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SENSITIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF VITAMINS (C AND E)

Ahmed Mahdi Saeed [*1], Mohammed Jassim Hamzah [2] and Noor Jassim Mohammed Ali [1]

Department of Chemistry [1], College of Education for Pure Science, Diyala University, Iraq.
Department Pharmaceutical Chemistry [2], Pharmacy College, Al-Nahrain University, Iraq.

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Correspondence to Author:
Dr. Ahmed Mahdi Saeed

Assistant Professor,
Department of Chemistry,
College of Education for Pure Science, Diyala University, Iraq.

E-mail: dr.ahmedalanbakey@yahoo.com

ABSTRACT: A sensitive, simple, accurate and fast method for vitamin C and E determination in pure and drug formulations using spectrophotometric was developed. The developed method is based on the formation of the charge transfer complex *via* the reaction between vitamins and Fe^{+3} [$FeNH_4(SO_4)_2.12H_2O$] in the presence of $K_3Fe(CN)_6$ which lead the formation of a blue-greenish colored product that has a maximum absorption at λ_{max}=743 nm. The optimum reaction conditions such as temperature, volume, reaction time and pH were studied. The linear dynamic range for the intensity versus vitamins concentrations are 0.05-28 and 0.5-28 µg/mL for vitamin C and E respectively, with LOD values of 0.01 and 0.09 µg/mL and LOQ values of 0.033 and 0.297 µg/mL. The correlation coefficient (R^2) is 0.9993, while the percentage linearity (%R^2) was 99.93%. %R.S.D for the repeatability (n=3) is < 0.3%. The method was applied successfully for the determination of vitamin C and E in pharmaceutical preparation. The new method can be accepted as an alternative analytical method for the determination of the mention vitamins in pure and dosage forms.

INTRODUCTION: Vitamins C or ascorbic acid is an essential water-soluble vitamin, which can't be synthesized endogenously in Human body. For this reason, people must get vitamin C from food and some other available supplements [1]. Vitamin C plays important role in the biosynthesis of L-carnitine, some neurotransmitter, protein and collagen fibers. The chemical formula for vitamin C is $C_6H_8O_6$ and has a molecular weight of 176.12. It is composed from six carbon atoms and one alcoholic molecules see **Fig. 1** [2, 3].

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Vitamin E is a fat-soluble vitamin that found in eight chemical different forms α-, β-, γ-, and α-tocopherol and α-, β-, γ-, and δ-tocotrienol, which have different biological activity. However, Alpha- (or α-) tocopherol **Fig. 2** is the only form that defined to meet people requirements [4, 5]. α-Tocopherol plays an important role in the breaking and cleaning free radicles from cell membrane and plasma lipoprotein. In addition α-Tocopherol enhances cell mediated immune functions. Therefore, vitamin E deficiency may lead to immune suppression, neurological disorders such as ataxia, brain malformation and peripheral neuropathy [6].

Few methods were adopted for the determination of both vitamins C and E, these were involved spectrophotometric methods [7-15], HPLC [16-18], Flow injection analysis [19], Ion selective electrodes [20] and

Titrimetric methods [21]. In this work, a rapid and sensitive method using spectrophotometric detection was proposed for measuring of vitamin C and E. Our adapted method is based on the charge transfer reaction of each vitamin with Fe^{+3} to form Fe^{+2} and subsequent reaction with potassium hexacyno-ferrate to form a colored complex that absorb at 743 nm. The suggested method has been successfully applied to the determination of vitamin C and E in pharmaceutical preparations. The method is safe, simple, sensitive, selective and accurate.

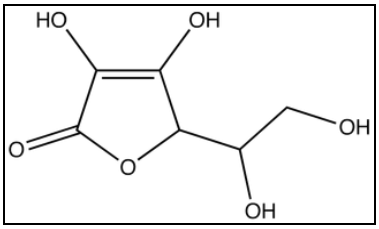


FIG. 1: CHEMICAL STRUCTURE OF VITAMIN C

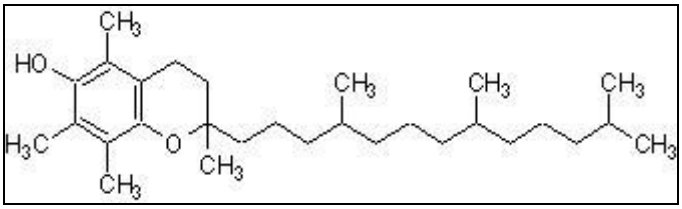


FIG. 2: CHEMICAL STRUCTURE OF VITAMIN E (α-TOCOPHEROL)

Experimental:

Instrument: A UV-VIS spectrophotometer (Jasco V-650 Japan) and 1 cm matched cells was used for electronic spectral measurements. Sartorius balance (Germany), Sonic bath (Korea), Shaking water bath (Taiwan) and Furnace (Germany) were also used throughout this research work.

Methods to Prepare Solutions in this Project: We used deionized water to prepare all the solutions except vitamin E was prepared in acetone. Standard solutions of vitamins (100 µg/mL) were prepared by dissolving 0.01 g of each vitamin in 100 mL standard flask. The working solutions of each vitamin were prepared using further dilution. A 100 µg/mL solutions of $K_3Fe(CN)_6$ and $[FeNH_4(SO_4)_2. 12H_2O]$ were prepared in water, 0.1M of HCl and 0.1M NaOH were also prepared and used for adjustment of pH.

Procedure: We used 10 mL calibrated flask to prepare a serial dilution starting from concentration 100 µg/mL of each vitamins solutions to cover the range of the calibration curve (0.05 – 28 µg/mL vitamin C) and (0.5 - 28 µg/ml vitamin E) in a final volume of 10 mL. For vitamin C, add 3 mL (100 µg/mL) of $K_3Fe(CN)_6$ and 2.5 mL (100 µg/mL) of $[FeNH_4(SO_4)_2. 12H_2O]$ then adjusting pH (pH=4) with HCl and finish the volume to 10 mL with distilled water.

Then shake the solution well and left the reaction at room temperature for 10 min. We used the absorbance at 743 nm against the reagent blank, which prepared in the same steps without adding vitamin C or vitamin E. For vitamin E added 4 mL (100 µg/mL) of $K_3Fe(CN)_6$ and 2 mL (100 µg/mL) of $[FeNH_4(SO_4)_2. 12H_2O]$, adjusting the solutions to pH = 4 and dilute the solutions to the mark with methanol. After 10 min measure the absorbance at 743 nm against reagent blank.

RESULTS AND DESICUSSION:

Absorption Spectra: The data we got from this work reveals that charge transfer reaction between vitamins (C or E) and $K_3Fe(CN)_6$ in the presence of $[FeNH_4(SO_4)_2. 12 H_2O]$ to get highly greenish-blue colored products can be apply as a convenient assay method for both vitamins. In **Fig. 3**, we are presenting the absorption spectra of the vitamins reaction colored products. The data in the figure suggests that a maximum absorbance was obtained at 743 nm and the effect of different reaction variables on the color development was tested to find the most agreeable conditions.

Optimization of the Reaction Experimental Condition: We optimized the effect various reaction concentrations on the color products absorption intensity. To get the optimal reaction, 10 µg/mL concentrations in final volume 10 mL of each vitamin E and vitamin C. Reaction medium effect on the intensity of the charge transfer complex was studied as shown in **Fig. 4**. The obtained results indicating that a maximum absorbance was obtained when using an acidic medium. Therefore, the reaction was carried out in all consequent experiments in acidic medium.

The effect of reactants order addition on the maximum absorbance of the formed product were examined. **Fig. 5** shows that addition of $K_3Fe(CN)_6$ to the vitamins followed using $[FeNH_4(SO4)_2. 12H_2O]$ is enough to obtain the maximum absorbance.

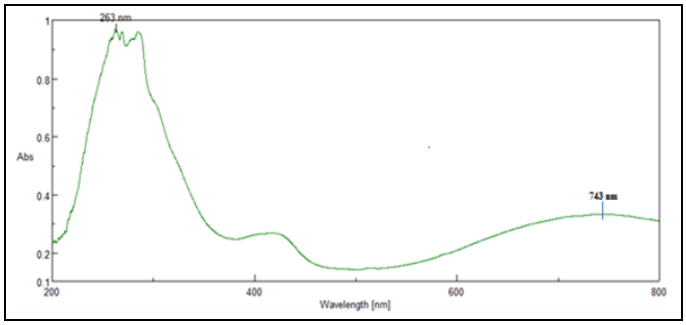


FIG. 3: ABSORPTION SPECTRA OF VITAMIN (C OR E), $K_3Fe(CN)_6$ AND $FeNH_4(SO_4)_2$ MIXTURE

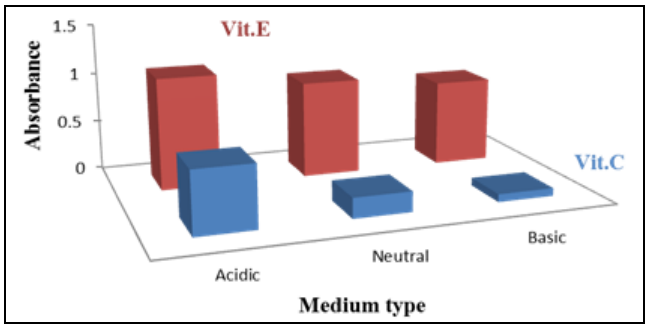


FIG. 4: EFFECT OF MEDIUM TYPE

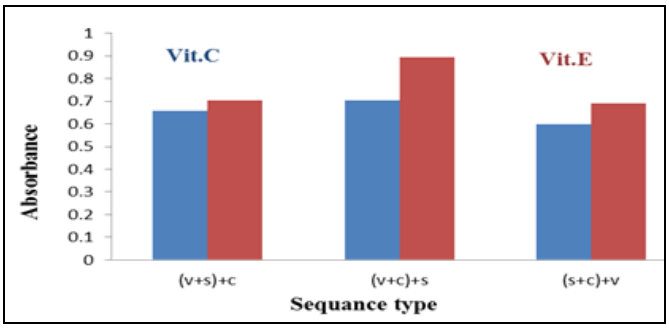


FIG. 5: SEQUENCE TYPE EFFECT

A various studies were carried out to established the optimum volume of 100 µg/mL $K_3Fe(CN)_6$. The obtained results indicating that 3 mL and 4 mL of 100 µg/mL $K_3Fe(CN)_6$ were the optimum volumes for vitamin C and E respectively as shown in **Fig. 6**.

The effect of $[FeNH_4(SO_4)_2.12H_2O]$(100 µg/mL) volume was optimized. The results shows that 2 mL and 2.5 mL are the optimum volumes to get the maximum absorbance as shown in **Fig. 7**.

The effect of pH (1 - 7) was also investigated. It was found that the charge transfer reaction may occur at pH 4. Therefore, this value of pH was used to adjust the reaction solutions.

Fig. 8 a and **b** represents changing at the colored products according to the temperature and time effects. In our modified method, the end of the charge transfer complexes consume 5 - 10 min, while, the optimum temperature was ambient temperature.

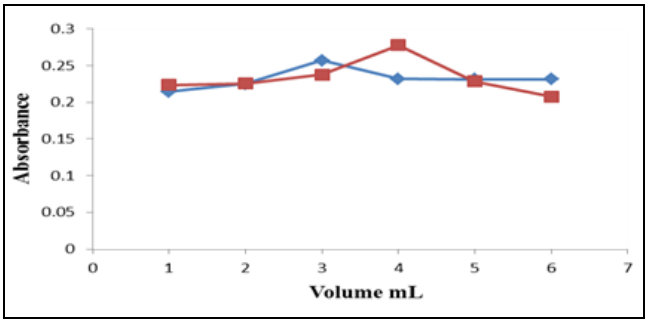


FIG. 6: EFFECT OF $K_3Fe(CN)_6$ VOLUME

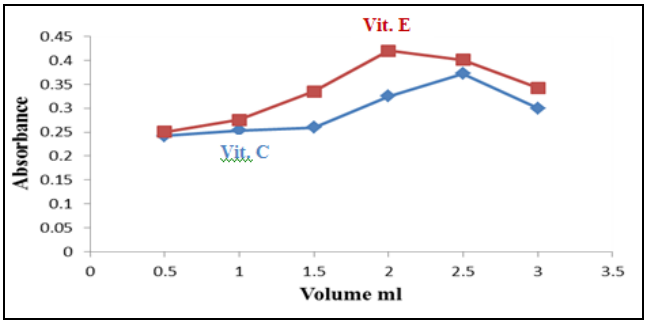
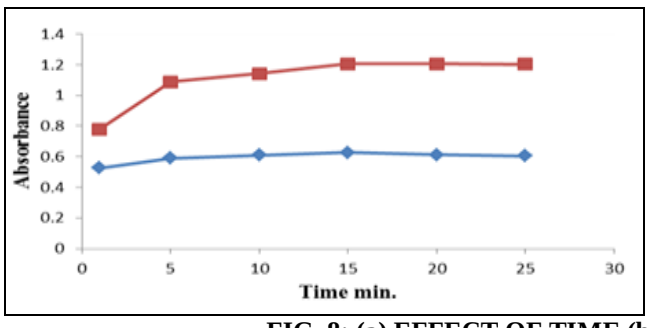


FIG. 7: EFFECT OF $[FeNH_4(SO_4)_2$. VOLUME



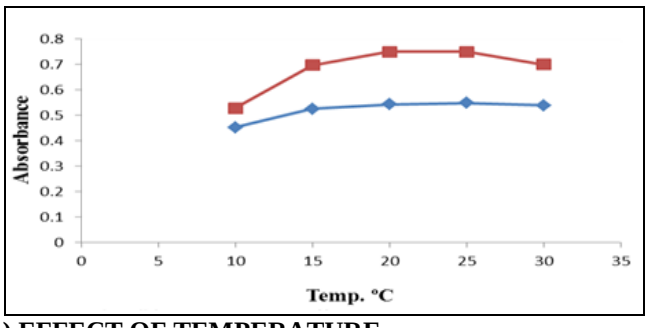


FIG. 8: (a) EFFECT OF TIME (b) EFFECT OF TEMPERATURE

Validity of Beer's Law: We described above the typical experimental conditions such as pH and temperature, which have to be used to design the calibration graphs to determine vitamins concentration. In **Table 1** we are presenting the results that we obtained from the analytical experiments, serial concentration range, relative standard deviations and regression equation for each vitamin. Beer's law was obeyed in the concentration ranges of 0.05-28, 0.5-28 µg/mL of vitamin C and E respectively. Above these limits, negative deviations were observed. The possible reason for the observation of negative deviation is association of the products formed through the

reaction in the solution to give the final colored products. R^2 value of the correlation coefficient is 0.9993 for both vitamins. While, LOD values are 0.01 and 0.09 µg/mL for vitamin C and E respectively and LOQ are 0.033 and 0.297. **Fig. 9** presents the calibration curve that we obtained for each vitamin.

Accuracy and Precision: We rated the accuracy of our suggested method using measuring the concentrations of vitamins E and C in replicates as in **Table 2**. The data suggests that the adopted method is indeed accurate as compare to the other analytical methods.

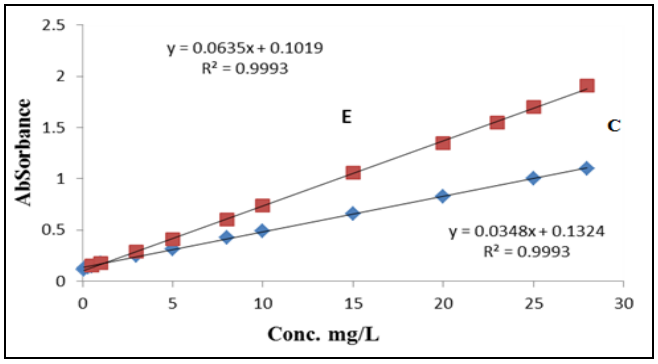


FIG. 9: CALIBRATION CURVE OF VITAMIN C AND E

TABLE 1: THE STATISTICAL PARAMETERS OF CALIBRATION CURVES OF VITAMIN C AND E

Parameters	Value	
	Vitamin C	Vitamin E
Linear equation	A=0.0348[C]+0.1324	A=0.0635[C]+0.1019
Slope(m)	0.0348	0.0635
Intercept(b)	0.1324	0.1019
Correlation Coefficient(R^2)	0.9993	0.9993
Percentage linearity (R^2%)	99.93%	99.93%
Intercept standard error	0.0104	0.0122
Intercept standard deviation	0.0360	0.0485
R.S.D	0.2877	0.2763
L.O.D (µg/mL)	0.01	0.09
L.O.Q (µg/mL)	0.033	0.297
Linearity range (µg/mL)	0.05-28	0.5-28

TABLE 2: STATISTICAL PARAMETERS TO EVALUATE THE ACCURACY OF THE ADOPTED METHOD

Method	Vitamin C(µg/mL)		% Recovery		% Error	% R.S.D
	Taken	Found				
UV-VIS	10	9.88	98.80	Mean = 100.34	1.20	0.91
	20	20.41	102.05	S.D = 1.97	2.05	0.26
	30	30.33	101.16		1.16	0.27
	Vitamin E(µg/mL)					
	10	10.29	102.90	Mean = 101.29	2.90	2.01
	20	20.36	101.80	S.D = 1.95	1.80	1.28
	30	29.75	99.17		0.83	0.69

Analysis of Dosage Forms: The proposed spectro photometric analysis method was used to measure the concentration of vitamins C and E in different pharmaceutical formulations from different companies. An amount from each vitamin of different kinds of pharmaceutical preparations was dissolved in its solvents and we used 100 mL calibrated flask to collect the solution. Then we finish the volume to the mark with distilled water. The flasks with its contents were shacked well and filtered. 0.75mL from each filtrate was taken to the measurements as described under general procedure. The obtained results were tabulated in **Table 3**, which confirms the applicability of the proposed method.

TABLE 3: ANALYSIS OF BOTH VITAMINS IN DIFFERENT DOSAGE FORMS

Method	Vitamin C	Label claim taken (mg/Tab)	Mean amount found (mg/Tab)	% Mean amount found	% R.S.D (n=3)
	Company				
UV-VIS	Furat pharma Tablet, Iraq	250	248.31	99.32	0.82
	Cetavit tablet, Alshaba, Syria	500	496.95	99.33	0.46
	Vitamin E				
	Philvitaie	400	387.44	96.86	1.33
	MVC	100	99.73	99.37	1.04

CONCLUSION: The suggested method is easy to apply, accurate and does not affect using heating or other drastic experimental conditions. However, we recommend adopting this method as alternative method to the existing spectrophotometric method. Furthermore, we suggest applying this method to evaluate of vitamin (C and E) in drug preparations to guarantee a high standard of quality control.

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CONFLICT OF INTEREST: None Declared.

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