International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

Spectrophotometric Determination of Vitamin C using Iron(II)- 4,7-Diphenyl-1,10-Phenanthroline Complex

***Ashwani Kumar, Parveen Kumar**
*Department of Chemistry,
Kurukshetra University, Kurukshetra , India

Introduction

A number of spectrophotometric methods have been developed using different reagents but are associated with some shortcomings for example methods using 2,2'-bipyridyl [1-4], 1,10-phenanthroline[5-8], ammonium molybdate[9,10], folin-coicalteu[11,12] etc. are time consuming; these methods need at least 1 h for a single determination. Methods based on ferrozine[13,14], dimethoxydiquinone[15] and 2,3,5-triphenyltetrazolium chloride[16,17] suffers from many interferences.

For the development of a method with improved characteristics, bathophenanthroline has been found to be a suitable reagent that forms a red colored complex with reduced iron(II) over the pH range 4.0-5.5. The method based on the extraction of Iron(II)-bathophenanthroline complex in dichloromethane provides the desirable features of simplicity and rapidity besides having better sensitivity and selectivity.

Experimental

Instrument

A systronics spectrophotometer (model-166) with a pair of matched 1cm quartz cells was used for absorbance measurements.

Reagents and solutions

All reagents were of analytical grade and double distilled water was used for preparing solutions.

Ammonium fluoride solution

Ammonium fluoride solution was prepared by dissolving 18.5 gram of salt in distilled water

Buffer solution

Acetate buffer solution (pH 5.0) was prepared by mixing 35.7 ml of 1M acetic acid and 64.3 ml of 1M sodium acetate (Trihydrate) solution.

Ascorbic acid

A fresh aqueous solution of ascorbic acid (100 μg ml^{-1}) was used. A lower concentration (10 μg ml^{-1}) was obtained by dilution of the stock solution.

Iron (III) solution

A (1 mg ml^{-1}) iron (III) solution was prepared by dissolving accurately weighed amount of ammonium ferric sulphate in 100 ml of deionised water containing 0.5 ml of concentrated sulphuric acid. A lower concentration (100 μg ml^{-1}) was obtained by dilution of the stock solution.

4,7-diphenyl-1,10-phenanthroline (Bathophenanthroline solution)

A 0.05% (w/v) solution was obtained by dissolving the reagent in ethanol.

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

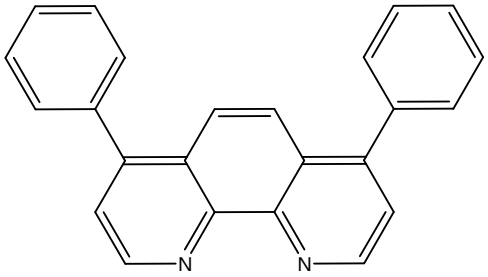

Fig 01 -4,7-diphenyl-1,10-phenanthroline

Procedure

Into a 100 ml seperatory funnel, 100 µg of iron (III) solution was pipetted and an aliquot of ascorbic acid was added followed by addition of 2ml of ammonium fluoride solution and 1ml of bathophenanthroline solution and 2 ml of acetate buffer solution for adjusting the pH in the range of 4.0-5.5. Enough water was added to make the aqueous phase to 10 ml. The resulting red colored complex was extracted for 30 sec with 10 ml of dichloromethane (DCM). The coloured extract was taken into a 10 ml volumetric flask and the volume was made up to the mark with DCM, if required. The absorbance of the red colored complex was measured at 485 nm against the reagent blank prepared similarly and the vitamin C contents is determined from the standard calibration curve prepared by taking different amounts of ascorbic acid up to 12 µg/ 10ml and using the optimum conditions of the procedure.

Determination of ascorbic acid in pharmaceutical products (tablets /capsules)

The tablets or capsules (5-10 items) were crushed to the powder form. An accurately weighed amount equivalent to 100 mg of ascorbic acid was dissolved in deionized water. The solution was filtered into a 100 ml volumetric flask and made up to mark with deionized water. The working solution of lower concentration (10 µg ml^{-1}) was obtained by suitable dilution of this solution. The diluted solution was analysed by the proposed procedure.

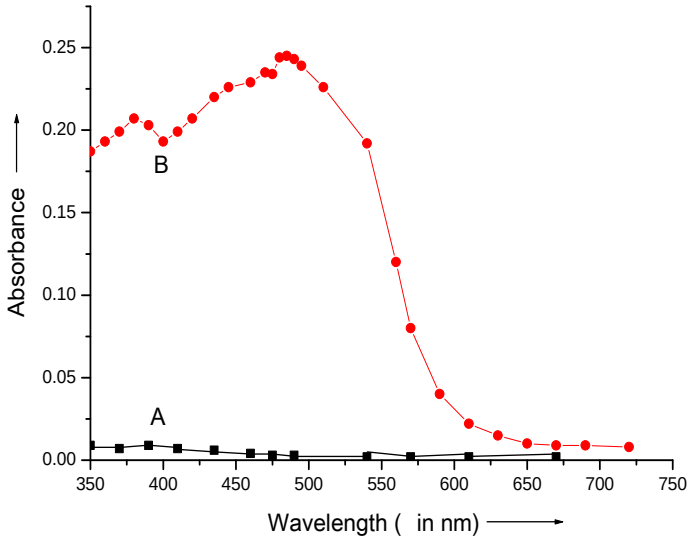

Fig. 02 – Absorption spectrum of Iron(II)-Bathophenanthroline complex (Conditions: Iron(III) =100 µg; Bathophenanthroline solution = 1.0 ml)

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

A – Reagent blank against dichloromethane

B – Complex against reagent blank.

Choice of solvent

Various solvents were tested to extract the Fe(II)-Bathophenanthroline complex as shown in Table 01. The solvents include dichloromethane, chloroform, carbon tetrachloride and benzene. However, n-Hexane, iso-Amyl acetate were found to extract the complex partially. Dichloromethane was chosen as an extractant because of the highest absorbance in this solvent.

Effects of reaction variables

The various parameters which can influence the extraction of the complex and absorbance were studied (Table 02). These include the change in concentration of the reagent, equilibration time and pH of the medium. In the study of each parameter, 10 ml of aqueous phase containing 100 µg of iron(III) and 10 µg of ascorbic acid was equilibrated with equal volume of dichloromethane.

Table 01

Extraction Behaviour of the complex in Different solvents

Solvent	Absorbance*
Dichloromethane	0.244
Chloroform	0.227
Carbon tetrachloride	0.196
Benzene	0.083
n-Hexane	0.025
iso-Amyl acetate	0.017

* Measured against respective blank

Effect of Bathophenanthroline concentration

An increase in the Bathophenanthroline concentration up to 0.8 ml of the reagent solution enhances the absorbance of the complex which thereafter remains the same up to 1.3 ml but there is a gradual decrease in absorbance above this concentration (Table 02, Fig. 03 Curve A). Hence, 1.0 ml of the Bathophenanthroline solution was used for further studies.

Effect of ammonium fluoride

Ammonium fluoride addition is very important as it prevents the extraction of Fe(III)-bathophenanthroline complex and 2ml solution is enough for complete masking.

Table 02 :Optimization of reaction variables

Reagent concentration (in ml)	0.4	0.6	0.8-1.3	1.5	2.0
Absorbance	0.127	0.217	0.244	0.242	0.239
Amount of buffer	0.0	0.5	1.0-2.5	3.0	
Absorbance	0.203	0.241	0.244	0.237	
pH	2.0	3.1	4.0-5.5	5.7	6.4
Absorbance	0.226	0.237	0.244	0.242	0.194
Equilibration time (in sec)	5	10	20	30-60	
Absorbance	0.069	0.153	0.208	0.244	

Conditions:Iron(III) (100 µg ml^{-1}) = 1 ml , Ascorbic acid (10 µg ml^{-1}) = 1 ml, Volume of aqueous phase = Volume of dichloromethane =10 ml , λ_{max} = 485 nm

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

Effect of pH

The extraction of the Fe(II)- Bathophenanthroline complex was studied over a pH range 2.0-6.4. It was observed that the complex gives maximum absorbance within range of pH 4.0-5.5 (Table 02, Fig. 03, Curve B). However, a decrease is observed on going to either side of the range.

Effect of the equilibration time

An increase in the time contact between two phases up to 30 sec enhances the extraction as observed by corresponding increase in the absorbance of the complex. It remains constant up to 60 sec of equilibration time (Table 02, Fig. 04). Therefore, equilibration time of 30 sec was chosen.

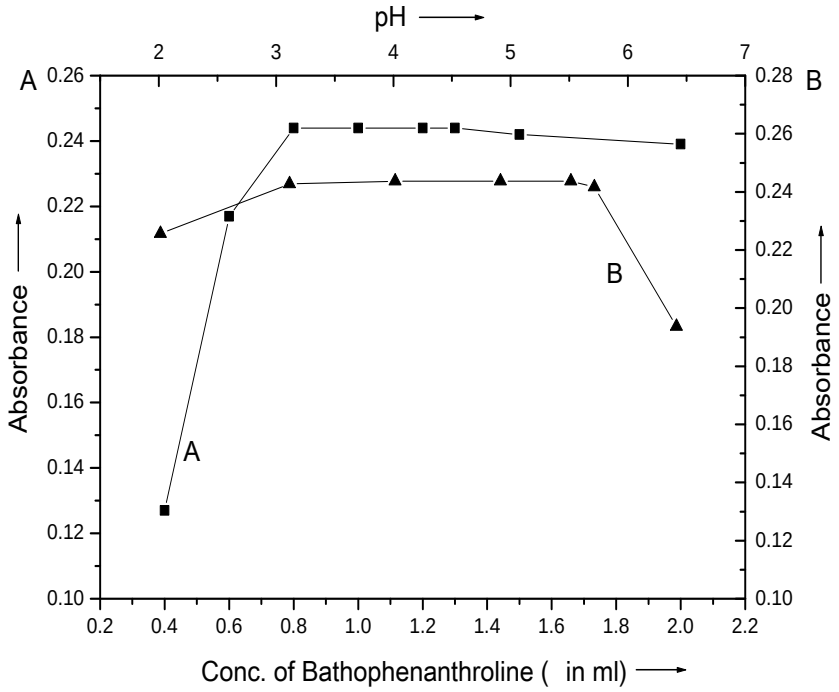

Fig. 03 A Effect of Bathophenanthroline concentration

B Effect of pH

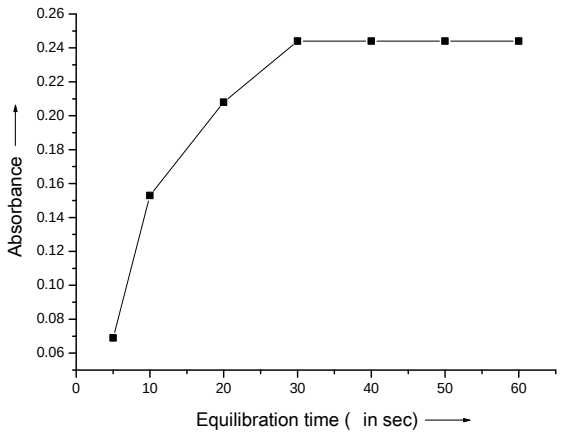

Fig.04 Effect of equilibration time

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

Calibration curve

A linear relationship between absorbance and concentration of the analyte was found to hold good within the range 0.0-1.2 µg ml^{-1} under the optimum conditions of the procedure (Table 03, Fig. 05). The calculated molar absorptivity and sandell's sensitivity are found to be 4.296×10^8 l mol^{-1} cm^{-1} and 0.41 µg cm^{-2} respectively.

Table 03

Absorbance Values at Different Concentration of Ascorbic Acid

Amount of ascorbic acid (µg/ 10ml)	Absorbance
2	0.042
4	0.097
6	0.146
8	0.192
10	0.245
12	0.294
14	0.316
16	0.320

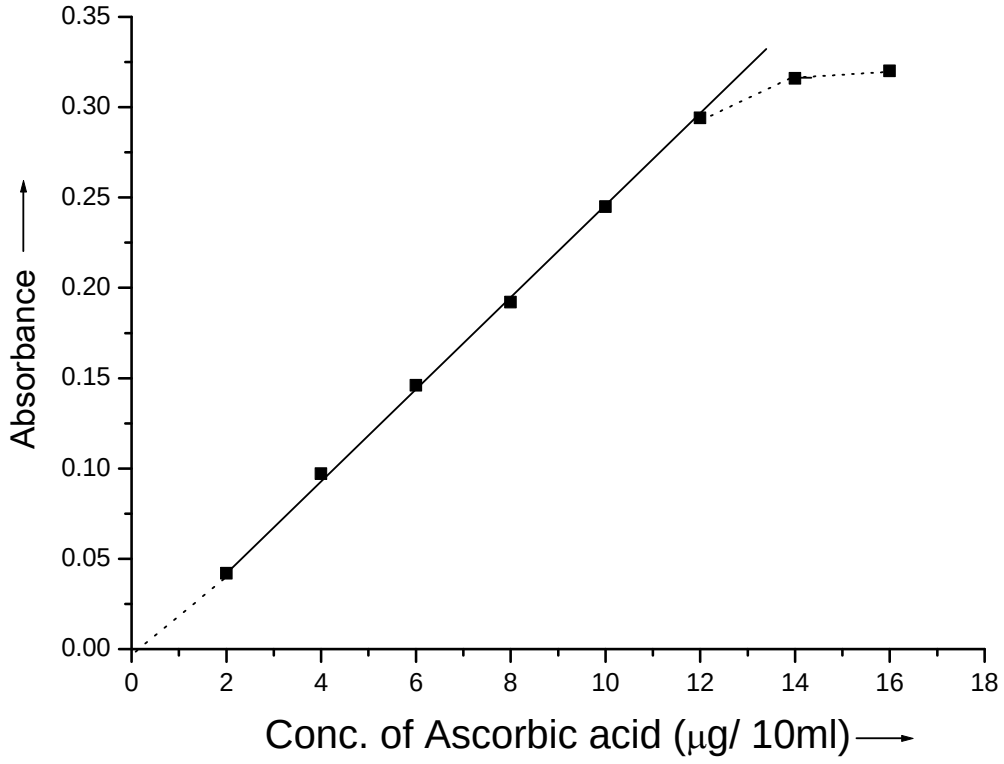

Fig. 05 Beer's law curve for ascorbic acid

International Journal of Engineering Technology Science and Research
IJETSR
www.ijetsr.com
ISSN 2394 – 3386
Volume 4, Issue 5
May 2017

Interference studies

The studies pertaining to the influence of possible constituents of vitamin C formulations were carried out and the resulting data are summarized in Table 04. In the determination of 10 μg ml^{-1} of ascorbic acid, different substances which are tolerated to different degrees include (mg in parenthesis) sugars, amino acids, vitamins, organic acids, inorganic cations and anions. Sugars are tolerated at the levels indicated but the tolerance limit for the studied vitamins and amino acids is not high especially with cysteine and riboflavin. Among the tested organic acids citric acid was found to interfere. For the tested cations and anions, Cu(II) and Al (III) are tolerated in traces whereas sodium sulphite and nitrate interfere seriously with the determination. Some other substances as mentioned under the heading 'miscellaneous' are also tolerated.

Table 04

Effect of diverse substances

	Substance added#	**Tolerance Limit (mg per 10ml)**
Sugars	Sucrose	200
	Glucose, Fructose, lactose	100
	Starch, mannose	50
	Xylose	20
Vitamins & Amino Acids		
	Asparatic acid	2.0
	Methionine, Thiamine	1.5
	Glutamic acid, Nicotinic acid	1.0
	Nicotinamide, Pyridoxine hydrochloride	0.5
	Cyanocobalamin	0.4
	Folic acid	0.2
	Riboflavin	0.03
	Cysteine	0.02
Organic acids		
	Benzoic acid	10
	Succinic acid	20
	Maleic acid	15
Tartaric acid		1.5
	Salicylic acid	2.0
	Citric acid	0.1
Cations and Anions		
	Ca(II), Mg(II)	20
	Al(III)	0.1
	Cu(II)	0.3
	Cl^-	60
	SO_3^{2-}	0.02
	NO_3^-	0.05
Miscellaneous		
	Formaldehyde	300
	Glycerol	250
	Urea, Thiourea	50

Substances were added prior to the addition of ascorbic acid.

Application

The proposed method for the determination of vitamin C is sufficiently sensitive and selective. The utility of the method was assessed by the analysis of the various pure form, multivitamin products (Table 05)

containing vitamin C as an ingredient. The values found were in close agreement to the prescribed values. The other features of the method include simplicity and rapidity.

Table 05

Analysis of pharmaceutical products

Sr.No.	Preparation	Ascorbic Acid Content per tablet (in mg)	
		Claimed	Found
1	Ascorbic acid (C-Mac) inj.	40	38.8
2	Ascorbic acid (Vetcee) 50		49.0
3	Limcee	500	498.7
4	Innergy-24	50	47.4
5	Tonikem plus	01	0.92

References

1 S. P. Arya and M. Mahajan, Proc. Natl. Acad. Sci., India, 67, (1997), 39.
2 M. Okamura, Clin. Chim. Acta, 115, (1981), 393.
3 Memon, Najma, Bhanger, M. Iqbal, M. Aslam, Pak. J. Anal. Chem.,4, (2003), 152.
4 Kleszczewska, Ewa, J. Trac. Micro. Tech., 21, (2003), 85.
5 X. Lu and Y. Xiao, LithuaJianyanHuaxue Fence, 27, (1991), 289.
6 N. Teshima, T. Nobuta, T. Sakai, Bunseki Kagaku, 57, (2008), 327.
7 Wei-xing Ma, Ou Shao, Hai-hongJia, Ying-hong Liu, Xing-you Xu, ShiyanJishu Yu Guanli, 25, (2008), 41.
8 E. Luque-Perez, A. Rios, M. Valcarcel, Fresenius J. Anal. Chem., 366,(2000), 857.
9 E. S. Elnenaey and R. Soliman, Talanta, 26, (1979), 1164.
10 K. L. Bajaj and G. Kaur, Analyst, 106, (1981), 117.
11 S. Si, ShengwuHuaxue Yu ShengwuWuli, 60, (1984), 80.
12 W. Si, LihuaJianyan, Huaxue Fence, 36, (2000), 212.
13 M. Suzuki, M. Takayanagi and T. Yashiro, Rinsho Kagaku, 14, (1985), 399.
14 A. Molina-Diaz, I. Ortega-Carmona, M. I. Pascual-Reguera, Talanta, 47, (1998), 531.
15 M. A. Eldawy, A. S. Tawfik and S. R. Elshabouri, Anal. Chem., 47, (1975), 461.
16 S. P. Arya, M. Mahajan, P. Jain, Proceeding of National Academy Of Sciences, 69,(1999), 419.
17 M. Xiao- Rong and M. Yang, XibeiShifanDaxueXuebao, ZiranKexueban, 35, (1999), 49.