The Determination of Tocopherols in Vegetable Oils by Square-Wave Voltammetry

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Square-wave voltammetry (SWV) has been applied to the determination of tocopherol concentrations in eight vegetable oils: canola, corn, olive, peanut, safflower, sesame, soybean, and sunflower. This technique has a lower limit of detection and somewhat better resolution than differential-pulse voltammetry (DPV) and is significantly faster than both DPV and high-performance liquid chromatography (HPLC), the technique most often used for tocopherol determinations. The tocopherols are oxidized at a stationary glassy carbon electrode as its potential is scanned from 0.40 to 1.00 V *vs* Ag/AgCl at a rate of 20 mV/s. The currents that arise from these oxidations are directly proportional to the tocopherols' concentrations. A standard addition method is used to determine the tocopherol contents of the oils listed. Under the conditions employed, the limit of detection is 10 mg/L tocopherol in oil.

KEY WORDS: Tocopherols, vegetable oils, voltammetry.

The levels of antioxidant materials in vegetable oil products are of great importance in determining the stability of these products. The tocopherols represent one of the largest groups of natural antioxidants. Therefore, measuring the tocopherol concentrations in vegetable oils is vital in ascertaining the oils' fitness for use in many applications.

Tocopherols are among the large number of naturally occurring substances that are known to be electrochemically active. The phenol groups of tocopherols can be easily, but irreversibly, oxidized at both carbon and platinum electrodes (1,2). The electrochemical activity of tocopherols has been used by a number of researchers to identify and quantitate these compounds in a variety of biological matrices (1–6). In particular, differential-pulse voltammetry (DPV) has been proposed as an alternative to standard high-performance liquid chromatography (HPLC) methods to decrease the time needed for tocopherol analyses (2,3).

The effectiveness of DPV methods, however, is often limited when the electrochemical reaction of the analyte is irreversible because this tends to lower the response, thereby increasing the limit of detection (7). In addition, the widths of DPV peaks also tend to increase under these circumstances, leading to decreased resolution of adjacent peaks. Another complication that can arise in DPV analyses of compounds in complex mixtures is the dependence of the analyte's electrochemical behavior on other components of the sample matrix. Therefore, any variation in the matrix can affect the DPV response observed for that analyte. Because solutions of standard materials and samples will have different matrices, this effect can limit the reliability of DPV procedures based on the use of analyte concentration *vs* peak current calibrations.

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Square-wave voltammetry (SWV), which uses a more complex excitation signal than DPV, has been shown to be superior to DPV in many applications (8). The chief advantage of SWV is the higher effective scan rates that are possible with this technique. As a consequence, SWV analyses can be upwards of 100 times faster than those based on DPV. The length of time needed to perform a DPV analysis is also detrimental to the analyte's response. Because the analyte's concentration at the electrode's surface is depleted during electrolysis, relatively long experiments, those lasting longer than one minute, tend to suffer from signal degradation. Typically, the degradation in the response is compensated for by rotating the electrode, which sweeps analyte past the surface, thereby replenishing the concentration near the electrode. Rotating electrodes, however, can be difficult to use and can suffer from irreproducible behavior. Since an SWV analysis occurs in a short period of time (30 s or less), the concentration of the analyte at the electrode's surface is not appreciably depleted, resulting in SWV signals at a stationary electrode comparable to those obtained from DPV experiments with an electrode rotating at 30 rps (4).

The sensitivity of SWV is also enhanced by its ability to reject background currents, which are usually not compensated for in DPV. These currents arise from solution phenomena like homogeneous chemical reactions, convection, and background electrochemical reactions. The current sampling routine used in SWV is also less affected by irreversible electrochemical behavior than that used in DPV. Therefore, SWV represents an improvement in both speed and sensitivity compared with those methods based on DPV. SWV methods are also considerably faster than typical HPLC methods.

In this work, SWV has been applied to the analysis of tocopherols in vegetable oils. A variety of conditions for SWV analysis have been investigated, and a procedure that balances speed, sensitivity, resolution and ease of operation has been developed.

MATERIALS AND METHODS

Equipment. A Model 384B Polarographic Analyzer and a Model 303A Static Mercury Drop Electrode (EG&G Princeton Applied Research Corp., Princeton, NJ) constituted the electroanalytical instrumentation. The 384B was preprogrammed by the manufacturer with SWV software. The 303A was equipped with a 3-mm diameter glassy carbon working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. The frequency for all SWV analyses was 10 Hz. The scan increment was 1, 2, 5, or 10 mV and was determined by the scan rate desired. The voltammograms were recorded on a DMP-40 plotter (Houston Instruments, Austin, TX).

Reagents. All reagents were used as purchased. α-, γ-, δ-tocopherol were supplied by Eastman Fine Chemicals (Rochester, NY) and were of the following purities—α: 99%; γ: 95%; and δ: 93%. Reagent-grade toluene and concentrated H_2SO_4 were from J.T. Baker (Phillipsburg, NJ),

while EM Science (Gibbstown, NJ) supplied the anhydrous, denatured ethanol. The vegetable oils studied were all commercial products and included canola, corn, olive, peanut, safflower, sesame, soybean, and sunflower oils.

Preparation of solutions. A standard solution containing 2500 mg/L of each tocopherol in ethanol was prepared from the pure materials. The background electrolyte consisted of 0.1 M H_2SO_4 in 1:1 ethanol/toluene. The oil samples were prepared as follows: Between 3 and 5 g of oil were accurately weighed into a 50-mL volumetric flask. Toluene (25 mL) was pipetted into the flask to dissolve the sample. This solution was diluted to volume with 0.2 M H_2SO_4 in ethanol.

Procedure. 10 mL of solution was placed into an electrolysis cell and purged for 90 s with argon gas. The working electrode's potential was scanned from 0.40 to 1.00 V *vs* Ag/AgCl while its current was measured. Between analyses, the electrodes were rinsed thoroughly with acetone and blotted dry with a tissue. A voltammogram of the background electrolyte alone was subtracted from the voltammograms of both the oil sample solution and the oil sample solution plus 100 μL of the 2500 mg/L tocopherol standard solution. Overall baselines were drawn on both the sample and sample-plus-standard voltammograms. The maximum current of each peak in these voltammograms was measured, and the concentration of tocopherols in the oil was calculated by the equation given by Meites (9),

$$C_u = (i_1 v C_s)/[i_2 v + (i_2 - i_1)V] \quad [1]$$

where C_u is the concentration of tocopherol in the sample; C_s is the concentration of tocopherol in the standard; i_1 and i_2 are the peak currents for the tocopherol's oxidation in the sample and the sample-plus-standard solutions, respectively; v is the volume of standard solution added; and V is the volume of sample solution in the electrolytic cell.

RESULTS AND DISCUSSION

Determination of experimental conditions. A number of electrolyte systems containing ethanol, toluene, and H_2SO_4 have been reported for the oxidation of tocopherols (2,5–7) and these were investigated for their efficacy in this method. There was no difference in the electrochemical responses of tocopherols when the acid's concentration was changed from 0.1 to 0.2 M. There also was no difference in the responses of samples analyzed in both 2:1 and 1:1 ethanol/toluene. An electrolyte system consisting of 0.1 M H_2SO_4 in 1:1 ethanol/toluene was chosen because this solution is somewhat less polar than those containing higher concentrations of acid and ethanol and, therefore, it dissolved samples more readily, especially crude vegetable oils and solid oil products like shortenings.

The sample sizes used provided peak currents of sufficient magnitude to make their measurement easy; currents between 0.05 and 2.00 μA were the most effective. For oils with naturally low tocopherol levels, increasing the sample size (or increasing the g oil/mL solution ratio), when possible, proved helpful in obtaining larger oxidation currents. For the particular electrochemical cell arrangement used herein, a minimum of 5 mL of solution was required.

A scan rate of 20 mV/s was chosen as a compromise between speed and resolution. Figure 1 shows the effect that different scan rates have on the resolution of the tocopherol peaks. The degradation of resolution above 20 mV/s indicates that the irreversible nature of the tocopherols' oxidation limits the speed at which SWV can be performed effectively on these materials. However, 20 mV/s represents a 10-fold increase in the scan rate compared

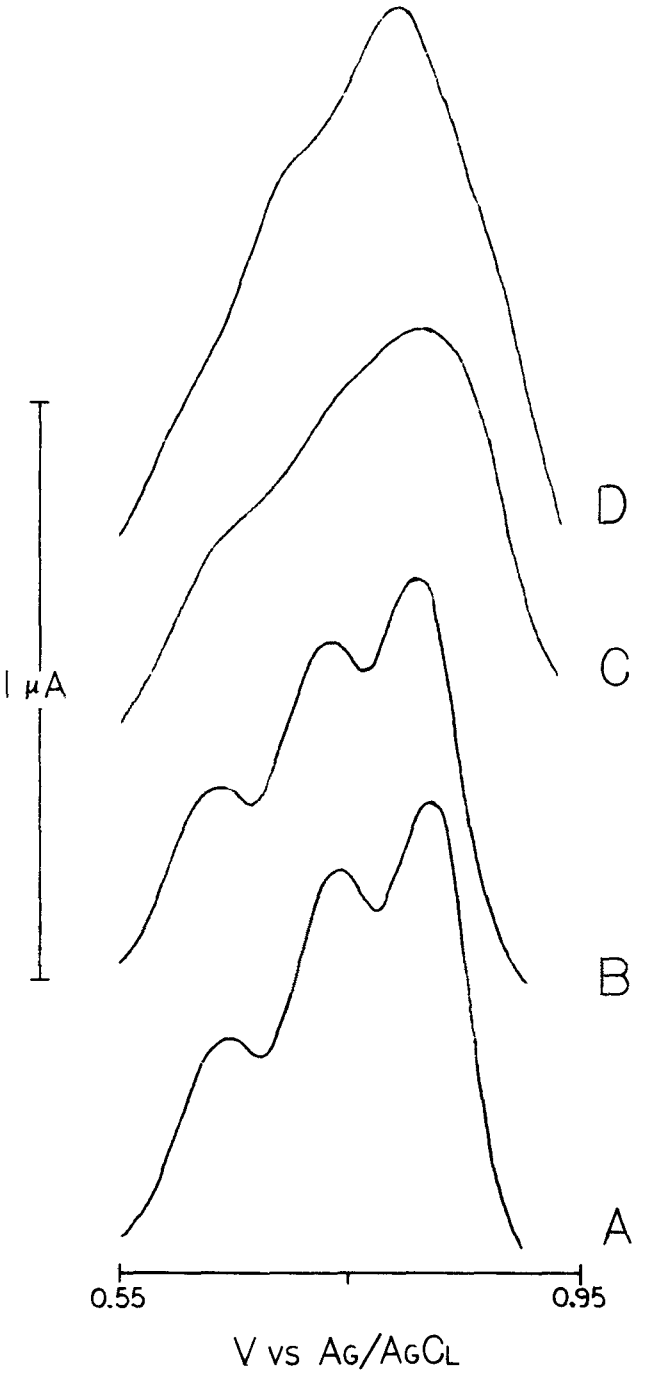

FIG. 1. SWV of tocopherol mixtures at different scan rates: (A) 10 mV/s; (B) 20 mV/s; (C) 50 mV/s; and (D) 100 mV/s. Solution contains 20 mg/L each of α-, γ-, and δ-tocopherol in 0.1 M H_2SO_4 in 1:1 ethanol/toluene.

to that normally used in DPV methods, thereby decreasing the overall time needed to perform an electroanalysis. Since typical DPV analyses have been shown to be six times faster than HPLC analyses, the time savings possible with SWV are even more advantageous.

Oxidation potentials of tocopherols. The oxidation potentials of tocopherols, under the experimental conditions used in this work, are given in Table 1. β-tocopherol was not included in this study since its oxidation potential is so close to that of γ-tocopherol that these compounds are, in effect, indistinguishable by voltammetry (2). Therefore, voltammetric methods quantitate both β- and γ-tocopherol as γ-tocopherol. It should be noted that the absolute values of the oxidation potentials are strongly dependent on the exact compositions of both the sample and the reference electrode solutions. However, the relative differences between the oxidation potentials of consecutive tocopherols remain relatively constant at 96 mV for the α-/γ-tocopherol pair and 77 mV for the γ-/δ-tocopherol pair. These relative differences can be used to identify quickly which tocopherols are present in a vegetable oil, even if the absolute values of the oxidation potentials are slightly different than previously determined.

Because the difference between any two adjacent tocopherol oxidation potentials is less than 200 mV, these peaks are not completely resolved in the voltammograms (Fig. 1). This is typical of the voltammetric responses of closely related compounds and, because oxidation potentials are related to the electronic properties of these compounds, it is difficult to significantly increase peak resolution by manipulating experimental conditions. Therefore, the accuracy and precision of SWV methods can be somewhat limited when compared to chromatographic methods such as HPLC (10), in which the parameters effecting peak separation can be manipulated more easily. However, as will be shown below, this lack of total resolution does not hinder either the rapid identification of the tocopherols present in a given oil or the measurement of their relative concentrations in different oils.

Under the experimental conditions used in this study, the oxidation potential of BHT (2,6-di-*t*-butyl-4-methylphenol), a common synthetic antioxidant, was 1.141 V *vs* Ag/AgCl. This is sufficiently more positive than the tocopherols' oxidation potentials so as not to interfere in the determination of tocopherol concentrations.

Oil analyses. Figure 2 shows the voltammograms for six of the oils studied. It is easy to distinguish the oils on the basis of their tocopherol contents—both by the identities and by the relative concentrations of the tocopherols present. The tocopherol distributions in these oils are given in Table 2. These values agree with those reported in the literature (1–3).

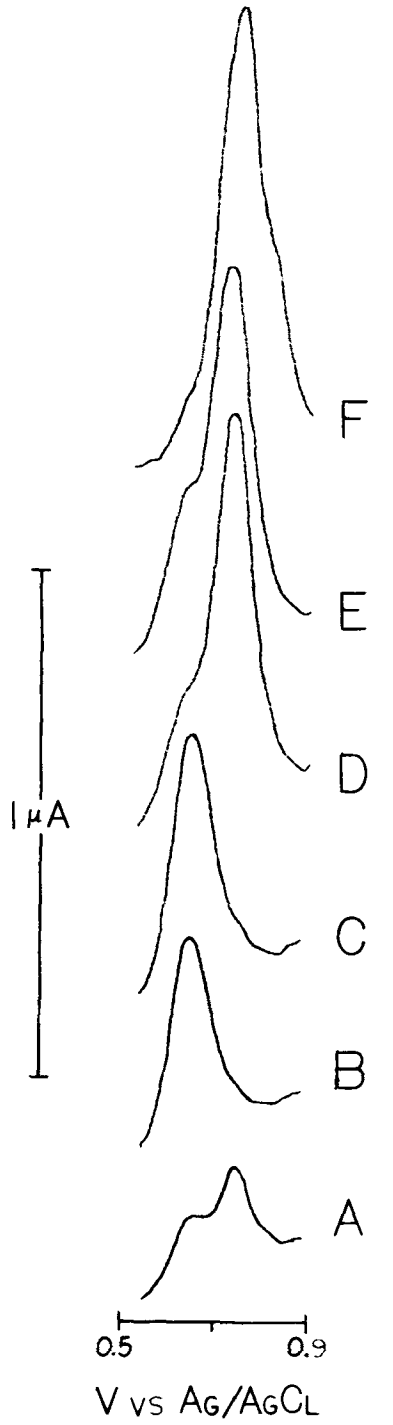

FIG. 2. SWV of vegetable oils in 0.1 M H_2SO_4 in 1:1 ethanol/toluene; scan rate: 20 mV/s. Oils: (A) peanut, 0.057 g/mL; (B) safflower, 0.046 g/mL; (C) sunflower, 0.048 g/mL; (D) corn, 0.061 g/mL; (E) canola, 0.052 g/mL; and (F) soybean, 0.054 g/mL.

TABLE 1

Oxidation Potentials of Tocopherols

Tocopherol	Potential (V *vs* Ag/AgCl)
α	0.610
γ	0.706
δ	0.783

The peak potentials in the voltammograms of olive and sesame oils, shown in Figure 3, are different from those for the other oils investigated. These peaks occur at 0.650 V and 0.770 V *vs* Ag/AgCl for olive oil and 0.776 V *vs* Ag/AgCl for sesame oil. These peaks are ascribed to the

TABLE 2

Tocopherol Content of Some Common Vegetable Oils

Oil	Tocopherol concentration (mg/L)			
	α	γ	δ	Total
Peanut	261.8	343.9	<20	650.7
Safflower	510.6	<20	<20	510.6
Sunflower	637.7	<20	<20	637.7
Canola	264.6	560.7	<20	825.3
Corn	142.0	496.7	<20	638.7
Soybean	127.6	728.7	221.7	1078.0

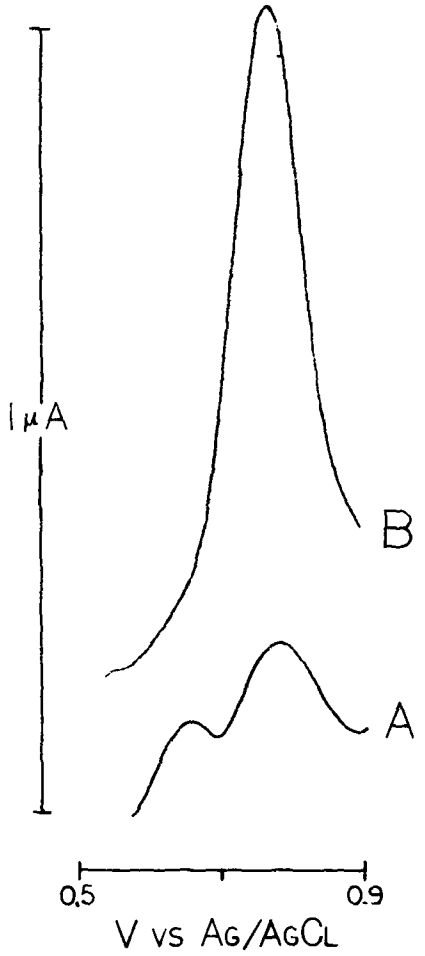

FIG. 3. SWV of tocotrienol-containing vegetable oils in 0.1 M H_2SO_4 in 1:1 ethanol/toluene; scan rate: 20 mV/s. Oils: (A) olive, 0.057 g/mL and (B) sesame, 0.043 g/mL.

oxidation of tocotrienols (2) and, therefore, were not quantitated in this work. However, the difference in oxidation potentials allows one to conclude quickly that these oils do not contain tocopherols as their primary antioxidant compounds, thus facilitating their rapid qualitative analysis.

The use of a standard addition method rather than one based on a concentration *vs* signal plot was not only faster, but also proved to be more accurate. A plot of α-tocopherol concentration *vs* peak current was used to analyze the content of this tocopherol in sunflower oil. The equation produced by linear regression of these data yielded a concentration of 312.2 mg/L α-tocopherol in oil, which is around 51% lower than that found by using the standard addition method and also significantly lower than values found by other workers from both DPV and HPLC (2,3). This is probably due to differences in the matrices of the oil samples and standard solutions, which causes the baselines of the oil solution voltammograms to be sloped differently than those of the standard solution voltammograms (Fig. 4). This difference in slopes leads to an underestimation of the tocopherol content of the oil.

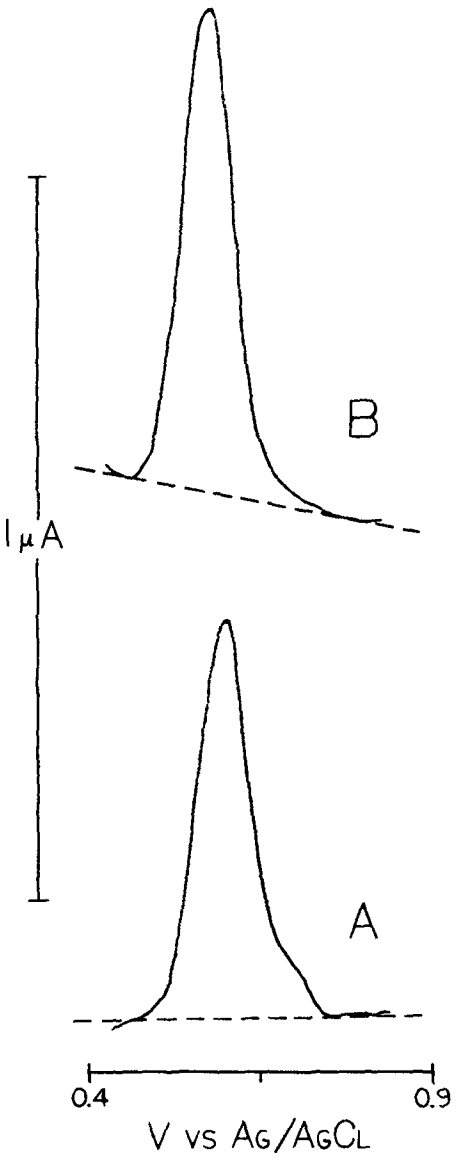

FIG. 4. Comparison of SWV baselines of oil and standard solutions. (A) Sunflower oil, 0.062 g/mL, containing 18.7 mg/L α-tocopherol (calculated by standard addition) and (B) 20 mg/L α-tocopherol standard. Conditions: 0.1 M H_2SO_4 in 1:1 ethanol/toluene; scan rate: 20 mV/s.

Precision, recovery, and limit of detection. A single analyst, using this method, analyzed a sample of sunflower oil twelve times over a period of three days. The average α-tocopherol concentration was found to be 637.7 ± 37.6 mg/L, which is a relative standard deviation of 5.9%. A portion of this oil was spiked with α-tocopherol and analyzed nine times to determine the recovery percentage of this method. This was found to be 98.6 ± 9.6%. The limit of detection for a single tocopherol was determined to be 0.6 mg/L in an oil sample solution. For the sample sizes used, this translates into approximately 10 mg/L tocopherol in the oil. For oils containing several tocopherols, particularly when one tocopherol is in far greater abundance than the others, the limits of detection for the less abundant compounds rise to 20 mg/L tocopherol in oil. The detection limits under these circumstances are clearly limited by the resolution of the voltammetric peaks, as discussed above, although identification of the tocopherols is still possible under these circumstances. Therefore, when it is desirous to quantitate <20 mg/L of low-level tocopherols in vegetable oil materials or when the measurement of β-tocopherol is necessary, HPLC is still the preferred method of analysis, even though it is significantly slower than SWV.

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