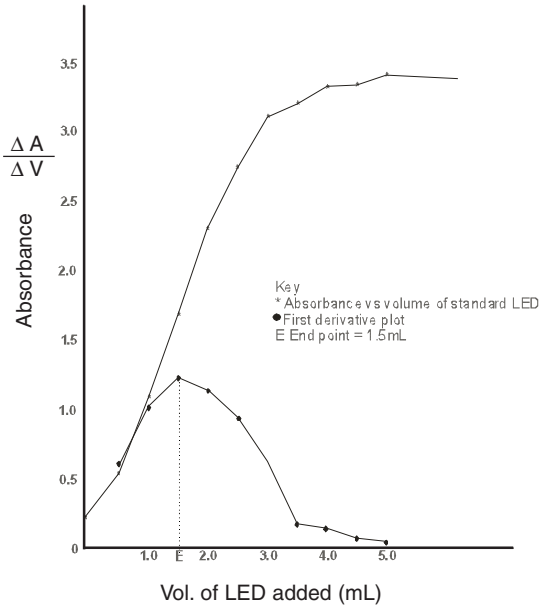

Fig. 2: A plot of reslut of spectro photometric titration of standard LED with Vit. C in 5 Alive juice.

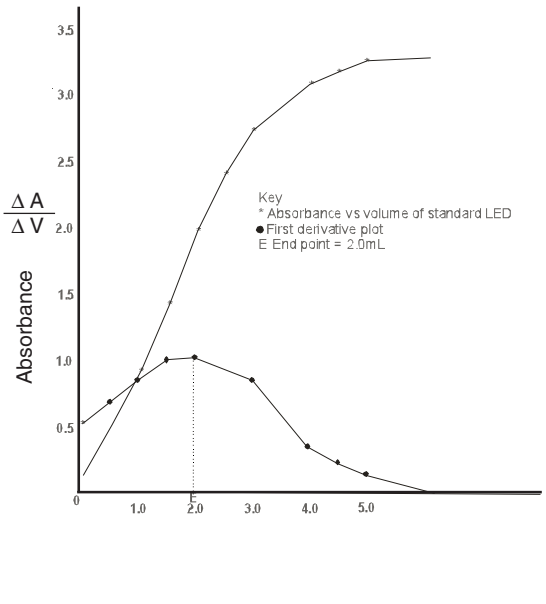

Fig. 3: A plot of reslut of spectro photometric titration of standard LED with Vitamin C in graphe fruit

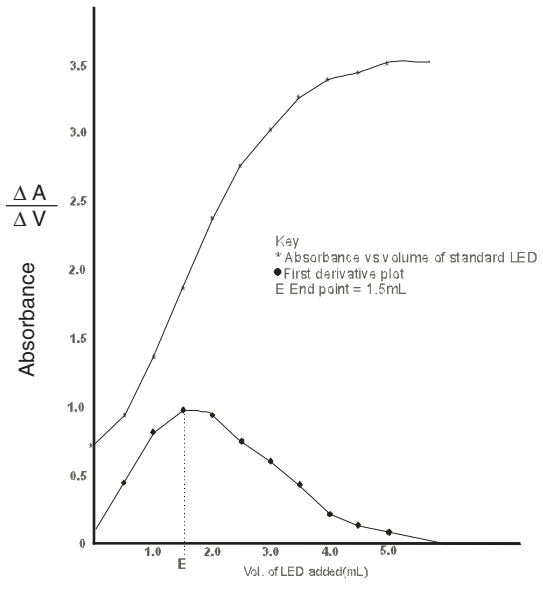

Fig. 4: A plot of reslut of spectro photometric titration of standard LED with Vitamin C in Emzor Vitamin C.tablet.

Table 3: Titration of 0.5mL samples with standard DCPIP

S. No.	Samples	Mean of triplicate endpoint (mL)	Amount of Vit. C present (mg/mL) X_1
Commercial fruit juice			
1	CA	0.49	0.30
2	CA + Acetic acid	0.54	0.33
3	LU	0.59	0.36
4	LU + Acetic acid	0.59	0.36
5	LU + Animal Charcoal	0.67	0.41
6	5A fruit juice	0.67	0.41
7	5A + Acetic acid	0.59	0.36
8	LB + Acetic acid	0.56	0.34
9	LB juice	0.70	0.43
10	LB + Animal Charcoal	0.74	0.45
Natural fruit samples			
11	Lime juice	0.74	0.45
12	Orange juice	0.70	0.43
13	Orange juice + Acetic Acid	0.67	0.41
14	Lime juice + Acetic Acid	0.70	0.41
15	Pineapple	0.60	0.37
16	Grape fruit	0.60	0.37
17	Tangerine fruit	0.62	0.38
18	Fresh palm wine	0.98	0.60
19	Emzor vitamin C tablet	0.54	0.94
20	Ulticare vitamin C tablet	1.50	0.92
21	Guava	1.85	1.13

The shapes of the titration curves obtained were very much in accordance with conventional titration curves. The main advantage of spectrophotometric method here is that it provided a simple means for determining minute quantities of vitamin C which otherwise could not be precisely determined by the visual titrimetric methods. With this method, it had been shown that the new locally extracted dye could detect vitamin C even as low as 0.06mg/ml in concentration (detection limit).

The maxima of the differential curves presented in figures 2, 3 and 4 gave better evaluation of the endpoint than does the plot of absorbance Vs volume of standard LED added. In the spectrophotometric titration of standard LED with vitamin C in juice (fig. 2), the endpoint is 1.5ml, but 1.0ml of standard LED was diluted to 20.0ml with distilled water.

20ml standard LED? 0.852mg of vitamin C (Titre value for LED)

1.5ml = 0.0639 mg of vitamin C. Using the same calculation in fig. 3 and 4 where endpoints were 2.0 and 1.5ml, amount of vitamin C determined equals 0.0852 and 0.0639mg respectively. It should be noted that at these concentrations, it would be very difficult to detect even with the conventional DCPIP using visual titrimetric method. Hence the spectrophotometric method developed in this research work serves as an alternative for determining vitamin C at a concentration as low as 10^{-2}mg in different samples.

Conclusion

This work has shown that a newly developed titrimetric method using locally extracted dye (LED) is capable of determining vitamin C content in samples under different conditions accurately. The method is simple and easy to use for the determination of vitamin C in fruit juices and other biological samples. It is more facile with a relative advantage in economy and time when compared to existing conventional methods.

It can be adapted to spectrophotometric titration method with high precision and detection limit (< 10^{-2}mg) for vitamin C content in plants and other samples. Vitamin C concentration in twenty-one (21) samples (fresh fruits, commercially packaged juices and vitamin C supplement tables) has been determined accurately with high precision and reproducibility.

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