JCBPS; Section A; May 2017 – July, 2017, Vol. 7, No. 3; 563-571. E- ISSN: 2249 –1929

[DOI: https: //doi.org/10.24214/jcbps.A.7.3.56371.

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT Research Article

Colorimetric determination of vitamin C using Fe(II)-5-Chloro-7-iodo-8-hydroxyquinoline complex

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Received: 19 May 2017**; Revised:** 01 June 2017; **Accepted:** 08 June 2017

Abstract: An extractive spectrophotometric procedure based on the complexation of reduced Iron (II) with 5-Chloro-7-iodo-8-hydroxyquinoline (CIHQ) for the estimation of micro amounts of vitamin C is described. The resulting complex is extracted into chloroform to give a reddish brown extract which shows an absorption band at 485 nm. Linear relationship between absorbance and concentration of ascorbic acid is observed up to 0.8 μg ml^{-1}. Interference studies of different substances including sugars, vitamins and amino acids, metal ions and organic acids were carried out. The utility of the method was tested by analysing some of the marketed products of vitamin C.

Keywords: Ascorbic acid, Absorption, Extraction, Spectrophotometry

INTRODUCTION

Many methods have been reported for the determination of ascorbic acid in pharmaceutical preparations and reviews have appeared on the results. These include indirect spectrophotometric methods based on the reduction of compounds such as DCIP[1-4], Iron(III)[5-10] , the ketone derivatisation method with o-phenylenediamine[11-13].Electrochemical[14-19],fluorimetric[20-23],kinetic[24,25],enzymatic[26,27],and chemiluminescence[28-31] methods have also been proposed. These methods have been used to increase the analytical sensitivity for ascorbic acid and some of them have been automated, but specialised equipment's are required for these procedures.

5-Chloro-7-iodo-8-hydroxyquinoline (CIHQ) complex has been found to form a coloured complex with Iron (II) and Iron (III) in slightly acidic medium. However, the reaction of Fe (III) with the 5-Chloro-7-iodo-8-hydroxyquinoline can be effectively masked by the addition of citrate. The Fe (II)- CIHQ complex is extracted into chloroform to give a reddish brown extract. The method based on the extraction of Iron (II)–CIHQ complex provides the desirable features of simplicity and rapidly besides having better sensitivity and selectivity. The detailed studies pertaining to the proposed method are presented here.

REAGENTS AND SOLUTIONS

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

(b).Reagents: All reagents were of analytical grade and double distilled water was used for preparing solutions.

(c).Iron (III) solution: A stock solution of iron (III) (1mg/ml) was prepared by dissolving accurately weighed amount (0.8632 g) 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 suitable dilution of the stock solution with distilled water.

(d). 5-Chloro-7-iodo-8-hydroxyquinoline (CIHQ) solution (Fig 01): A 0.05% (w/v) solution was obtained by dissolving the reagent in ethanol.

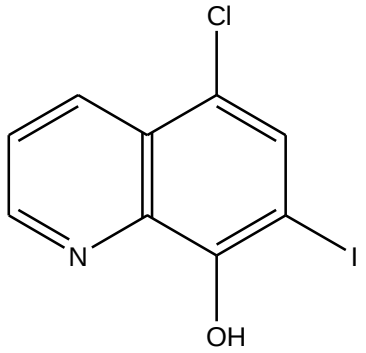

Fig 01: 5-Chloro-7-iodo-8-hydroxyquinoline

(e).Ascorbic acid: A fresh aqueous solution of ascorbic acid (50 µg ml^{-1}) was used.

(f).Potassium Citrate solution: A 5% (w/v) aqueous solution of Potassium Citrate was prepared in distilled water.

Procedure: Into a 100 ml separating funnel, 1ml of 100 µg Iron (III) solution was taken and an aliquot of ascorbic acid were added. After swirling contents, 2 ml of potassium citrate was added followed by addition of 1.5 ml of 0.05% CIHQ solution. The volume was made to 10 ml with distilled water. The brown coloured complex was extracted in to 10 ml chloroform for 45 sec. The extract was then transferred to 10 ml volumetric flask and its volume was made up with chloroform. The absorbance of coloured complex was measured at 485 nm against the reagent blank similarly. The content of ascorbic

acid were computed from the standard calibration curve prepared by taking different amounts of ascorbic acid up to 8.0 µg/ 10ml and using the conditions of the procedure.

Analysis of tablets/ capsules: A known number (5-10) of vitamin C tablets/ capsules were ground to powder form. An accurately weighed amount equivalent to 100 mg of Ascorbic acid was dissolved in water. The solution was filtered and the filtrate was transferred to 100 ml volumetric flask. The volume was made up to mark with water. The working solution (10 µg/ ml) was prepared by dilution. A known volume of the prepared solution was analysed for ascorbic acid contents by the recommended procedure.

RESULT AND DISCUSSION

Spectral Studies: Iron (III) gets reduced easily with Ascorbic acid to iron (II) which forms an extractable coloured complex with CIHQ reagent. The electronic spectrum of Fe (II)-CIHQ in $CHCl_3$ was studied along with that of reagent blank over the range 370-760 nm (Fig 02), which shows two absorption bands in the region of 482-488 nm and 595-600 nm. The complex absorbs strongly at 485 nm where the absorption due to reagent blank is negligibly small. Hence, all absorbance measurements were carried out at 485 nm. While the Fe (II)-CIHQ complex having absorption maxima below 370 nm which was not interfere in the determination.

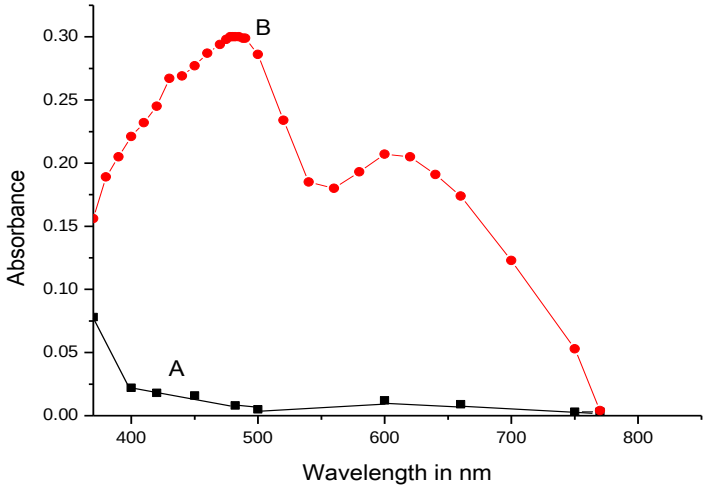

Fig. 02: Absorption spectrum of Iron (III)-CIHQ complex in chloroform.

(Conditions: Fe (III) =100 µg; CIHQ solution = 1.5 ml)

A – Reagent blank against chloroform. B – Complex against reagent blank

Effect of reaction variables: The various parameters which can influence the extraction of the complex and absorbance as well were studied. These include the changes in concentration of the reagent, effect of pH, effect of equilibration time and potassium citrate were studied.

Effect of reagent concentration: The increase in the reagent (CIHQ) concentration through 1.5 ml of CIHQ solution leads to increase in the absorbance which remains constant up to 2.0 ml of CIHQ solution

(Table 1, Fig. 03, Curve A). However, further increase in its concentration causes a gradual decrease in the absorbance. Hence, 1.5 ml of the CIHQ solution was used for further studies.

Effect of potassium citrate solution: Potassium citrate addition is very important as it prevents the extraction of Fe (III)-CIHQ complex and maintains optimum pH (5.5-6.5) for the procedure. **(Table 1, Fig. 04, Curve B).**

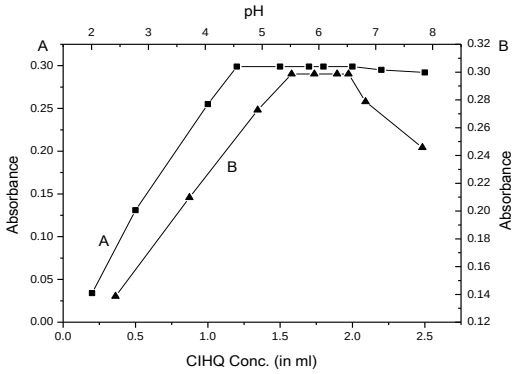

Fig. 03: A Effect of CIHQ concentration

B Effect of pH

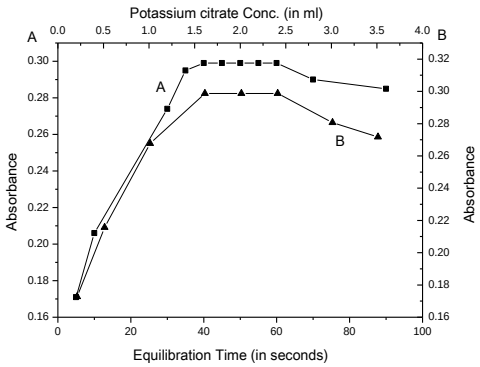

Fig.04: A Effect of equilibration time

B Effect of Potassium citrate concentration

Effect of equilibration time: An increase in the contact time between two phases up to 45 sec enhances the extraction as evidenced by corresponding increase in the absorbance of the complex. It remains constant up to 1.0 min. of equilibration time (Table 1, Fig.04, Curve A). Therefore, equilibration time of 45 sec was chosen.

Table 1: Optimization of reaction variables

CIHQ vol.(in ml)	0.2	0.5	1.0	1.5-2.0	2.2	2.5
Absorbance	0.034	0.131	0.255	0.299	0.295	0.292
Equilibration time (in sec.)	5	10	30	35	40-60	
Absorbance	0.171	0.206	0.274	0.295	0.299	
Potassium citrate (in ml)	0.2	0.5	1.0	1.6-2.4	3.0	3.5
Absorbance	0.173	0.216	0.268	0.299	0.281	0.272
pH	2.4	3.7	4.9	5.5-6.5	6.8	7.8
Absorbance	0.139	0.210	0.273	0.299	0.279	0.246

Effect of pH: The extraction of the Fe (III)-CIHQ complex was studied over a pH range 2.4-7.8. However, the mentioned lower pH value was adjusted by addition of 0.1N HCl solution. It was observed that the complex gives maximum absorbance within range of 5.5-6.5 (Table 1, Fig. 03, Curve B). However, a decrease is observed on going to either side of the range.

Choice of extractant: The extraction behaviour of the complex in different solvents was studied as shown in Table 2. The complex gets extracted into chloroform, dichloromethane, carbon tetrachloride, benzene, to give a brown coloured extract in each case (extraction decreases in that order). While it was little extracted into Ethyl acetate and n-hexane. As the absorbance in chloroform is maximum, hence, it was chosen as an extractant.

Table 2: Extraction Behaviour of the complex in Different solvents

Solvent	Absorbance*
Chloroform	0.300
Dichloromethane	0.283
Carbon tetrachloride	0.242
Benzene	0.156
Ethyl acetate	0.098
n-Hexane	0.010

* Measured against respective blank

Beer's law: A linear relationship between absorbance and the concentration of ascorbic acid was observed upto 8.0 µg/ 10ml (Table 3, Fig. 05). The molar absorptivity and sandell's sensitivity at 485 nm are found to be 8.5 x 10^5 $Lmol^{-1}cm^{-1}$ and 0.2072 x 10^{-3} µg cm^{-2}.

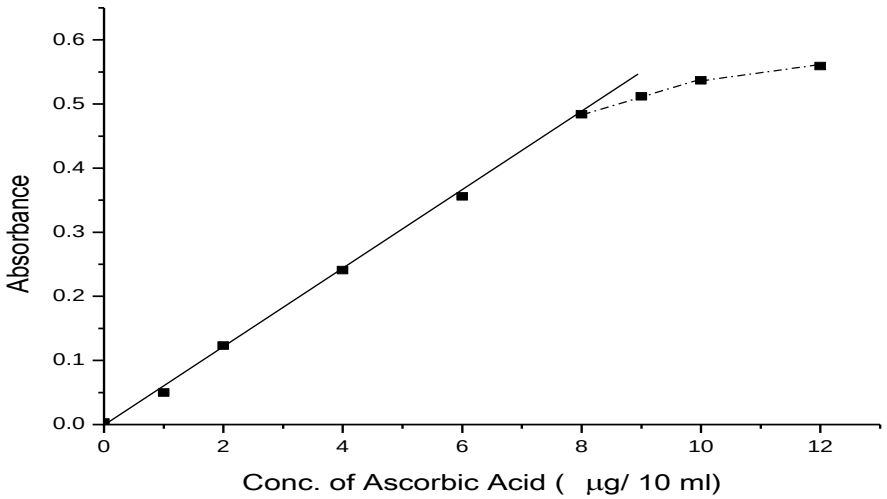

Fig 05: Beer's law curve for ascorbic acid

Table 3: Absorbance Values at Different Concentration of Ascorbic Acid

Amount of ascorbic acid (µg/10ml)	Absorbance
0	0.003
1	0.057
2	0.123
4	0.241
6	0.356
8	0.484
9	0.512
10	0.537
12	0.559

Interference studies: The tolerance limits of diverse substances commonly encountered in vitamin C formulations were investigated. The results are listed in the Table 4. All of the tested vitamins and amino acids, except cysteine, are tolerated, but to varying degrees (Table 4). No interferences was observed from sugars, inorganic cations Mg (II), Ca(II) and anions Cl^-, $SO4^{2-}$. Some of the organic compounds, such as formaldehyde, glycerol, urea, benzoic acid succinic acid, maleic acid, citric acid and tartaric acid, were also found to be tolerated to a good amount in the analysis.

Table 4: Effect of Diverse Substances

Substance added[#]	**Tolerance Limit (mg/10ml)**
Sugars	
Sucrose	150
Fructose	100
Glucose	100
Xylose	25
Lactose	100
Starch	20
Vitamins & Amino Acids	
Aspartic Acid	1.5
Methionine	1.0
Glutamic Acid	1.5
Nicotinic Acid	0.5
Riboflavin	0.1
Nicotinamide	1.0
Folic Acid	0.3
Cyanocobalamin	0.4
Thiamine	1.5
Cysteine	0.05
Pyridoxine hydrochloride	1.0

Organic Acids	
Benzoic Acid	10
Succinic Acid	15
Maleic Acid	10
Citric acid	2.0
Tartaric Acid	1.5
Metal Ions	
Mg(II)	5.0
Ca(II)	3.0
Cl^-	250
$SO4^{2-}$	100
Miscellaneous	
Formaldehyde	600
Glycerol	400
Urea	75

 # Substances were added prior to the addition of ascorbic acid

Applications: The proposed procedure was applied to the determination of the ascorbic acid contents in various pharmaceutical preparations such as Limcee tablets in pure and dosage forms, C-mac, Vetcee injection in pure form; multivitamin products such as Innergy and toniken plus (Table 5). All these products were collected from the market. The amount of vitamin C obtained in each case was almost same as provided with specified value as claimed by the manufacturers.

Table 5: Analysis of pharmaceutical products

Sr. No.	Preparation	Ascorbic Acid Content per tablet/cap./inj. (in mg)	
		Claimed	Found
1	C-mac (inj.)	40	38.5
2	Vetcee (inj.)	50	47.8
3	Limcee	500	496.9
4	Innergy	50	48.3
5	Toniken Plus	1.0	0.90

ACKNOWLEDGEMENT

The authors wish to thank the Chairman, Department of Chemistry, Kurukshetra University, for necessary facilities.

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On line publication Date: 8.6.2017