ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 51 (2001) 267–272

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

Colorimetric determination of tocopheryl acetate (vitamin E) in pure form and in multi-vitamin capsules

Alaa S. Amin*

Chemistry Department, Benha University, Egypt

Received 15 March 2000; accepted in revised form 30 October 2000

Abstract

A simple, accurate and sensitive method for the determination of micro amounts of vitamin E (V_E) in pure form and in multi-vitamin capsules is described. The method is based on the reduction of tetrazolium blue in slightly alkaline medium by vitamin E after extraction from aqueous EDTA medium with petroleum ether and transesterification. The oxidation reduction reaction occurs after 10 min of heating in a water bath at 90 ± 2°C, leading to the formation of a highly coloured formazan derivative. Different variables affecting the colour development were investigated and optimized. Absorbance measurements were made at 526 nm and the calibration graph was linear for 0.2–11.0 μg/ml of (V_E) with relative precision of about 0.7–1.5% when the standard additions method is used. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Vitamin E determination; Colorimetry; Tetrazolium blue; Capsules analysis

1. Introduction

At least seven different types of vitamin E have been identified namely, α, β, γ, δ, ε, ξ, and η tocopherol. McBride and Evans [1] identified α-, γ- and δ tocopherol by a rapid voltammetric method in their work on estimation of tocopherols and antioxidants in oils and fats, but the peak of β- tocopherol was superimposed on that of γ- tocopherol. An analytical methods committee panel [2] found that although γ- and η tocopherols were inseparable by two-dimensional paper chromatography and so were β-and ε-tocopherols, α-tocopherol gave a separate spot.

Most published assay for tocopherol are suitable for its determination in feeds, aquatic organisms, biological fluids and in dosage forms by high performance liquid chromatography (HPLC) [3–5], HPLC-mass spectra [6], inductively coupled plasma atomic emission spectrometry [7], and chronopotentiometry [8]. The chemical determination of tocopherols in food and other material is rather complicated [9,10]. The general method involves the extraction of lipids containing tocopherols, removal of the interfering substances and determination of the tocopherols. Most procedures follow the Emmirie and Engel method [11], which is regarded as the most suitable for the determination of purified tocopherols. The method adopted in this work avoids the conventional complicated procedures, because of the absence of lipids in the multi–vitamins being analysed.

The aim of the present work was to develop a colorimetric method for the determination of V_E in pure and in multi-vitamin capsules based on the reduction of tetrazolium blue by V_E to form a highly coloured red formazan derivative. In the procedure described in this paper, a method of analysis for V_E in multi-vitamin capsules containing iron and other minerals is developed. Our method was compared with the official [12] and other spectrophotometric methods reported earlier [13,14] showing a good agreement between the final results.

2. Material and methods

2.1. Reagents

All reagents used were of analytical-reagent grade. Tetrazolium blue. A 0.01 M was prepared by dissolving 0.7277 g of tetrazolium blue (Aldrich product) in water and diluting to the mark in a 100-ml calibrated flask.

2.1.1. d-α-tocopheryl acetate standard solution

A stock solution was prepared by dissolving 0.1769 d-α-tocophery1 acetate in 100 ml of methanol. It was stored in a refrigerator and renewed monthly. Dilute solutions were prepared by taking 250, 200, 100, 50 and 25 μl aliquots

* Chemistry Department, Faculty of Science, Benha University, Benha, Egypt. Tel.: +2-055-350996; fax: +2-013-225494.

0939-6411/00/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0939-6411(00)00148-X

of the stock solution and placing them in 25-ml calibrated flasks.

2.1.2. EDTA solution

0.008 M anhydrous disodium ethylenediaminetetracetate (0.2982 g) was dissolved in 100 ml of bidistilled water. Sodium hydroxide, 2.0 M was prepared by dissolving 2.0 g of NaOH in bidistilled water and completing to the mark in a 250-ml calibrated flask.

2.2. Procedure

2.2.1. Transesterification

Tocopheryl acetate was converted into tocopherol by modification of the procedure described by Sheikh et al. [15] and Campbell et al. [16]. Standards were prepared by taking 25, 50, 100, 250 and 500 μl portions of stock solution in 25-ml calibrated flasks, adding a drop of sulphuric acid (act as a catalyst) and 20 ml of methanol to each, covering the necks of the flasks with aluminum foil perforated to permit the escape of methyl acetate and excess of methanol, and heating the flasks at 70–80°C in a water-bath for 90–105 min; within this period, the flask contents were reduced almost to dryness. The end-product of transesterification was dissolved in 15 ml of methanol. 0.5 ml of 0.01 M of the reagent was then added, 5.0 ml of 0.2 M NaOH and complete to the mark with methanol. The absorbance was measured after 10 min of heating in a water-bath of 90 ± 2°C at λ_{max} 526 nm, and the calibration curve was drown in the usual way.

2.2.2. Samples

A multi-vitamin solution was prepared according to the method suggested in a collaborative study by the AOAC [10]. Capsules were ground in a morter into a very fine powder and an exact amount was weighed into a 100-ml calibrated flask, dissolved and diluted to volume with 8×10^{-3} M EDTA. Suitable (5.0 or 10 ml) aliquots of the EDTA–vitamin solution were taken for analysis. The tocophery1 acetate was extracted with two 10-ml portions of petroleum ether (b.p 40–60°C). The resulting combined extract was evaporated almost to dryness and the residue was thoroughly washed with methanol into a 25-ml standard flask. This solution was then acidified, transesterified and analysed by the procedure given above for the standards.

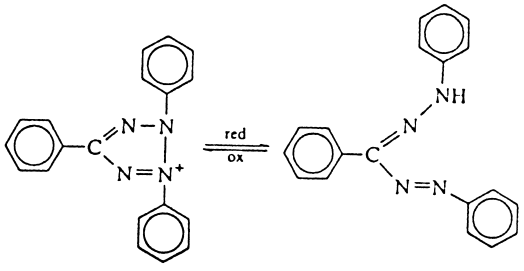
For the standard additions method three separatory funnels were used. To the first was added a 5.0-ml aliquot of the EDTA-vitamin solution. A mixture of a 5.0-ml aliquot of the EDTA-vitamin solution and 0.10 ml of standard solution (measured with a micropipette) was placed in the second. The third contained 100 μl of the standard solution alone. The tocopheryl acetate in each solution was extracted with two 10-ml portions of petroleum ether and the extracts were treated as just described for the samples. The standard additions method was repeated with 10-ml aliquots. The tocophery1 acetate content in mg/g of dry multi-vitamin (I.U.) is calculated from the standard additions method results as follows

$$I.U. = \left[\frac{\text{mg of vitamin E standard taken} \times A_{\text{Unk}}/(A_{\text{Unk+Std}} - A_{\text{Unk}})}{W} \right]$$

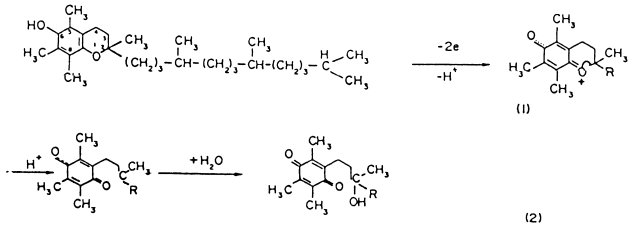
which A_{Unk} is the absorbance of the unknown, $A_{\text{Unk+Std}}$ is that of the unknown with the standard added, and W is the weight of dry multi-vitamin (in g) in aliquot taken for analysis.

3. Results and discussion

Tetrazolium salts have been used for the colorimetric determination of corticosteroids and modified corticosteroids having an α-keto group [17–19]. It was also used for the determination of some compounds having other ketonic groups which can reduce tetrazolium salts to the highly coloured red formazan [20,21]. In our previous work [21], the reduction of tetrazolium blue is represented by the following scheme



The oxidation of α-tocopherol to the quinone by tetrazolium is summarized in the reaction below.



The rate of reaction is indicated by the rate of colour development, which depends on the rate of reduction of tetrazolium blue by tocopherol. To optimize conditions, we have investigated a number of parameters such as alkalinity, temperature, time, reagent concentration, solvent, transesterification and order of additions.

3.1. Effect of alkalinity

Previously our work on tetrazolium blue [21] established that the optimum basic media was 5.0 ml of 0.2 M NaOH. In this work, our investigation showed that the reaction of tocopherol with tetrazolium blue will proceed in a similar

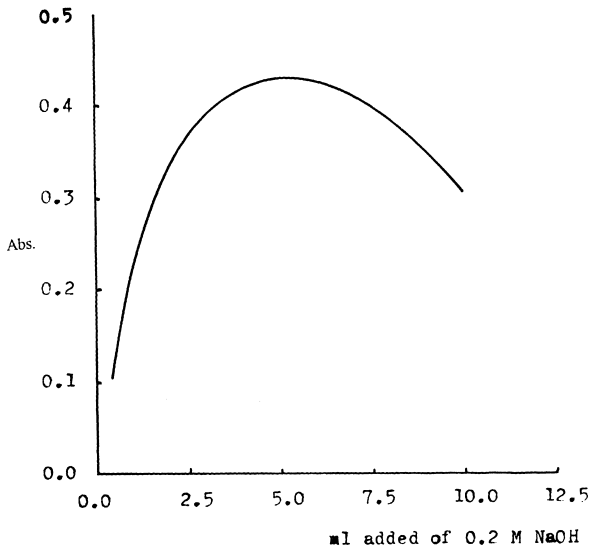


Fig. 1. Effect of addition of 0.2 M NaOH on the absorbance of 5.0 μg/ml of tocopherol in presence of 5×10^{-4} M tetrazolium blue in 10 ml final assay solution after heating in water bath of 90 ± 2°C for 10 min.($n = 6$, SD = 0.022).

alkaline medium. It was found that the oxidation reduction reaction does not occur in acidic and neutral medium. At pH >7.5 gradual increase in absorbance of the reduction product (formazan) until addition of 5.0 ml of 0.2 M NaOH solution. Moreover a constant and stable colour development was observed at this medium. Increasing the addition of NaOH, a gradual decrease in absorbance occurs (Fig. 1).

3.2. Effect of temperature and time

A preliminary investigation showed that the reduction of tetrazolium blue to formazan at room temperature required 3.0 h for completion. Raising temperature, the time for complete colour development is reduced. Maximum colour was obtained by heating on a water-bath at 90 ± 2°C for 10 min. Prolonged heating decreased the colour intensity, as represented in Fig. 2, so the reaction time should be very accurately controlled. The developed colour was stable for 3.0 h as shown in Fig. 3.

3.3. Effect of reagent concentration

Various volumes of 0.01 M solution of tetrazolium blue were investigated to select the optimum reagent concentration. In the reaction with tocopherol 0.5 ml was sufficient to produce maximum colour intensity.

3.4. Effect of solvent

As an assay solvent, methanol afforded maximum sensitivity. For the other solvents tested (ethanol, acetone, dioxane, propanol and dimethylformamide), the colour development does not achieve the same wavelength on using methanol, in addition to lower colour intensity. Seventy five percent methanol–water mixture was found to be the best ratio for full colour development. No change in the absorbance of the coloured product occurred on increasing the ratio of methanol.

3.5. Transesterification

The transesterification process is so slow that a catalyst is essential to make the reaction go faster. We add a drop of concentrated sulphuric acid to the mixture in each flask, and the sulphuric acid has been found to be a very satisfactory catalyst.

3.6. Order of addition

We have observed that the reagent must be added after the transesterification of tocopherol and before the addition of NaOH solution, to ensure maximum reproducibility. Other orders uses gave low colour intensity and not achieved to the same λ_{max} indicating incomplete oxidation-reduction reaction.

3.7. Prevention of photochemical reaction

Tocopherol is photochemically active and easily oxidized by air. Tsen [13] has made several suggestions for dealing with this, including determination under dim artificial light in a darkened room and use of a brown glass flask in the determination. Covering the flask with aluminum foil provides a darkened environment for the reaction.

3.8. Interferences

To eliminate interference of any metal present in the

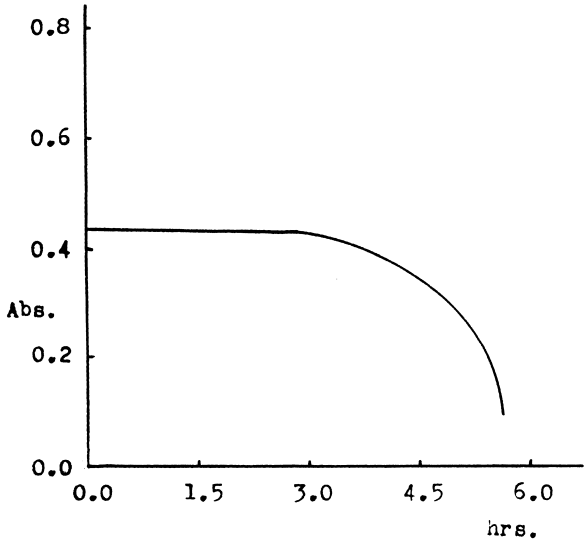


Fig. 2. Effect of time to complete colour development of 5 μg/ml tocopherol using 5×10^{-4} M tetrazolium blue in presence of 0.1 M NaOH in 10 ml final assay solution placed in water bath of 90 ± 2°C ($n = 6$, SD = 0.036).

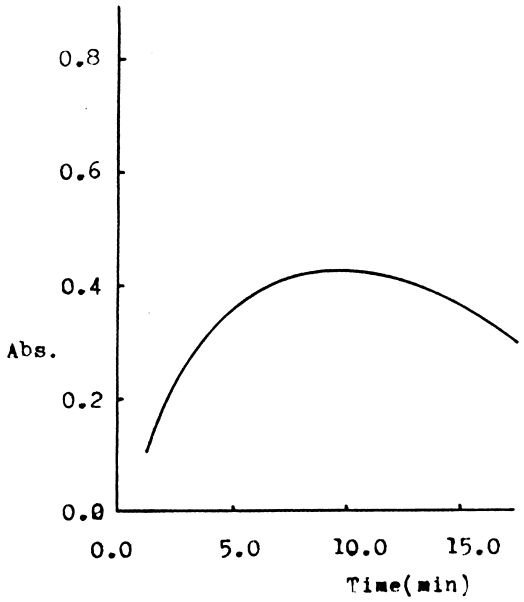


Fig. 3. Stability of the coloured product on oxidation of 5 μg/ml tocopherol with 5×10^{-4} M tetrazolium blue in present of 0.1 M NaOH in 10 ml final solution after heating in a water bath of 90 ± 2°C for 10 min ($n = 6$, SD = 0.074).

multi-vitamin preparations, the sample solution is prepared in EDTA medium, to complex the metal ions. The interference of vitamin C or a component of the multi-vitamins which is capable of reducing tetrazolium blue can be eliminated by extracting the tocopheryl acetate with petroleum ether, a solvent in which vitamin E, but no other vitamin, is highly soluble. The effect of errors arising in the transesterification can be reduced by using the standard additions method. Since all solutions are prepared in the same manner, experimental errors for the solutions of vitamin extract or the extract containing the standard increment are minimized.

3.9. Precautions in transesterification

During transesterification the calibrated flasks containing the methanol-tocopherol solution should be gradually heated in the water-bath. If the flasks are immersed directly in a hot bath, spurting will occur, with a consequent loss of some of the contents. Transesterification should be continued until the solution in each flask is almost evaporated to dryness. Incomplete transesterification results in a low absorbance value.

3.10. Analytical data

A linear correlation was found between absorbance and concentration in the range 0.2–11.0 μg/ml of vitamin E. For more accurate results, Ringbom optimum concentration range was found in the range 0.6–10.2 μg ml^{-1}. The correlation data for tocopherol are calculated using the least-squares method. The reproducibility of the procedure was determined by running six replicate samples, each containing 7.0 μg/ml in the final assay solution. At this concentration, the relative standard deviation was 1.37%. The performance of the proposed procedure was assessed by calculation of the *t*- and *f*-values compared with the official method [12] (depending on gas chromatography). Mean values were obtained in a student's *t*- and *f*-test and 95% confidence limits for five degrees of freedom [22], and the results (Table 1) showed that the calculated *t*- and *f*-values did not exceed the theoretical values.

3.11. Sensitivity, accuracy and precision

The mean molar absorptivity and Sandell sensitivity as calculated from Beer's law are presented in Table 1. In order to determine the accuracy and precision of the method, solutions containing six different concentrations of tocopherol were prepared and analysed in quintuplicate. The measured standard deviations (s), relative standard deviation (S_r), the standard analytical errors and confidence limits (Table 2) can be considered satisfactory, at least for the levels of concentration examined.

Comparison of the results obtained by the proposed procedure using tetrazolium blue with those obtained using iron(III)-ferrozine-reagent [14] for the determination of vitamin E showed a wider range of determination, higher accuracy and precision. Although the spectrophotometric

Table 1
Quantitative parameters for the determination of vitamin E

Parameter	Value	Parameter	Value
λ_{max} (nm)	526	Regression equation[a]	
Beer's law limits/μg/ml	0.2–11.0	Slope (a)	0.086
Ringbom range/μg/ml	0.6–10.2	Intercept (b)	0.003
Molar absorptivity l/mol cm	3.72×10^4	Correlation coefficient (r)	0.9996
Sandell sensitivity/μg/cm^2	0.012	*t*-test (2.57)[b]	1.37
Range of error/%	± 1.80	*F*-test (5.05)[b]	2.41
Standard deviation/%	0.96	Relative standard deviation (%)	1.37

[a] $A = a + bC$ where C in μg/ml.
[b] Theoretical values for five degrees of freedom and 95% confidence limit.

Table 2
Evaluation of accuracy and precision of the proposed procedure

Taken	Found/μg/ml[a]				Standard error	Confidence limits
	Proposed	Official	S	S_r (%)		
1.0	0.99	0.97	0.03	0.669	0.012	0.99 ± 0.035
2.0	2.03	1.93	0.05	1.06	0.020	2.03 ± 0.060
4.0	3.95	4.10	0.08	1.47	0.033	3.95 ± 0.095
6.0	6.10	5.85	0.10	1.50	0.041	6.10 ± 0.012
8.0	8.08	8.17	0.07	1.28	0.029	80.8 ± 0.080
10.0	9.84	10.30	0.12	1.56	0.050	9.84 ± 0.140

[a] Average of six determinations.

method [14] is more sensitive ($\varepsilon = 5.35 \times 10^4$ l/mol cm), the proposed method does not need any treatment to prevent the photochemical reaction of ferric iron.

Comparison of the recovery obtained with the proposed procedure with the purity of the studied drug as determined according to the British pharmacopoeia [12] showed a high accuracy of the present method. The proposed procedure is simpler, low cost, less time consuming and more sensitive than the official method [12]. Moreover, the proposed procedure could be used for the routine determination of vitamin E in pure form and in multi-vitamin samples.

3.12. Analytical application

The proposed procedure was applied to some multi-vitamin capsules containing vitamin E, using the standard additions method in which variable amounts of pure vitamin were added to the previously analysed portion of mulit-vitamin capsules. Results are shown in Table 3 and confirm that the proposed method is not liable to interference by metal ions. The results seemed to be accurate and the method was successfully applied to determine V_E in the presence of excipients such as lactose, maltose, glucose and starch, which are usually added during the preparation of capsules. The presence of prepared wheat-germ oil, vitamin B_1, B_2, B_6, B_{12}, and vitamin A together with V_E in their capsules did not interfere with the results. The proposed procedure is highly sensitive, therefore it could be easily used for the routine analysis of pure vitamin E and multi-vitamin capsules.

The performance of the proposed procedure was assessed by calculation of *t*- and *f*-values compared with the official method [12] (depending on gas chromatography) for 95% confidence limits with five degrees of freedom [22]. The

Table 3
Determination of vitamin E in different multi-vitamin capsules

Capsules	Composition	Taken	Added	Found (mg)[a]	
		(mg)	(mg)	Proposed	Official [12]
Vitagreen-E[b]	Specially prepared wheat-germ oil 200 mg	2.0	–	1.97	2.06
			2.0	4.02	4.10
	d-α-tocopherol 298 IU		4.0	6.6	5.90
			6.0	7.95	8.15
Vitamin A & E[c]	d-α-tocopheryl acetate 400 mg	3.0	–	3.05	2.93
			3.0	6.20	5.95
			6.0	8.9	9.15
			8.0	10.90	11.25
Di-Viton A & E[d]	d-α-tocopheryl acetate 400 mg	2.5	–	2.53	2.045
			2.5	5.05	4.92
			5.0	7.46	7.57
			7.5	9.85	10.26
E- Viton[d]	d-α-tocopheryl acetate 100 mg	4.0	–	3.96	4.07
			4.0	8.05	8.15
			8.0	12.10	11.80
			12.0	15.90	15.75

[a] Average of six determinations.
[b] Sekem Company for Natural Product, Cairo, Egypt.
[c] Pharco Pharmaceuticals Company, Alexandria, Egypt.
[d] Kahira Pharmaceutical & Chemical Industries Company, Cairo, Egypt.

results showed that the *t*- value was 1.68, whereas the theoretical value is 2.57. The *f*-value was 2.79, whereas the theoretical value is 5.05. These results indicated that there was no significant difference between the proposed and official methods.

4. Conclusion

The proposed procedure is fairly simple, low cost, time consuming and more sensitive than the official [12] and previously reported methods [13,14]. Although the colour development at room temperature required 3.0 h for completion, this can be shortened to 10 min by raising the temperature to 90 ± 2°C. The proposed method is suitable for the determination of V_E in pure and in multi-vitamins capsules without interferences from excipients, additives and other vitamins present in formulations, suggesting applications in routine determination of V_E in pure and in multi-vitamin capsules.

References

[1] H.D. McBride, D.H. Evans, Anal. Chem. 45 (1973) 446.
[2] Society for Analytical Chemistry, Analytical Methods Committee, Analyst 84 (1959) 356.
[3] S. Wielinski, A. Olszanowski, Chromatogrophia 50 (1999) 104.
[4] K.E. Sharpless, M. Arce-Osuna, J.B. Thomas, L.M. Gill, AoAC Int. 82 (1999) 288.
[5] H.Z. Huo, H.J. Nelis, P. lavens, P. Sorgeloos, A.P. De Leenheer, J Chromatogr. Biomed. Appl. 724 (1999) 249.
[6] S. Strohschein, C. Rentel, T. Locker, E. Bayer, K. Albert, Anal. Chem. 71 (1999) 1780.
[7] P. Masson, Spectrochem. Acta 54B (1999) 603.
[8] Z.J. Suturovic, N.J. Marjanovic, Electroanalysis 11 (1999) 207.
[9] A.A. Christie, A.C. Deam, B.A. Milburn, Analyst 98 (1973) 161.
[10] A.J. Shepperd, W.D. Hubbard, J. Assoc. Anal. Chem. 52 (1969) 442.
[11] A. Emmerie, C. Engel, Rev. Trav., Chim. 58 (1937) 283.
[12] Her-Majesty's Stationary Office, British Pharmacopoeia, Her-Majesty's Stationary Office, London, 1998.
[13] C.C. Tsen, Anal. Chem. 33 (1961) 849.
[14] W. Adeniy, B. Jaselskis, Talanta 27 (1980) 993.
[15] J. Sheikh, J. Dolezal, J. Zyka, Microchem. J. 16 (1971) 395.
[16] E.E. Reid, F.O. Cockerille, Org Syntheses Collective 3 (1978) 468.
[17] R.B. Burton, A. Zafferopni, E.H. Keutmann, J. Biol.Chem. 188 (1951) 763.
[18] C. Chem, J. Wheeler, H.E. Tewell, J. Lab. Clin. Med. 42 (1953) 749.
[19] W.J. Mader, R.R. Buck, Anal. Chem. 24 (1952) 666.
[20] A.S. Meyers, M.C. Lindberg, Anal. Chem. 27 (1955) 813.
[21] A.S. Amin, Mikrochim Acta 126 (1997) 1045.
[22] J.C. Miller, J.N. Miller, Statistics in analytical chemistry, 3rd Edition, Ellis Horwood, Chichester, 1993.