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SENSITIVE SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN C IN HERBAL PRODUCTS AND PLANT SAMPLES.

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ABSTRACT

A Simple, sensitive and accurate spectrophotometric method has been developed for determination of Vitamin C in synthetic mixtures and real samples. This method is based on the oxidation of Vitamin C by Known excess of Te(IV) in hydrochloric acid medium. The unreacted Te(IV) reacts with iodide in the same acid medium to liberate iodine, which then reacts with starch to yield a blue coloured starch-iodine complex, which shows maximum absorbance at 480 nm. The reacted oxidant corresponds to vitamin C content. This reaction system is the basis for the indirect spectrophotometric determination of vitamin C. vitamin C when added in increasing amounts, consume Te(IV) and decreases the concentration of Te(IV). The absorbance is found to decrease linearly with increase in concentration of vitamin C. A 1.0 mL of 2.0 mol L^{-1} concentrationof HCl was found to be optimum for the oxidation of vitamin C within 10 min, and hence the same concentration was employed for the determination. The volumes of 1.5 mL of 0.5% KI and 1.0 mL of 0.5 % starch solution in a total volume of 10 mL of reaction mixture were found to be suitable for the analysis. The Beer's range is obeyed in the concentration range of 0.2-4.0ug/cm^3. The molar absorptivity ans Sandell's sensitivity values are found to be 0.0745 L $mol^{-1}cm^{-2}$ and 0.0197 µg cm^{-2} respectively. The cofficient of co-relation is found to be 0.967. The Regression equation is Y=0.18X +0.197. The method is successfully applied for the determination of vitamin C in orange peel powder,Mint leaves and spinach.

KEYWORDS: Vitamin C, Tellerium, Herbal samples, plant material, spectrophotometer.

INTRODUCTION

Ascorbic acid (vitamin C), is the dienol form of γ-lactone of 2-deoxy-2-keto-L-gulonic acid, which is present in citrus fruits, vegetables, milk, beverages and pharmaceutical products. Ascorbic acid occurs in different concentrations in a variety of natural samples. It is added to several pharmaceutical products as an essential ingredient, a stabilizer for vitamin B complex, and as an anti-oxidant. Consequent upon its desirable effects, it is widely used in the treatment of certain diseases such as scurvy, anaemia, haemorrhagic disorders *etc* Ascorbic acid is widely required in the metabolism of living beings. It is considered essential for the development and regeneration of muscles, bones, teeth and skin. Also it has been identified as a radical scavenger *in vivo*. The therapeutic importance of AA (**AA** stands for Ascorbic acid /vitamin C) has prompted many researchers to develop methods for its determination in real samples as well as in pharmaceuticals and these methods have been reviewed.[1-3] The importance of ascorbic acid for the organism as well as the problems caused by excess of vitaminC have been investigated in detail.[4-6] There is some evidence that large doses of vitamin C increase lymphocyte blast genesis,which is associated with prognosis of cancer.[7] Ascorbicacid, which increases intestinal absorption of iron, may alsoincrease absorption of heavy metals such as lead and mercury,accelerating the development of toxicity from thesemetals.[8]

Many analytical techniques are available for the determinationof ascorbic acid in different matrices, i.e. HPLC,[9] AAS,[10] Flow Injection Analysis,[11-12] Ion exchange,[13] Turbidimetric method,[14] etc. A number of organic and inorganic reagents1,2,4-dinitrophenyl hydrazine,[15] 2-mercaptoetanol,[16] fast red AL salt[17] have been reported for the spectrophotometric determination of ascorbic acid.

The methods used for AA determination include titrimetric, spectrophotometric, fluorimetric, electrochemical,chromatographic, kinetic and chemiluminescens procedures. But due to their inherent limitations, these techniques (except titrimetric and spectrophotometric) are not commonlyused for routine analysis. However, photometric methods are particularly attractive because of ease in accessibility and their quick applicability to routine analysis. Manyspectrophotometric methods suggested for the determination of AA have been based onreduction of iron(III) to iron(II) with AA, followed by the complexation of reduced iron(II)with different reagents such as 1,10- phenanthroline,[18] bipyridine[19], and *p*carboxyphenylfluorone.[20] The reduction of Cu(II) to Cu(I) with AA, the formed Cu(I)

interacts with neocuproine reagent was the basis for its AA determination.[21] Some of these methods sufferfrom many disadvantages like use of heating step and 20 min for full colour development ,low sensitivity and poorer selectivity.Few indirect spectrophotometric methods have been reported for analysis of AAutilizing iron(III) – thiocyanate complex[22] and ferrozine[23] Even these procedures are unsuitable for routine analysis, since the iron(III) – thiocyanate method required expensive experimental set-up. To overcome these limitations in the existing methods, there is still a need for a sensitive and cost-effective method for the determination of ascorbic acid that can be employed for the routine analysis of it in pharmaceuticals as well as in real samples. A simple and highly sensitive method is proposed for the determination of ascorbic acid. The method is free from the interferences of a number of substances commonly found in fruits, beverages and pharmaceuticals and has been applied to the determination of ascorbic acid in fruits, beverages and pharmaceuticals samples.

The proposed methods utilized Te(IV) with starch-iodine for the determination of micro amounts of ascorbic acid in different samples.

MATERIALS AND METHODS

Apparatus: All glasswares used for the experimental purpose were made up of Pyrex or Borosil glass. The burette, pipette and standard flasks were calibrated by the method described by Vogel.[24]

The absorption measurements were carried out on a spectrophotometer, model EQ-822, supplied by Equiptronics, Powai using 1-cm matched glass cells. The spectrophotometer was calibrated by measuring the absorption spectra of potassium chromate in potassium hydroxide solution and that of potassium permanganate in sulphuric acid solution.[25]

A digital pH meter model EQ-610, supplied by Equiptronics, Powai having an accuracy of ±0.02 pH and resolution of 0.01 pH was used to measure the pH of the solutions. The pH meter was calibrated with standard buffer solutions of pH 7.0, 4.0 and 9.2. A single pan digital analytical balance of series CA-223, supplied by Contech, having sensitivity of 0.001 g was used for weighing chemicals, reagents and samples.

Reagents: All the chemicals used were of A.R. grade. Distilled water was used for preparing standard solutions as well as for all experimental work.

Aqueous solutions of potassium iodide[0.5 %], starch [0.5 %], 2.0 M hydrochloric acid were prepared.

Preparation of solutions

1.Tellerium(IV) [0.01 mol L-1]: Prepared by dissolving 0.868 g of sodium telleurite in HCl(mol/dm3) and diluted to 500 mL distilled water. A working standard solution was prepared by a suitable dilution of standard solution.

2. *Standard solution*

Aqueous solution of vitamin C/ascorbic acid (AA) [100 µg mL^{-1}] was prepared by dissolving 0.01 g of it in 100 cm^3 of distilled water. Working solution was prepared as required by dilution.

Proposed method

Different aliquots of the standard solution of vitamin C containing 0.2 – 4.0 µg mL-1 were transfered into a series of 10 mL standard flasks. Then, a volume of 0.5 mL of 1000 µg ml^{-1} Te(IV) solution was added to each flask followed by acidification by 1.0 mL of 2.0 mol L-1 hydrochloricacid. After 10 min, 1.5 ml of 0.5 % KI was added to each flask. After 2.0 min, 0.1 mL of 0.5 % starch was added and the contents were diluted to the mark with distilled water and mixed well. The absorbance of the colored complex was measured at 580 nm against distilled water after 5.0 min. Blank was prepared similarly omitting the AA and its absorbance was measured against distilled water. The decrease in absorbance corresponding to consumed Te(IV) and in turn, to AA concentration, obtained by subtracting the absorbance of AA solution from the corresponding blank. The calibration graph was drawn by plotting the difference in absorbance against the concentration of AA, and the amount of AA was computed from the calibration curve. Fig1.

Analysis of vitamin C in Real samples

1)1g of orange peel powder collected from Dombivali area was used .The extraction was carried out in Soxhlet extractor by using 70% (150 ml) acetone for 4 hrs. 10 cm^3 of sample was used for the analysis.

2) The different samples of Mint leaves, were collected from local area in Thane. Preparation of plant extracts in different solvents: Mint leaves were air dried for few days and grinded

into fine powder. Five grams of sample of mint powder was extracted in70 % acetone(300 ml) and 100% methanol(300ml) by using soxhlet extractor for4 hrs.

3) Extraction and determination of Vitamin C in spinach (Spinacia oleracea).
7 g of sample powder was extracted in 350 cm^3 of the extracting solvent i.e. 70% acetone for 5½ to 6 hours using soxhlet extractor. Calcium and zinc interfered seriously in the determination of vitamin C.

25 cm^3 of the crude plant extract was used to remove calcium[26] and zinc[27] gravimetrically prior to analysis. The resulting filtrate, now free of calcium as well as zinc, was made upto 100 cm^3 with distilled water. An aliquot of this sample solution was transferred into a 10 cm^3 standard volumetric flask and the vitamin C content was determined by the present method. The results were found to be in good agreement with those obtained by reference method.[28]

RESULTS AND DISCUSSION

This method involves the oxidation of AA by Te(IV) in an acid medium. The unreactedTe(IV) reacts with iodide in the same acid medium to liberate iodine, which then reacts withstarch to yield a blue coloured starch-iodine complex. This reaction system is the basis forthe indirect spectrophotometric determination of AA. AA when added in increasingamounts, consume Te(IV) and decreases the concentration of Te(IV).

The absorbance is found decrease linearly with increase in concentration of AA (Figure 2)**.**Hydrochloric acid was the medium of choice for oxidation of AA by Te(IV) as well asthe latter's determination with iodine-starch reagent. A 1.0 mL of 2.0 mol L–1 concentrationof HCl was found to be optimum for the oxidation of AA within 10 min, and hence the sameconcentration was employed for the determination of AA with Te(IV)- iodine-starch reagent.The volumes of 1.5 mL of 0.5% KI and 1.0 mL of 0.5 % starch solution in a total volume of10 mL of reaction mixture were found to be suitable for the analysis.

Analytical data

The Beer's law limit, molar absorptivity, Sandell's sensitivity, correlation coefficient, Of the results are given in Table 1.

Table 1

Parameter	Values
Absorption maxima	580 nm
Beer's range	0.2-4.0 ppm
Molar absorptivity	0.0745 L $mol^{-1}cm^{-2}$
Sandell's sensitivity	0.0197 µg cm^{-2}
Regression equation	*Y=0.18X +0.197
Slope	0.18
Intercept	0.197
Co orelation coefficient	0.967

*Y= a+bx, where x is the concentration in µg mL-1

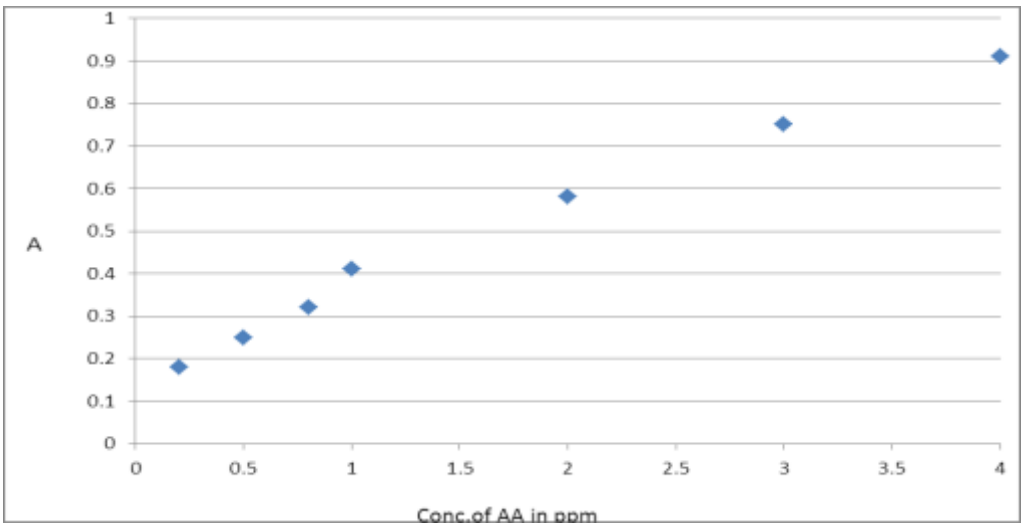

Fig1: Calibration graph

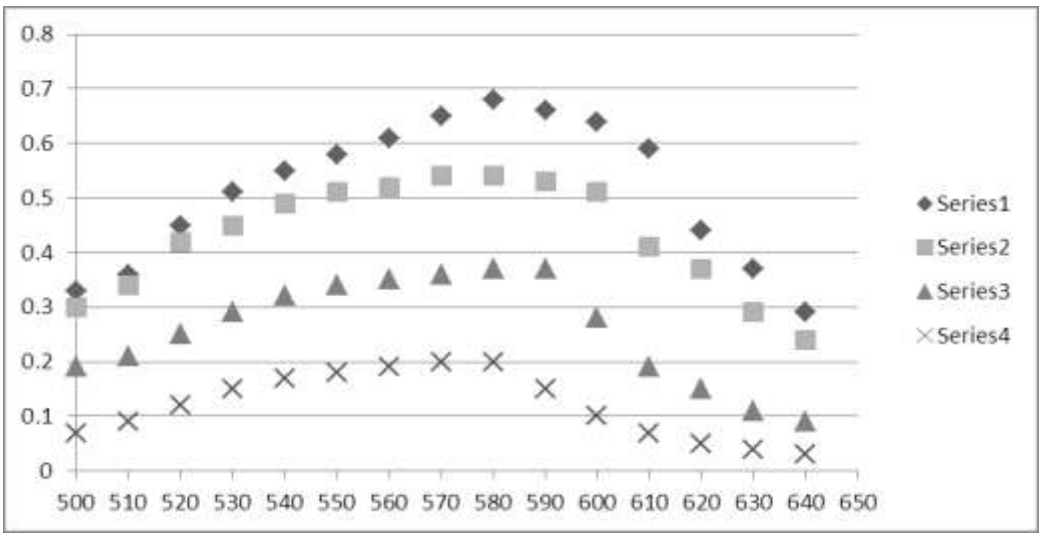

Figure 2. Absorption spectra of the Te(IV) – iodine -starch complex with ascorbic acid (1) Blank (without AA) (2) 1.0 µg mL-1 (3) 2.0 µg mL-1 (4) 3.0 µg mL-1 against water.

Applications to Real samples

The proposed methods were applied to the quantitative determination of AA orange herbal powder,Mint leaves in organic solvent.Table 2.

Table 2

Real sample	Sample in (70% acetone)	# Proposed method	# Reference Method[28]
1	Orange peel powder	5.2± 0.450	4.99± 0.75
2	Mint powder	2.0± 0.408	2.07± 0.64
3	Spinach leaves	6.3± 0.578	5.98± 0.77

 # Average of three determinations

CONCLUSION

The proposed spectrophotometric methods for the determination of AA are simple, selective and offer the advantage of sensitivity without the need for extraction or heating. The assay methods do not involve any stringent reaction conditions, and non interference from associated substances in the dosage forms and real samples. The methods developed have been utilized to determine vitamin C in real samples.

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REFERENCES

1. Arya S P, Mahajan P and Jain, Anal.Chim.Acta., 2000; 417: 1.
2. Arya S P, Mahajan P and Jain, Anal. Sci., 1998; 14: 889
3. Ana-Maria Hossu,V. Magearu, , Roum.Biotech.Lett., 2004; 9: 1497.
4. Fernandes, J. C. B.; Oliveira-Neto, G. D.; Kubota, L. T.Anal. Chim. Acta., 1998; 366: 11.
5. Wong, D. W. S. Quimica de los Alimentos. Mecanismos y Teoria; Acribia: Zaragoza, 1995.
6. Belitz, H. D.; Grosch,W. Quimica de los Alimentos; Acribia:Zaragoza, 1998.
7. Caeron, E.; Pauling, L. Int. J. Environ. Stud., 1977; 10: 303.

8. Wyngaarded, J. B.; Smith, L. H. CECIL Text Book of Medicine;Sounders W.B. Company, Harcourt Bruce Jovanovich,Inc.: London, 1987.
9. Nayyssonen, K.; Parviainen, S.; Parviainen, M. T.;Heinonenk, K.; Mononen, I. J. Liq. Chromatogr., 1988; 11: 1717.
10. Yebra, M. C.; Cespon, R. M.; Morencid, A. Anal. Chim. Acta., 1994; 298: 157.
11. Leon, L. E.; Catapano, J. Anal. Lett, 1993; 26: 1741.
12. Teshima, N.; Nabuta, T.; Sakai, T. Anal. Chim. Acta., 2001; 438: 21.
13. Chang, E. E.; Hsu, T. J.; Kao, W. Y.; Yang, C. C. J. ChinesChem. Soc., 1982; 29: 93.
14. Farajzadeh, M. A.; Nagizadeh, S. J. Chinese Chem. Soc., 2002; 49: 949.
15. Dabrowski, K.; Hinterlaitner, S. Analyst., 1989; 114: 83.
16. Verma, K. K.; Jain, A.; Verma, A.; Chaurasia, A. Analyst, 1991; 116: 641.
17. Backheet, E. Y.; Emara, K. M.; Askal, H. F.; Saleh, G. A. Analyst, 1991; 116: 861.
18. Anwar J, Farooqi M I, Nagra S A and Khan A M, J.Chem..Soc.Pak., 1990; 12: 75.
19. Fathi M R, Elahi R and Hashemi , Chem.Anal(Warsaw)., 2005; 50: 1069.
20. Fujita Y, Mori I, Yamaguchi M, Hoshino M, Shigemura Y and Shimano M, Anal. Sci., 2001; 17: 853.
21. Kubilay G, Kevser S, Esma T, Mustafa O and Resat A, Talanta, 2005; 65: 1226.
22. Noroozifar M, Khorasani–Motlagh M and Rahim A, Acta. Chim. Slov., 2004; 51: 717.
23. Molina-Diaz.A, Ortega-Carmona I and Pascual-Reguera M , Tlanta, 1998; 47: 531.
24. G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denney, A.I. Vogel's Text Book of Quantitative Inorganic Analysis including Elementary Instrumental Analysis, ELBS and Longman, New York, 1978; 4th ed: 47-82.
25. E.B. Sandell, Colorimetric Determination of Traces of metals, Interscience Publishers, New York, 1965; 3rd ed.
26. Vogel AI. A text book of quantitative inorganic analysis including elementary instrumental analysis. 3rd ed. New York(NY): ELBS and Longman Press., 1962; 551.
27. Vogel AI. A text book of quantitative inorganic analysis including elementary instrumental analysis. 3rd ed. New York(NY): ELBS and Longman Press., 1962; 533.
28. H.D.Revanassiddappa and M.A.Veena,E-journal of chemistry, 2008; 5: 10-15.