Spectrophotometric Analysis of Vitamin E Using Cu(I)-Bathocuproine or/and Fe(II)-2,4,6-tris-(2'-pyridyl)-s-triazine Complexes

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Summary: Vitamin E (tocopherols and tocotrienols) antioxidants are determined by reducing Cu(II) to Cu(I) or Fe(III) to Fe(II) in presence of vitamin E and subsequent complexation of Cu(I) with bathocuproine and /or Fe(II) with 2,4,6-tris-(2'-pyridyl) -s-triazine (TPTZ).
Both the reactions are monitored separately, Cu(I)-bathocuproine at 479 nm where as, Fe(II)-(TPTZ) at 595 nm spectrophotometrically. Linear calibration curves are achieved for both complexes between 1 to 5μg ml^{-1} for vitamin E.
The methods were applied for the determination of vitamin E in pharmaceutical preparations and edible oils. Vitamin E, from edible oils, was solvent extracted into n-hexane prior to saponification.
Furthermore, a single lined flow injection manifold was also examined. A large excess of Cu(II) or Fe(III) with different concentrations of vitamin E in buffer pH 4 was run on the line and constant amounts of reagent bathocuproine or TPTZ in each case was injected through the injector. The peak height shows a linear relationship for vitamin E between 0.5 to 2.5 μg ml^{-1} for both complexes.

Introduction

Vitamin E is a generic term which includes the mixture α,β, γ and δ tocopherols and trocotrienols. These are fat soluble and biological active anti-oxidants, where, 6-hydroxychroman ring of vitamin E oxidizes to corresponding quinone in the presence of oxidizing agents [1]. Vitamin E protects low density lipids (LDL) molecules, where, each LDL molecule is surrounded by six tocopherol molecules [2]. The major antioxidative function of tocopherol is the reaction between peroxy radical and tocopherol.

$$LOO\bullet + \alpha - TOH \rightarrow LOOH + \alpha\text{-}TO\bullet$$

$$LOO\bullet + \alpha - TO\bullet \rightarrow (\alpha - TO)\ OOL.$$

This inhibits free radical chain autooxidation of polyunsaturated fatty acid esters (linoleic acid ester) (Loo·). Thus, tocopherol is highly effective antioxi-dant protecting lipids of cell membrane, capable of breaking the chain reaction by scavenging a peroxy radical [3] and prevent the damage to cell membrane and plasma polyunsaturated lipids [4]. Vitamin E is one of the antioxidants which depletes in the blood (lymphocytes and plasma) of seropositive, AIDS and atheroscalerosis patients [4]

Vitamin E has been determined by Voltametric [5], G.C-mass [6], liquid chromatographic with particular reference to HPLC [7-12] and spectrophotometric [1, 13 -16] methods.

Among these methods, spectrophotometric methods are simple and involve less expensive equipment. The determination of sulphide, sulphite, isoniazid, ascorbic acid, vitamin K_3 and cysteine based on the reduction of Cu(II) to Cu(I) or Fe(III) to Fe(II) followed by color development with an appropriate chromogenic reagent have been reported [16–21]. Tsen [12] has examined TPTZ as spectrophotometric reagent for tocopherols. However, for the determination of tocopherols and tocotrienols in real samples of oil did not apply TPTZ.

The present work reports the determination of total vitamin E in pharmaceutical preparations and edible oils. The methods are based on the quantitative reduction of Cu(II) to Cu(I) or Fe(III) to Fe(II) in slightly acidic medium and subsequent complexation with bathocuproine or TPTZ followed by spectrophotometry.

Results and Discussion

Bathocuproine and TPTZ have been reported as spectrophotometric reagents for the determination of Cu (I) and Fe(II) respectively. In the present work the methods applied are based on the indirect determination of total vitamin E by the quantitative reduction of Cu(II) to Cu(I) or Fe(III) to Fe(II) and subsequent formation of yellow complex with

bathocuproine and that of intense blue with TPTZ and these reactions were examined spectrophotometrically. The effects of pH within 1-10, reagent final concentration between $1.3x10^{-4}$ to $1.1x10^{-3}$ M for bathocuproine and $1.6x10^{-4}$ to$1.28x10^{-3}$M/liter for TPTZ, metal ion concentration $3.9x10^{-4}$ to $2.3x10^{-3}$ for copper(II) and $4.47x10^{-4}$ to 2.68 $x10^{-3}$M/liter for iron(III), time of reaction after formation of complex upto 30 minutes and temperature from 10- to 70°C on the color reactions were examined. Acetate buffer pH 4 was considered optimal with maximum color development (Fig. 1). The final concentration of bathocuproine or TPTZ used was $5.5x10^{-4}$ or $6.6x10^{-4}$ respectively, copper (II) and iron (III) concentration were optimized $7.8x10^{-4}$ M/liter and $8.9x10^{-4}$M/liter respectively. The maximum color developed was within five minutes at 30°C (Fig. 2). The measurements for different concentrations were made when stable color was obtained (Fig. 3). The molar absorptivities calculated for vitamin E with Cu-bathocuproine and with Fe(II)-TPTZ were 3.3 x 10^4 L mol^{-1} cm^{-1} and 2.8 x 10^4 L mol^{-1} cm^{-1} respectively (Table-1). Linear relationship was obtained between 1 to 5 μg ml^{-1} both for Cu (I)-bathocuproine and Fe(II)-TPTZ, with the coefficient of correlation of 0.9941 and 0.9988 and slope sensitivities of 0.073x and 0.0667x respectively (Fig. 4). The detection limit measuresed as the concentration limit of indicating the absorbance of 0.01 and observed as 0.2μg/ml of vitamin E.

Six test samples of vitamin E prepared were analyzed and relative percent error obtained was within ± 0.5 to 4%. The reproducibility of analysis of 5 μg ml^{-1} of vitamin E (n=6) was measured and relative standard deviation (RSD) was calculated to be 2 %. The methods were applied for the determination of vitamin E in capsules and the amount found was 397 mg per capsule where as, the amount reported was 400mg per capsule. Similarly vitamin E was determined in different brands of edible oils available in the market. The results of analysis are summarized in (Table-2) with RSD 1.9 %.

Additionally, the use of flow injection for the analysis of vitamin E was also examined. A single

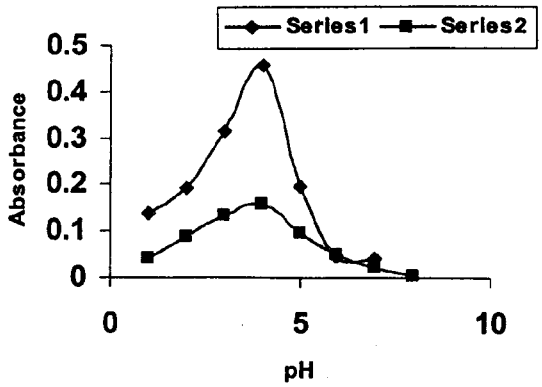

Fig.1: Effect of pH on the absorbance of (♦) Cu (I)-bathocuprine and (■) Fe (II)-TPTZ after reduction with vitamin E.

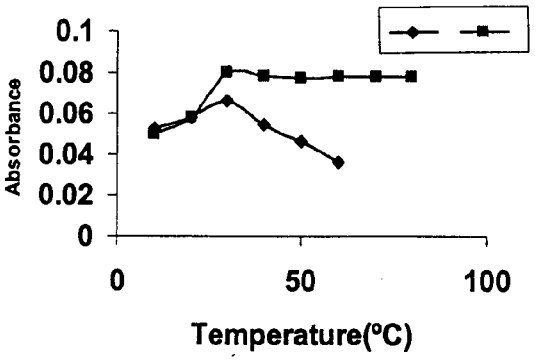

Fig. 2: Effect of temperature on the absorbance of (♦) Cu (I)-bathocuprine and (■) Fe (II)-TPTZ after reduction with vitamin E.

lined manifold was only considered. The option of injecting vitamin E or reagent solutions was considered. Being inexpensive, vitamin E was run on the peristaltic pump together with Fe(III) and buffer pH 4, the reagent was injected through the injector. An average peak height of at least three determinations was measured and linear relationship was obtained between 0.5 to 2.5 μg ml^{-1} for vitamin E.

Experimental

Reagent solutions of bathocuproine or 2, 4, 6-tris-(2'-pyridyl)-s-triazine (TPTZ) (E.Merck), and

Table-1:Spectrophotometric data of the determination of Vitamin E using Cu(I)-Bathocuproine and Fe(II)-TPTZ complex.

S. No.	Reducing agent	Metal	λ-max (nm)	(L.$mole^{-1}$ cm^{-1})	Calibration range μg/ ml	RSD% (n=3)	R Error% (n=6) (for test samples)
1	Vitamin E	Cu(I)	479	$3.3X10^4$	1-5	±2.2	0.54
2	Vitamin E	Fe(II)	595	$2.8X10^4$	1-5	±2.1	3.92

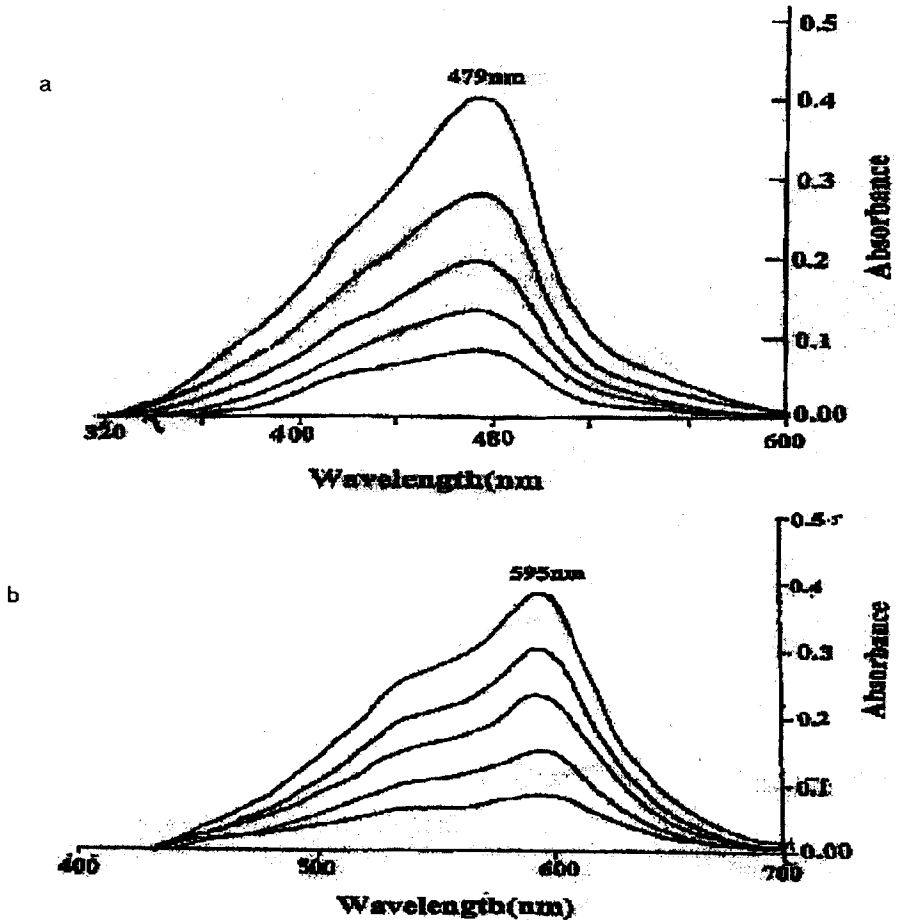

Fig. 3 Effect of Concentration from 1 to5 μg /ml on the absorbance of (a) Cu (I) and (b) Fe (II) after reduction with vitamin E.

Table 2: Analysis of Vitamin E in Edible oils and Ghees

Manufacturer	Total amount of Vitamin E (Naturally occurring and added)
Habib Oil	147.5
Habib Ghee	111.7
Evion oil	298.00
Tullo light	81.00
Butter (domestic)	4.00
Rafan corn oil	223.5
Blue band margarine	81.00

Pharmaceutical preparations

Sample	Amount found In (mg/Capsule)	Amount reported in (mg/Capsule)
Avion Capsules	397	400

vitamin E (E.Merck) used were prepared in ethanol. Cu (II) and Fe (III) solutions containing 1 mg ml^{-1} in each case were prepared from their chloride salts (E.Merck) in double distilled water.

A) Spectrophotometric Determination

Cu(II) or Fe(III) solution (0.5 ml,1 mg ml^{-1}) was transferred into a 10 ml volumetric flask containing bathocuproine or TPTZ (2 ml 0.1 % w/v) solution, and acetate buffer pH 4(1ml) followed by an appropriate volume of vitamin E ,to adjust the final concentration of vitamin E between 1 to 5 μg ml^{-1}, the volume was made up to the mark using ethanol. Reagent blank was also prepared following the above procedure without the addition of vitamin E. The absorbance for each of the standard was recorded against the reagent blank on Hitachi model 220 spectrophotometer and the wavelength was fixed at

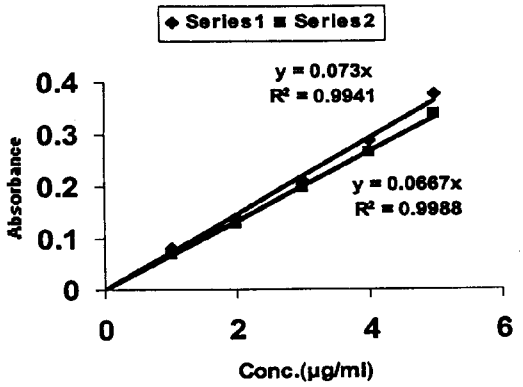

Fig. 4 Calibration curve of (♦) Cu (I) – bathocuprine and (■) Fe (II)-TPTZ after reduction with vitamin E.

479 nm for Cu(I)-bathocuproine and that of for Fe(II)-TPTZ at 595 nm.

B) Analysis of Vitamin E in Pharmaceutical Preparations

Six capsules containing 400 mg vitamin E (Avion) (E.Merck) were mixed thoroughly and 0.1g of this was dissolved in 100 ml of ethanol. 1 ml of this solution was further diluted to 10 ml using ethanol and (0.1-0.5ml) was used for analysis following the above procedure. The amount of vitamin E was calculated from the calibration plot.

C) Analysis of Vitamin E in Branded Edible Oils and Ghee Samples

Five grams of oil or ghee was dissolved in 10 ml of ethanol-water (1:1) and vitamin E was extracted by continuously shaking for ten minutes into 25ml of n-hexane. The extraction was repeated twice, the fractions were mixed together and n-hexane was evaporated on rotary evaporator. The residue (vitamin E) was transferred into ethanol (10ml) for saponification with KOH (2N 10ml) at 70° C on water bath, for 30 minutes. After saponification vitamin E was back extracted into n-hexane (10ml, three times), the fractions were mixed together and washed with water to remove an excess of KOH, and finally n-hexane was removed on rotary evaporator. The residue was dissolved in ethanol for hydrolysis with 0.25ml H_2SO_4 (conc) and a few drops of water at 90°C for 90 min. The final volume was adjusted to 50 ml using ethanol. From this solution 2ml was used for spectrophotometric measurements according to the above mentioned procedure (A).

D) Flow Injection Analysis Procedure

A single lined manifold with Gillson's M312 miniplus peristaltic pump coupled with Spectronic-21 spectrophotometer, Rehodyne 7125 injector and a Hitachi model 561 recorder were used. The length of reaction coil was three meters. Vitamin E (5 to 25µg) solutions were transferred into a 10 ml volumetric flask, containing Cu(II)/Fe(III) (0.5 ml, 1mg ml^{-1}) solution and acetate buffer pH 4 (1ml) were added and the final volume was adjusted to 10ml to get 0.5-2.5µg ml^{-1} using ethanol. These contents were run on FIA system and 20µl of bathocuproine or TPTZ (0.1% in ethanol w/v) was injected through the injector. The spectrophotometer was fixed at 479 nm for Cu(II) - bathocuproine and at 595 nm for Fe(II) - TPTZ complex.

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