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Exploitation of a simple redox reaction between manganese(III) and o-tolidine for a highly sensitive spectrophotometric determination of ascorbic acid

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ABSTRACT

A simple and highly sensitive spectrophotometric method for the determination of ascorbic acid is described. The developed method is based on a redox reaction in that Mn(III) generated electrolytically is taken in excess, which is oxidizing a known but a less quantity of vitamin C and the unreacted oxidant will oxidize further o-tolidine to produce a orange yellow quinonedi imine absorbing cation(λmax. 455nm). Therefore, in principle, the decrease in colour intensity of the absorbing system is proportional to the concentration of vitamin C. The stoichiometry between Mn(III) and o-tolidine and stability of the complex were determined by Job's method, the corresponding values obtained were 2:1 and 1.58×10^5 $lmol^{-1}$. The system was found to be obeying Lambert-Beer's law in the range, 0.08-0.8mg l^{-1} of ascorbic acid. Molar absorbtivity, correlation coefficient, and Sandell's sensitivity values were also calculated and found to be 4.4359×10^3, 0.9987 and 0.0397μg/cm^{-2} respectively. The reliability of the new method was tested by parallel determination of ascorbic acid in standard, pharmaceutical and fruit samples using a standard method. The results of vitamin C obtained from both the methods were comparable with one another and the reliability and reproducibility of the new method were concluded by F and t test values. Therefore, the new method could be employed effectively for the determination of ascorbic acid in standard and other samples either as an independent high sensitive spectrophotometric method or a complimentary method to official method.

KEYWORDS

Vitamin C;
Redox reaction;
Mn(III);
o-Tolidine;
Spectrophotometry.

INTRODUCTION

Vitamin C, known by its chemical name ascorbic acid, is an essential water soluble nutrient required for optimal health[1] with daily recommended intake 70 mg[2]. It is also considered essential for the development and regeneration of muscles, bones, teeth and skin[3]. It participates in a variety of biological events concerning electron transport reaction, hydroxylation and the oxidative catabolism of aromatic amino acids with antioxidant properties, which prevent scurvy[3]. Vitamin C occurs naturally in different concentrations in most fresh fruits[4] and is added during the manufacture of juices and soft drinks[5]. It is added to the several

pharmaceutical products as an essential ingredient, for example, a stabilizer for vitamin B complex and also as an antioxidant[6]. As a consequence of its desirable effects[7], it is widely used in the treatment of certain diseases such as scurvy, common cold, anemia, haemor rhageic disorders, wound healing and even infertility.

Vitamin C degrades quickly and therefore, there is a special concern regarding the shelf life of those fortified foods[3], and also considering its importance[1,2,4,5], several analytical methods are being reported frequently[9-21] which include, titrimetry[8,9], spectrophotometry[10-15], chromatography[16], HPLC[17], polarogrphy [18], fluorimetry[19], voltammetry[20], chemiluminescence[21], and potentiometry[22], Among such methods[5,15], most frequently used method is a titrimetry involving 2,6 dichlorophenolindophenol[5] as titrant, the method claimed to be rapid but the reagent is unstable[15]. The most sensitive methods for it include chemiluminescence [21], fluorimetry[19], and HPLC[12,17], however, the equipments cost restrict the practice of such methods. Hence, the spectrophotometric method involving 2, 6-dichlorophenolindophenol[8, 20, 23] is appeared to be most commonly used. However, as the reagent[8,20,23] is a dye, obviously requiring strict control of pH, and colour is appeared to be stable only for about 20min. In addition, the method also recommends the storage of the reagent in a refrigerator[23]. As a consequence[8,20,23], and also considering the importance of ascorbic acid[1-7] and also the limitations associated with reported methods[12,17,19],particularly in the spectrophotometric method[20, 23] and also encouraged by earlier work with Mn(III)[31] a new spectrophotometric method is developed here for the determination of ascorbic acid. The method is based on a redox reaction in that Mn(III) generated electrolytically[24,25] is taken in excess, oxidizing a known but a lesser quantity of vitamin C and the unreacted oxidant will oxidize further o-tolidine to produce an orange yellow, quinonedi imine absorbing cation[29]. Therefore, in principle, the decrease in colour intensity of the absorbing system is proportional to the concentration of vitamin C.

EXPERIMENTAL

Apparatus and reagents

Spectrophotometer: ELICO SL 171mini. Spec. with 1cm matched quartz cells were used for absorbance measurements.

Ascorbic acid(E, Merck India), sulfuric acid, sodium thiosulphate, potassium iodide and starch, manganous sulphate(SD fine chemicals, Boisar India), o-tolidine(Sigma-Aldrich chemie Gmbh, Steinheim, Germany), were used.

Preparation of manganese(III) solution

10ml of 2M manganese(II) sulphate solution was diluted to about 100ml with 5M sulfuric acid and electrolyzed the solution for about 2h[24,25]. Concentration of the prepared Mn(III)solution was determined iodometrically[26] and the solution was found to be 0.015M with respect to Mn(III). From this, an aliquot, 1.3ml was further diluted to 100ml in a volumetric flask with 5M sulfuric acid, the solution so obtained was 0.0002M(cal.).

Preparation of standard o-tolidine solution

Accurately weighed amount, 20mg of o-tolidine sample was dissolved in a clean beaker containing about 10ml of ethanol and the solution was transferred into a 100ml volumetric flask. The beaker was washed with water and washings were also transferred into the flask. Then, the solution was diluted to the mark with water. The prepared solution was 0.001M(cal.), from this solution; an aliquot of 10ml was further diluted to 50ml with water to obtain 0.0002M(cal.) solution.

Standard sample preparation

Ascorbic acid solution(20mg/lit) was prepared by dissolving 0.020g of ascorbic acid in 100ml of water.

Pharmaceutical sample preparation[27]

A known weight of finely ground powdered tablet equivalent to 100mg of ascorbic acid (500mg for celien) was stirred for 2-3minutes with about 50ml distilled water. The mixture was filtered through a Whatman No. 1 filter paper, then, the filtered solution was transferred into a 250ml volumetric flask and diluted to the mark with distilled water. This solution was further diluted by transferring 2.5ml into a 100ml volumetric flask and made up to the mark with distilled water. Then, an aliquot of 0.2ml, that sample solution was analyzed as described under general procedure and also by the stan-

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dard method[23]. The results obtained by both the methods are given in TABLE 2.

Syrups/Juices [27]

An accurately measured, 0.5ml of the solution was transferred into a 250ml volumetric flask and made up to mark with distilled water. This solution was further diluted by transferring 2.0ml into a 100ml volumetric flask and made up to the mark with distilled water. Then, an aliquot of 0.4ml of that sample solution was analyzed as described under general procedure and also by the standard method[23]. The results obtained by both the methods are given in TABLE 2.

Fruits[27]

About 5g portion of each sample was squeezed or homogenized mechanistically for about five minutes and extracted into distilled water. Extract was filtered into 100ml volumetric flask and made up to mark with distilled water. Then, an aliquot of the sample solution was analyzed for vitamin C both by the new method through the described general procedure and also by the standard method[22]. The results obtained from both the methods are given in TABLE 2.

TABLE 1 : Optical parameters

Parameters	Values
Wave length (nm)	455
Beer's law limit (ppm)	0.08-0.8
Molar absorptivity(10^3 l mol/cm^{-1})	4.4359
Sandell's sensitivity (μg cm^{-2}per 0.001A)	0.0397
Slope	0.3270
Intercept	-0.0425
Correlation coefficient	0.9987
Stoichiometry, tolidine : Mn(III)	1:2

Tabulated values at 95% confidence limit are 2.365 and 6.39 for t-test and F-test, respectively; *Mean value ±standard deviation (n=5).

TABLE 2 : Determination of ascorbic acid in different samples

Sample	Amount* found in μgml^{-1} by		F-test	t-test
	Proposed method	Standard method		
Pharmaceutical				
Celine	0.195(± 0.008)	0.197 (±0.007)	1.306	0.40
Limcee	0.196(±0.009)	0.198(±0.007)	1.600	0.20
Cecon	0.160(±0.007)	0.162(±0.009)	1.600	0.39
Fruits:Apple	0.200 (±0.020)	0.190(±0.020)	1.000	1.00
Orange	0.430 (±0.040)	0.400(±0.030)	1.910	0.91

TABLE 3 : Effect of foreign ion experimental solution containing 0.4ppm of ascorbic acid

Interferon	Tolerance limit(μg ml^{-1})	Interferon	Tolerance limit(μg ml^{-1})
Caffeine	300	Ca^{+2} ($CaCl_2$)	200
Vitamin-B	100	Mg^{+2}($MgSO_4$)	300
Glucose	130	Na^{+}(NaCl)	270
Fructose	130	$Pb^{+2}Pb(NO_3)_2$	023
Formaldehyde	015	Zn^{+2}($ZnSO_4$)	100
Hydrogen sulphide(Na_2S)	1.50	Fe^{+2} ($FeSO_4$)	020
		Fe^{+3}($FeSO_4$)$_2$	003

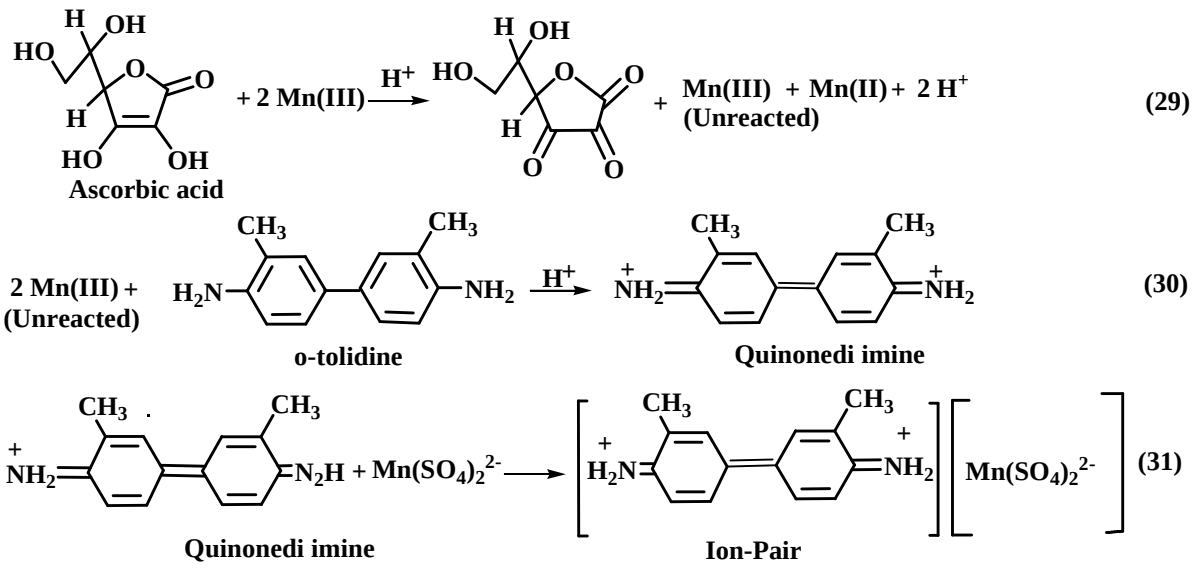
Procedure

Construction of the calibration graph

A series of labeled 10ml volumetric flasks were arranged. 1.0ml of 0.0002M Mn(III) solution was added to each flask. A known but various volumes, 0.1-1.5ml of standard ascorbic acid (8ppm) solutions were added to each flask and the solutions were kept aside for about 2min to ensure the completion of reaction. Then, 1.0ml of standard o-tolidine(0.0001M) solution was added to each flask and the solution in each one of them was diluted to the mark with water. The solutions were mixed well and kept aside for about 10min. The absorbance of each solution was measured at 455nm against water. The calibration graph obtained from the values was as given in figure 1.

RESULTS AND DISCUSSION

The method is based on a redox reaction in that Mn(III) generated electrolytically[24,25] taken in excess, is oxidizing a known but a lesser quantity of vitamin C then the unreacted oxidant will oxidize further o-tolidine to produce an orange yellow, quinonediimine absorbing cation[29]. Therefore, in principle, the decrease in colour intensity of the absorbing system is proportional to the concentration of vitamin C. The colour of the system was found to be stable for more than 2h. The stoichiometry between the o-tolidine and Mn(III) was determined by Job's method[28] and the values were found to be 1:2 with respect to o-tolidine and Mn(III). The stability constant of the system was also calculated and found to be 1.58×10^5 l mol^{-1}. The proposed method was applied for the determination of ascorbic acid present in standard, pharmaceutical samples, and also present in fruit juices. The optical characteristic parameters were calculated from experimental data and are given in TABLE 1. The reliability of the method was tested by parallel determination of ascorbic acid using a standard method[23]. The reliability and reproducibility of the new method was concluded by F and t test values as given in TABLE 2. A probable reaction mecha-



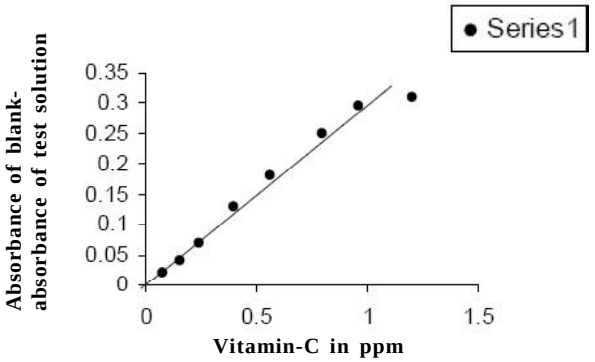


Figure 1: Calibration graph

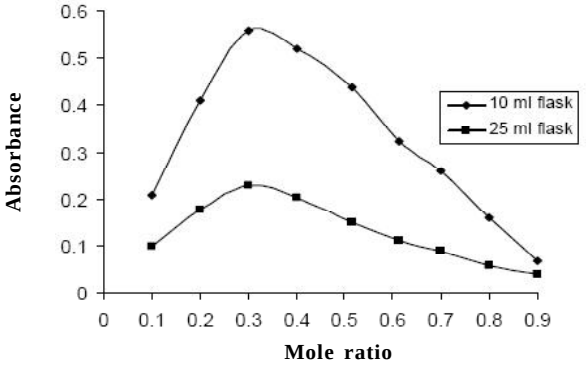


Figure 2 : Determination of stoichiometry and stability constant

nism of the method is also given.

Effect of foreign ions

Tolerance limit of the common foreign ions on experimental solution containing 0.4ppm of ascorbic acid was tested by studying the effects of these on the absorbance of the experimental solution. An error of less than ±5% in the absorbance values was considered to be tolerable. The tolerance limit of the foreign ions tested is given in TABLE 3

Determination of stoichiometry and stability constant

The composition between Mn(III) and o-tolidine was studied by modified Job's method of continuous variation[28]. The concentrations of aqueous solutions of Mn(III) and o-tolidine both of them were 0.0001M. Nine solutions were prepared in 10 ml flasks containing Mn(III) and o-tolidine in various molar ratio's so that their volumes were always amounted to 5ml. The results obtained were used in plotting the graph, figure 2, that accounts for 2:1 stoichiometry between Mn(III) and o-tolidine respectively. Similarly, the experiment was carried out for other set of solution following the above procedure but with the solutions diluted to 25ml instead of 10ml. The results obtained were used for the construction of the graph, figure 1, which again accounts for 2:1 stoichiometry between Mn(III) and o-tolidine respectively. The stability constant of the complex was calculated[28] and found to be 1.58×10^{5} l mol^{-1}.

CONCLUSION

The developed new method was employed effec-

tively for the determination of ascorbic acid in the standard solution, pharmaceutical samples and also in fruit juices. Electrolytically generated manganese(III)[24,25] was found to be stable for more than one month . The developed method is simple[17-22], rapid and highly sensitive[32-34] and yields reproducible and accurate results without involving critical maintaince of experimental condition. The reproducibility of the new developed method was established by comparing the results of the new method with those of a standard method[23]. The standard deviation as well as calculated t-test and F-test values reveal that the accuracy and precision are affordable[26] by the method. Therefore, the new method could serve either as an alternative or a parallel to the strandard methods for the determination of ascorbic acid.

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