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Analytica Chimica Acta 441 (2001) 201–206

ANALYTICA CHIMICA ACTA

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Voltammetric behavior of α-tocopherol and its determination using surfactant + ethanol + water and surfactant + acetonitrile + water mixed solvent systems

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Received 17 January 2001; received in revised form 2 May 2001; accepted 10 May 2001

Abstract

Cyclic voltammograms, at the carbon paste electrode were recorded for α-tocopherol dissolved in surfactant + ethanol + water and surfactant + acetonitrile + water mixed solvent systems containing ammonia. The oxidation potential of the vitamin was found to vary with a change in the surfactant, being more positive in cationic surfactants as compared to the anionic or nonionic surfactants. Differential pulse voltammetric technique was utilized for the determination of the vitamin in the above solvent systems. The limit of detection and the linear range varied in different surfactants, the lowest being in the Triton X-100 + acetonitrile + water mixtures. Simultaneous determination of α-tocopherol and ascorbic acid was carried out. Multivitamin–multimineral pharmaceutical preparations analyzed gave satisfactory results.

Keywords: α-Tocopherol; Surfactants; Triton X-100; Cyclic voltammetry; Differential pulse voltammetry; Ascorbic acid

1. Introduction

α-Tocopherol is the most common of the group of tocopherols, the others being β-, γ-, and δ-tocopherol. The tocopherols are viscous oils at room temperature, insoluble in water but soluble in certain organic solvents such as ethanol and acetonitrile. The main sources of the vitamin are vegetable oils obtained from cottonseed, lettuce, rice germ olive and sunflower. Nuts and cereal grain products such as filberts and almonds, milk, eggs, meat and fish are also rich in their α-tocopherol content [1]. The electrochemical activity of the E-vitamers is associated with the phenolic hydroxy group, which readily undergoes oxidation. Polarography has been employed for the determination of α-tocopherol in the presence of β- and γ-vitamers [2]. McBride and Evans [3] have used a glassy carbon electrode and a linear sweep waveform for the measurement of the tocopherol content of vegetable oils dissolved in ethanol + benzene mixtures. Shiozaki and others have also used a glassy carbon electrode for the analysis of Vitamin E; in this case however the determination was carried out in a nonaqueous supporting electrolyte consisting of 0.5 M $LiClO_4$ dissolved in acetonitrile [4]. Other more commonly employed methods are the direct and modified Emmerie–Engel colorimetric [5] and spectrophotometric methods.

Surfactants play a very important role in increasing the solubility of an organic substance, which is either insoluble or sparingly soluble in water. Early work in the field of solubilization has been adequately

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0003-2670/01/$ – see front matter
PII: S0003-2670(01)01119-9

reviewed by Klevens amongst others [6]. Formation of micelles is a prerequisite for solubilization in surfactant systems. The distribution of a solubilizate between the micelles and the intermicellar fluid is a complex phenomenon [7]. It has been shown that bile salts and phospholipids efficiently solubilize tocopherol by the formation of micelles [8]. As investigations in aqueous micellar systems mimic the microenvironment analogous to that of the active and binding sites of enzymes in physiological systems [9], the purpose of our work was to analyze the vitamin in a predominantly aqueous environment as against a completely nonaqueous environment as has been done earlier. We have attempted to dissolve α-tocopherol in 50% (v/v) ethanol + water and 40% (v/v) acetonitrile + water mixed solvent systems with the help of certain ionic surfactants, viz. cetyl trimethyl ammonium bromide (CTAB), cetyl pyridinium chloride (CPC), and sodium dodecyl sulphate (SDS) and a nonionic surfactant Triton X-100 (TX-100). α-Tocopherol, which is a strong antioxidant, occurs in several pharmaceutical preparations in conjunction with another strong antioxidant, which is ascorbic acid. They are also found together in many biological fluids and enzymes and in the adrenal gland of the human body [1]. In combination with each other they may either inhibit or catalyze certain reactions occurring inside the human body. Hence, it is important to monitor both these vitamins in the presence of each other. Simultaneous determination of ascorbic acid (water-soluble vitamin) and α-tocopherol (fat-soluble vitamin) has therefore been taken up in the present work.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical reagent grade. Double distilled, deionized water was used for preparation of all solutions. Triple-distilled ethanol and acetonitrile (over molecular sieves) containing less than 0.1% water (as determined by Karl Fischer titration), were used to prepare ethanol+water and acetonitrile+water mixtures, respectively. Both the solvents were stored in sealed containers to prevent atmospheric contamination. All the voltammetric studies were carried out in 0.1 M ammonia solution at $25 \pm 0.2^{\circ}$C.

2.2. Apparatus

The voltammetric system used for the studies was EG&G Princeton Applied Research 264A potentiostat with model 303A electrode assembly and *X–Y* chart recorder RE0089. The carbon paste electrodes were prepared as before [10]. It was used in conjunction with a saturated calomel reference electrode and a platinum counter electrode.

2.3. Procedure

Initially cyclic voltammograms were recorded for a solution containing α-tocopherol (2.53×10^{-4} M) in absolute ethanol containing 0.1 M ammonia. Then cyclic voltammograms were recorded for the same concentration of the vitamin in 0.1 M surfactant+50% (v/v) ethanol + water mixed solvent systems. Similar experiments were performed in 0.1 M surfactant+40% (v/v) acetonitrile + water. Voltammograms in all the media were recorded at different scan rates.

Differential pulse voltammetry (DPV) was utilized for the quantification of α-tocopherol in all the above media. The surface of the electrode was periodically renewed by pressing out a small amount of the paste, scraping of the excess and polishing the tip on a zero grade polishing paper until the surface had a shiny appearance. Between successive runs, the solutions were purged with dry nitrogen for 2 min. DPV was also used for the quantification of ascorbic acid in the TX-100 + acetonitrile + water mixtures.

Simultaneous determination of α-tocopherol and ascorbic acid was then taken up. Synthetic samples having compositions of the two vitamins, viz. α-tocopherol:ascorbic acid in the ratios 75:200, 60:100 and $50\,\mu g\,cm^{-3}$:$100\,\mu g\,cm^{-3}$ were prepared and their differential pulse voltammograms recorded.

2.4. Determination of α-tocopherol and ascorbic acid in the multivitamin preparations

The pharmaceutical preparations analyzed for their α-tocopherol content were Co-vita 200 (Savita pharmaceuticals, India) and Vita X (Ferni pharmaceuticals) capsules which contained α-tocopherol extracted from natural vegetable oils. Simultaneous analysis of α-tocopherol and ascorbic acid in Sofox (Savita pharmaceuticals, India) capsules was also taken up, which

contained riboflavin, copper, manganese, zinc and selenium along with the binding agents in addition to α-tocopherol and ascorbic acid.

Each capsule was cut open to ooze out its contents. The matrix was then dissolved in 0.1 M TX-100+40% (v/v) acetonitrile + water mixed solvent systems containing 0.1 M ammonia. The solution was then filtered through a Qualigens (615) filter paper to remove traces of any undissolved matter. The standard addition method was employed for the quantification of the vitamins, wherein 0.3 cm^3 of the filtrate was taken into a cell containing 10.0 cm^3 of the supporting electrolyte and additions of 0.2 cm^3 α-tocopherol:ascorbic acid (100 $\mu g\,cm^{-3}$:500 $\mu g\,cm^{-3}$) were done. DPV with pulse amplitude of 50 mV and scan rate of 20 $mV\,s^{-1}$ was used to record the peaks.

3. Results and discussions

α-Tocopherol is a fat-soluble vitamin, soluble only in purely organic solvents such as pure ethanol and pure acetonitrile. The addition of even a small quantity of water causes the solution to become turbid. However, in the presence of about 0.1 M surfactant (CTAB/CPC/SDS/TX-100), the vitamin remains in solution even when the major portion of the solvent is the aqueous phase. Formation of micelles of surfactant monomers is not restricted to aqueous solutions. It has been observed in a variety of solvents whose polarity ranges from completely polar through dipolar aprotic [11,12]. To promote micellar aggregation it is required that the solvent must possess a combination of two main factors, i.e. high solvent polarity and high hydrogen bonding ability. Pure acetonitrile being dipolar aprotic, lacks the ability to form hydrogen bonds which leads to weak cohesive forces in the solvent; and pure ethanol, though can extensively hydrogen bond with itself, lacks the high polarity of organizing solvents. Hence, pure ethanol and pure acetonitrile do not support aggregation of surfactant monomers [13]. Nevertheless, micellization of several amphiphiles has been reported in solvents such as acetonitrile, dimethyl sulphoxide and acetone where hydrogen bonding ability is either nonexistent or minimal [14]. The presence of water as an equal or major portion leads to the formation of ternary systems comprising of organic solvent + water + surfactant. In the case of alcohols it has been shown that the alcohol itself distributes itself between the aqueous and micellar phases and gets accumulated both in the palisade layer and inside the micellar hydrophobic core, thus favoring the stability of the system by decreasing the critical micellization concentration (CMC) and causing the micelles to swell [15]. Thus, incorporation of the alcohol in the micelles produces noticeable changes in the micellar shape and its transport properties. Besides this, in the case of both ethanol and acetonitrile, increase in the water content causes an increase in the dielectric constant of the solvent system. It has been shown that an increase in the dielectric constant of a solvent system leads to the onset of micellization [16]. The CMC of the surfactants is however higher in the nonaqueous–aqueous solvent mixtures as compared to that in purely aqueous solutions. Thus, one may say that solubilization of α-tocopherol is promoted in the nonaqueous–aqueous solvent mixtures due to micellization of the surfactants.

Cyclic voltammograms recorded for α-tocopherol (2.53×10^{-4} M) in ethanol and acetonitrile (containing 0.1 M ammonia) at a scan rate of 100 $mV\,s^{-1}$ showed a single irreversible oxidation wave for the vitamin with the oxidation potential being 0.25 and 0.28 V in each of the solvents, respectively, and the peak current being 5.7 μA in both the solvents. Fig. 1 shows a comparative view of the cyclic voltammograms for the same concentration of α-tocopherol dissolved in pure ethanol and the vitamin dissolved in 0.1 M CPC+50% (v/v) ethanol + water mixture. A shift in the peak

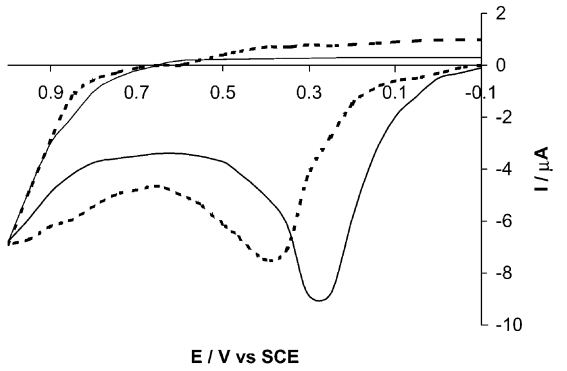

Fig. 1. Cyclic voltammograms for 2.53×10^{-4} M α-tocopherol (—) in pure ethanol and (- - -) 0.1 M CPC + 50% (v/v) ethanol + water mixed solvent systems.

Table 1
Effect of medium on the cyclic voltammetric peak potentials (E_p vs. SCE) and peak currents (I_p) for 2.53×10^{-4} M α-tocopherol at a scan rate of 100 mV s^{-1}

Surfactants (0.1 M)	50% (v/v) Ethanol + water		40% (v/v) Acetonitrile + water	
	E_p (V)	I_p (μA)	E_p (V)	I_p (μA)
CPC	0.36	4.1	0.38	4.0
CTAB	0.34	4.5	0.36	4.3
SDS	0.29	5.2	0.32	5.2
TX-100	0.28	5.6	0.31	5.6

potential was observed in the case of α-tocopherol dissolved in ethanol + water with the help of CPC, toward more positive values (0.38 V), along with a slight decrease in peak current. Both, the shift in oxidation potential and the decrease in peak currents in the presence of the surfactant indicate that the vitamin molecules have penetrated into the hydrophobic core of the micelles. This explains the higher energy required for oxidation and since these large micellar aggregates transport the solubilized substance to the electrode more slowly as compared to the free solubilizate, a decrease in peak current is quite obvious. Similar observations were reported by Kirchoff et al. [17] in their studies on rhenium and technetium complexes in micellar media. In each of the surfactants voltammograms were recorded at scan rates of 20, 50, 100 and 200 mV s^{-1}. The plots of current (I_p) versus square root of scan rate ($v^{1/2}$) were found to be linear in all the media indicating that the oxidation of the vitamin was governed by diffusion. Table 1 shows the effect on the peak potentials (E_p) and peak currents (I_p) for α-tocopherol in different surfactants. From the table it is seen that the TX-100+nonaqueous+aqueous solvent mixtures show the least difference in both the peak potentials and peak currents of the vitamin as compared to that in pure ethanol and pure acetonitrile containing 0.1 M ammonia, the values of which are given earlier. This could be because the solubilized species does not enter right into the core of these micelles but remains solubilized in the palisade layer and also the diffusion of TX-100 micelles could be faster as compared with other surfactants.

DPV was used for the quantification of the vitamin in all the above media. The limit of detection and linear concentration range observed was different in each system as can be seen from Tables 2 and 3. The lowest limit of detection was observed in the TX-100 + nonaqueous + aqueous solvent mixtures. The 0.1 M TX-100 + 40% (v/v) acetonitrile + water mixed solvent systems were used for all further analyses as the percentage of water in these systems was more. The detection limit observed, for the vitamin in this system was 0.6 μg cm^{-3} (R.S.D. = 4.3%) and the linear working range was observed to be between 1.5 and 200 μg cm^{-3} (coefficient of correlation = 0.9921), at a scan rate of 20 mV s^{-1} and pulse amplitude of 50 mV. By lowering the scan rate to 10 and 5 mV s^{-1} and increasing the pulse amplitude, sharper peaks were obtained and a detection limit of 0.09 μg cm^{-3} for α-tocopherol was possible. Table 4

Table 2
Linear concentration range and limit of detection observed in surfactant + ethanol + water mixed solvent systems by DPV at scan rate 20 mV s^{-1} and pulse height of 50 mV

Surfactant	50% (v/v) Ethanol + water			
	Detection limit (μg cm^{-3})	R.S.D. (%)	Linear concentration range (μg cm^{-3})	Coefficient of correlation
CPC	1.1	4.0	1–200	0.9967
CTAB	1.3	4.2	2–200	0.9921
SDS	2.0	4.0	4–200	0.9926
TX-100	0.8	4.3	1.5–200	0.9932

Table 3
Linear concentration range and limit of detection observed in surfactant + acetonitrile + water mixed solvent systems by DPV at scan rate 20 mV s^{-1} and pulse height of 50 mV

Surfactant	40% (v/v) Acetonitrile + water			
	Detection limit ($\mu g\,cm^{-3}$)	R.S.D. (%)	Linear concentration range ($\mu g\,cm^{-3}$)	Coefficient of correlation
CPC	1.1	4.1	1–200	0.9922
CTAB	1.5	4.0	2–200	0.9937
SDS	1.5	4.0	3–200	0.9922
TX-100	0.6	4.3	1.5–200	0.9921

shows the accuracy and precision of the method used for the analysis of some pharmaceutical preparations at a scan rate of 20 mV s^{-1} and pulse amplitude of 50 mV. In the same medium, a linear working range from 50 to 500 $\mu g\,cm^{-3}$ of ascorbic acid (coefficient of correlation = 0.9927), at scan rate of 20 mV s^{-1} and pulse amplitude 50 mV was observed.

Simultaneous determination of α-tocopherol and ascorbic acid was then carried out. The differential pulse voltammograms for the simultaneous determination of α-tocopherol and ascorbic acid at a scan rate of 20 mV s^{-1} and pulse amplitude 50 mV showed poor resolution. Both the peaks, though visible, seemed to merge with each other. For better resolution the scan rate was decreased and pulse height was increased. Increase in pulse height to 100 mV and a drop in scan rate to 2 mV s^{-1}, gave the best separation as shown in Fig. 2. Synthetic samples of different compositions of the two vitamins were analyzed and gave good results. Table 5 compares the actual and observed content obtained for both the vitamins in the mixtures of different proportions. Maintaining the same experimental conditions, a pharmaceutical preparation, containing both the vitamins, viz. Sofox capsules were analyzed and the results were satisfactory.

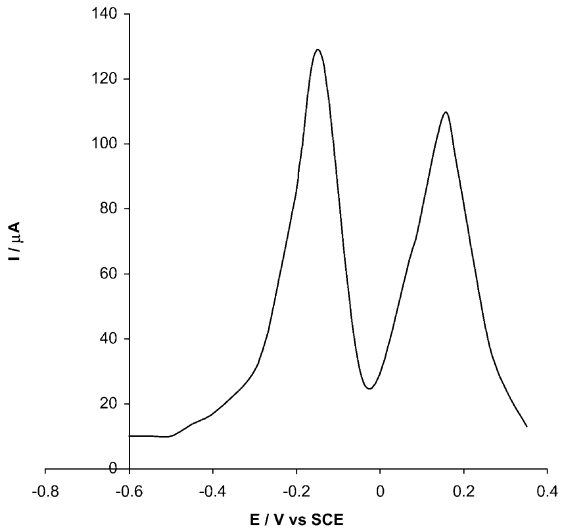

Fig. 2. Simultaneous determination of ascorbic acid and α-tocopherol (200 $\mu g\,cm^{-3}$:75 $\mu g\,cm^{-3}$) in 0.1 M TX-100 + 40% (v/v) acetonitrile + water mixed solvent systems at a scan rate of 2 mV s^{-1} and pulse height 100 mV.

Table 4
Results of the pharmaceutical analysis in 0.1 M TX-100 + 40% (v/v) acetonitrile + water mixed solvent systems

Vitamin preparation	Quoted content (mg)	Observed content ($n = 5$)
Co-vita 200	134.2	133.1 ± 2.0
Vita X	134.2	133.0 ± 2.0
E-vitamers	140.0	140.0 ± 2.1

Table 5
Accuracy and precision of the method for simultaneous determination of α-tocopherol and ascorbic acid in 0.1 M TX-100 + 40% (v/v) acetonitrile + water mixed solvent systems

Synthetic sample (ascorbic acid:α-tocopherol)	Observed content ($n = 5$)	
	Ascorbic acid	α-Tocopherol
200 $\mu g\,cm^{-3}$:75 $\mu g\,cm^{-3}$	199.8 ± 2.2	72.8 ± 2.1
150 $\mu g\,cm^{-3}$:60 $\mu g\,cm^{-3}$	145.8 ± 2.2	58.20 ± 3.1
100 $\mu g\,cm^{-3}$:50 $\mu g\,cm^{-3}$	99.4 ± 2.1	49.3 ± 2.9
Real sample (Sofox capsules) 10 mg:100 mg	9.7 ± 0.3	97.8 ± 2.1

4. Conclusion

It has been observed that the presence of surfactants causes the dissolution of α-tocopherol, which is essentially a fat-soluble vitamin, in a solvent system where an equal or major portion is the aqueous phase. This is possible due to the incorporation of the vitamin in the micelles of the surfactants. Analysis of α-tocopherol, is therefore possible in a nonaqueous + aqueous mixed solvent system as against the completely nonaqueous systems used for analysis until recently. This gives tremendous scope of understanding the behavior of the vitamin in certain biological fluids such as bile salts and pancreatic juices, which form micelles inside the body, and in which the vitamin readily dissolves. The detection limits observed by us were almost comparable or lower than those observed by other more commonly employed methods such as colorimetry and spectrophotometry for the analysis of the vitamin. Simultaneous determination of a hydrophilic vitamin and a lipophilic vitamin, viz. ascorbic acid and α-tocopherol in a single scan without prior separation, has thus been made possible.

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