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Quantitation of Vitamin E in Pharmaceutical Formulations and Oil Samples Using Spectrophotometric Method

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A novel, rapid and facile procedure is described for the determination of vitamin E. It involves the reduction of Fe(III) to Fe(II) with vitamin E that results the formation of pink colored complex of reduced Fe(II) with dimethyl glyoxime in presence of pyridine in pH range between 6.5-7.2. The absorbance of the complex was measured at 512 nm. Beer's law was obeyed in the range 2.0 to 5.2 μg/mL of vitamin E with molar absorptivity of 1.23×10^4 L mol^{-1} cm^{-1} and the Sandell's sensitivity was 0.0351 μg/cm^2. The coefficient of determination was $r^2 = 0.997$ with the relative standard deviation 1.1 %. The various factors which affect the resulting complex were optimized to obtain maximum absorption. The proposed method is satisfactorily utilized for pure as well as various dosage forms of vitamin E in pharmaceutical formulations which contains common reductants by pre-treatment of the samples.

Keywords: Spectrophotometer, Vitamin E, Ferric chloride, Dimethyl glyoxime.

INTRODUCTION

Tocopherol is the term used for fat soluble vitamins *i.e.* vitamin E, which includes the mixture of α, β, γ and δ-tocopherol and tocotrienols [1]. Out of all tocopherols, α-tocopherol is the most biologically active member of vitamin E family. The prime biological function of vitamin E is to protect the polyunsaturated fatty acids of cell membranes from free-radical damage therefore it acts as an antioxidant and supplementation of human diet with vitamin E is significant to prevent chronic diseases [2,3]. Vitamin E can also be used as active antiageing agent [4,5] in cosmetics as it has an protective effects on free radical reactions and also food industries used vitamin E concentrates as natural source antioxidant. As tocopherols and tocotrienols are readily oxidized when subjected to heat, light and alkaline conditions therefore commercial products are prepared like complexes *e.g.* with cyclodextrin [6,7]. Due to these vital functions of vitamin E there is a need of quantification of vitamin E in various pharmaceutical samples and oil products. A variety of methods has been developed for the analysis and quantification of α-tocopherol in food and supplement matrices. But there is also a continued interest in the development of sensitive and reproducible methods for its determination in various natural and synthetic products. Subsequent improvements to vitamin E assay include different techniques such as HPLC [8-13], voltametry [14], UPLC [15], gas chromatography [16,17], spectroflourimetry [18], liquid chromatography [19], RP-HPLC [20,21] and spectrophotometry [22-29]. In literature only a few methods are reported for spectrophotometric quantitation of vitamin E.

In the present work, vitamin E is determined by the use of oxidizability of 6-hydroxychroman ring of α-tocopherol to the corresponding quinine *i.e.* α-tocopherylquinone by the oxidizing agent finally giving coloured products.The method is based on the quantitative reduction of Fe(III) to Fe(II) and subsequent complexation with reagent (Fig. 1) in between the pH range 6.5-7.2 and the absorbance of the complex is measured at 512 nm by spectrophotometry. The purpose of this study was to quantify α-tocopherol in different pharmaceutical formulations and edible oils by complexation of reduced Fe(II) with dimethylglyoxime in the presence of pyridine and the absorbance of the resulting complex was measured at 512 nm spectrophotometrically.

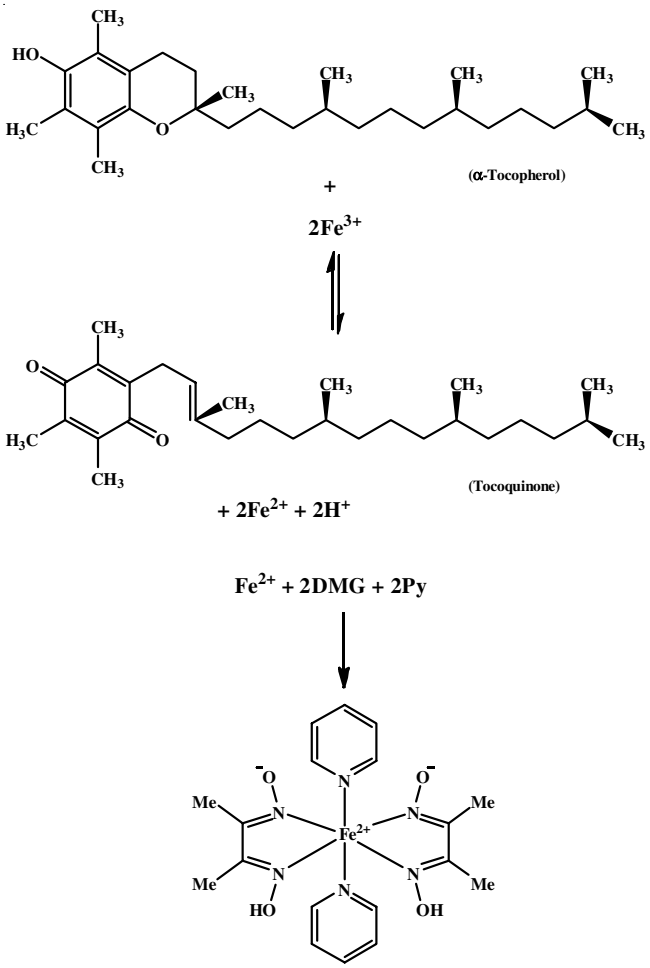

Fig. 1. Complex of Fe(II)-DMG in presence of pyridine

EXPERIMENTAL

α-Tocopherol (Sigma-Aldrich) solution (1 mg/mL) was prepared in absolute ethanol. Absolute ethanol and light petroleum were prepared from 95 % alcohol and ether used was of analytical grade. Reagent solution of dimethyl glyoxime (DMG) (E. Merck) also prepared in absolute ethanol. Fe(III) solution of 1000 ppm were prepared from their chloride salts (E. Merck) in 0.01 N HCl. All the remaining chemicals and reagents were of analytical grade.

A Systronics UV-visible spectrophotometer 117 equipped with quartz cell of 1 cm light path was used to record the molecular absorption spectra and absorbance at selected wavelengths. A Hanna-H-198100 pH meter was used to measure the pH of solutions.

Iron(III) solution (1 mg/mL) was prepared by dissolving 0.1 g of iron using ferric chloride in 100 mL of double distilled water containing 1 mL of 0.01 N HCl. Dimethyl glyoxime solution (1 % w/v) was prepared by dissolving 1 g of DMG in 100 mL of absolute ethanol by heating it for 5 min at 40 to 50 ºC. Pyridine solution (2 % v/v) was prepared by diluting 2 mL of pyridine with 100 mL of double distilled water.

Determination of α-tocopherol using Fe(II)-dimethyl glyoxime method: Iron(III) solution (100 μg) was taken in a 10 mL volumetric flask. An aliquot of vitamin E solution was added followed by appropriate addition of 2 mL of 1 % dimethyl glyoxime and 2 mL of 2 % pyridine. The concentration of α-tocopherol was adjusted between 2.0 to 5.2 μg/mL and the volume was made upto the mark using absolute ethanol. Reagent blank was also prepared following the above procedure without the addition of vitamin E. The absorbance for each standard was recorded against the reagent blank on systronics model 117 UV-visible spectrophotometer and the wavelength was fixed at 512 nm for all observations made in the recommended procedure.

Analysis of vitamin E in pharmaceutical formulations

Sample preparation: Five capsules containing 400 mg of vitamin E were mixed thoroughly and 0.1 g of this oily mass was transferred into 100 mL round bottom flask. In round bottom flask, 2 mL of KOH solution (50 %,w/v), 10 mL glycerol and 25 mL absolute ethanol was added and reflux it for 50 min at 70 ºC. Cool it at room temperature and then transferred the solution into separating funnel and extract with 30 mL of ether for 10 min. Discard the aqueous layer and evaporate the etheral layer to dryness and then dissolved the dried mass in 100 mL of absolute ethanol.

Procedure: The amount of vitamin E was determined by following the same procedure that was used for vitamin E determination in pure form as described above.

Analysis of vitamin E in edible oils

Sample preparation: Edible oil (5 mL) was dissolved in 15 mL of ethanol-water (1:1) and extraction of vitamin E was done by continous shaking for 15 min in 30 mL of *n*-hexane. The extraction was repeated twice and then the fractions were mixed together and *n*-hexane was evaporated using rotatory evaporator. The saponification of the residue obtained was done by dissolving it in 25 mL of absolute ethanol followed by the addition of 2 mL KOH (50 %, w/v) and 10 mL glycerol by refluxing it at 70 ºC. Allow the solution to cool at room temperature and then extracted back in *n*-hexane. Evaporate *n*-hexane and then dissolved the dried mass in 100 mL of absolute ethanol for the analysis of vitamin E by the recommended procedure.

RESULTS AND DISCUSSION

In the present work, the method applied is based upon the indirect determination of total vitamin E by quantitative reduction of Fe(III) to Fe(II) and subsequent formation of pink coloured complex with dimethyl glyoxime (DMG) in the presence of pyridine. The absorption spectra of Fe(II)-DMG-pyridine complex was recorded against reagent blank prepared under similar conditions. The colour of Fe(II)-DMG-pyridine complex (*i.e.* absorbance at 512 nm against the reagent blank) formed as a result of Vitamin E oxidation and the complex was stable upto 90 min. It showed a maximum absorption at 512 nm and that was used for the determination of vitamin E in various samples. The UV/visible spectra of α-tocopherol in ethanol is shown in Fig. 2.

Optimization of reaction variables: The reaction variables which affect the formation of colored complex were studied.

Effect of dimethyl glyoxime concentration: As the amount of dimethyl glyoxime (DMG) increases there was corresponding increase in absorbance from 0.5 mL to 2.0 mL (Table-1) and thereafter the absorbance remained unaffected upto 2.5 mL. Hence, 2 mL of 1 % DMG was found optimum concentration.

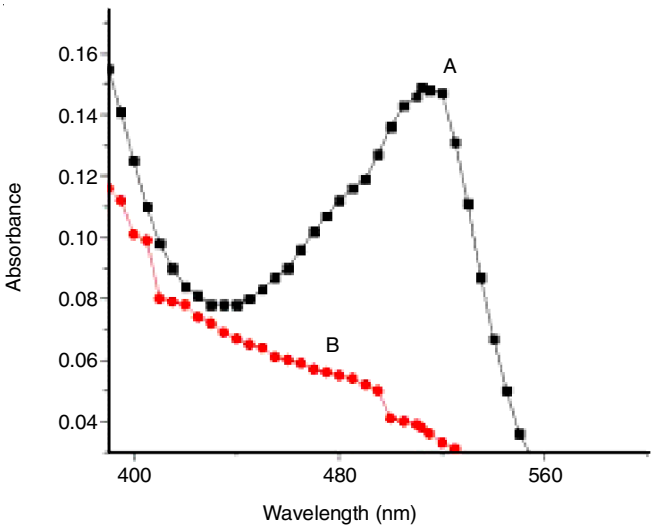

Fig. 2. Absorption spectrum of Fe(II)-DMG-pyridine complex (Conditions: Fe(III) = 100 µg, vitamin E = 50 µg, DMG solution = 2.0 mL, pyridine = 2.0 mL), A = Measured against blank, B = Blank measured against water

TABLE-1
OPTIMIZATION OF REACTION VARIABLES

DMG (1 %w/v, in mL)	Absorbance	Pyridine (2 %, v/v, in mL)	Absorbance
0	0	0	0
0.5	0.019	0.5	0.041
1.0	0.043	1.0	0.066
1.5	0.088	1.5	0.083
2.0	0.149	2.0	0.149
2.5	0.132	2.2	0.148
3.0	0.125	2.5	0.147

Effect of pyridine: Amount of pyridine greatly influence the complexation of Fe(II) with dimethyl glyoxime. Therefore, it was necessary to optimize the concentration of pyridine which gives the maximum absorption of the complex formed (Table-1). It was found that with an increase in the amount of pyridine, the absorption increases up to 2 mL (2 %, v/v), after that absorption starts decreasing on further addition. Hence, 2 mL of pyridine was used for the analysis of vitamin E in various samples.

A calibration curve was constructed under the optimized conditions. It has been observed that there is a linear relationship between the concentration of vitamin E and absorbance over the concentration range 2.0 to 5.2 µg/mL (Table-2) as shown in Fig. 3. The correlation coefficient was showing good linearity of calibration curve.

TABLE-2
EFFECT OF VITAMIN E CONCENTRATION ON ABSORBANCE OF COMPLEX

Vitamin E (µg/mL)	Absorbance	Vitamin E (µg/mL)	Absorbance
2.0	0.022	4.4	0.116
2.4	0.038	4.8	0.135
2.8	0.052	5.2	0.149
3.2	0.066	5.6	0.147
3.6	0.082	6.0	0.147
4.0	0.104	–	–

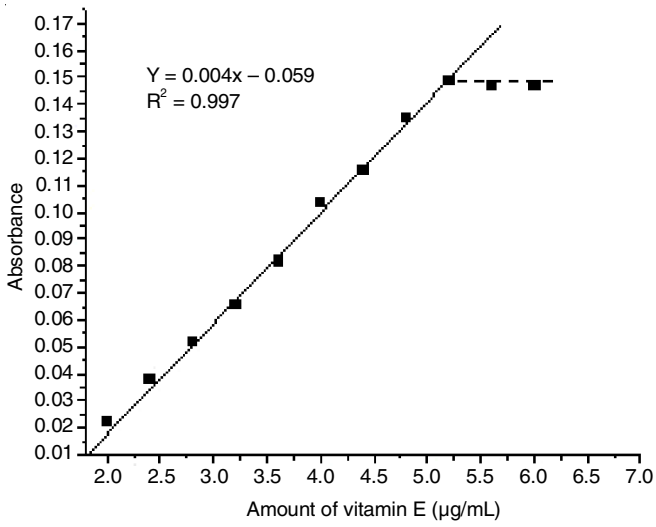

Fig. 3. Calibration curve of Fe-DMG-pyridine after reduction with vitamin E

Statistical data of calibration curve of Fe(II)-DMG-Py with different concentrations: Different parameters such as λ_{max}, Beer′s limit, molar absorptivity, standard deviation, relative standard deviation, Sandell′s sensitivity, correlation coefficient, regression equation and stability of coloured complex are summarized in Table-3.

TABLE-3
STATISTICAL DATA OF CALLIBRATION CURVE

Parameters	Vitamin E
λ_{max} (nm)	512
Beer's limit (µg mL^{-1})	2.0-5.2
Molar absorptivity (L $mol^{-1}cm^{-1}$)	1.23×10^4
Regression equation	Y = 0.004x – 0.059
Slope	0.004
Intercept	0.059
Correlation coefficient	0.997
Sandell's sensitivity (µg cm^{-2} per 0.001 Abs. unit)	0.0351
Stability of colored complex (min)	90
Standard deviation	0.041473
Relative standard deviation (%)	0.011520

Applications: The method is successfully applied in the pharmaceutical formulations (Table-4) and in edible oils

TABLE-4
APPLICATION TO PHARMACEUTICAL FORMULATIONS

Sample	Amount taken	Added[a]	Found[a]
E-Rich	20	0	19.8 ± 0.4
		2.0	22.2 ± 0.3
		2.5	23.0 ± 0.1
		2.8	23.5 ± 0.2
Native Forte	20	0	20.2 ± 0.1
		2.0	21.8 ± 0.6
		2.5	22.6 ± 0.3
		2.8	22.5 ± 0.4
Evion	20	0	20.2 ± 0.3
		2.0	21.6 ± 0.2
		2.5	21.8 ± 0.1
		2.8	21.6 ± 0.7
Enew	20	0	19.8 ± 0.3
		2.0	21.8 ± 0.3
		2.5	22.3 ± 0.2
		2.8	22.4 ± 0.6

a =Average of four observations.

TABLE 6
COMPARISON OF THE PROPOSED METHOD WITH THE EXISTING METHOD

S. No.	Metal-ligand system	λ_{max} (nm)	Time for complexation	Conditions required	Ref.
1	Fe(II)-2,2'-bipyridine	407	4 min	Neutral medium	[27]
2	Cu(I)-neocuproine	450	30 min	Buffer required	[28]
3	Fe(II)-2,4,6-tripyridyl-*s*-triazine	595	60 s	Acidic medium required (pH 4)	[29]
4	Fe(II)-bathophenanthroline	534	3 min	Buffer required	[28]
5	Cu(I)-bathocuproine	479	5 min	Acidic medium required (pH 4)	[29]
6	Fe(II)-DMG/pyridine	512	10 s	Neutral medium (pH 6.5-7.2)	**Proposed method**

(Table-5) within the acceptable range. Moreover, this method is found to be very fast in the determination of vitamin E when compared to the other reported methods (Table-6).

TABLE-5
ANALYSIS OF OIL SAMPLES

Sample	Vitamin E (µg/mL)
Mustard oil	0.02
Olive oil	0.05

Conclusion

A novel, simple, selective and highly accurate UV-visible spectrophotometric method for determination of vitamin E in pharmaceutical formulations and edible oils was developed and validated.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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