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Rapid Determination of Antioxidants with an Electrochemical Micro Flow-Through Detector

Part I: Determination of Tocopherols

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Schnellbestimmung von Antioxidanten mit einem elektrochemischen Durchflußdetektor

I. Bestimmung der Tocopherole

Zusammenfassung. Eine elektrochemische Methode zur Bestimmung von Tocopherolen in Fetten, Ölen und fetthaltigen Lebensmitteln wird vorgeschlagen. Die Besonderheit der vorgestellten Methode liegt darin, daß ein elektrochemischer Detektor benutzt wird, der es erlaubt, mit sehr kleinen Probemengen auszukommen. Überdies hat sich gezeigt, daß die untere Bestimmungsgrenze bei 10^{-8} mol/l Tocopherol liegt.

Summary. An electrochemical method for determining tocopherols in oils, fats, and fat-containing foods is presented. The use of an electrochemical detector brings the detection limit down to 10^{-8} mole/l tocopherol.

At present there is an urgent need for a reliable, rapid and sensitive method for the determination of antioxidants in foods and food products. The determination of phenolic antioxidants is very important for the oil-processing and oil-consuming industries. Many food processors and food legislators need an analytical method for antioxidant concentration evaluations. Vegetable oils and fats frequently contain considerable concentrations of natural antioxidants (e.g. vitamin E group). Determinations of the concentration of different antioxidants present can give valuable information of the keeping-quality of oils.

A large number of methods for determining tocopherols has been published. Most procedures require considerable time for sample preparation. They have been reviewed by Bunnell [1]. Nowadays probably the classical method most often used involves alkaline saponification, extraction of unsaponifiables, separation of tocopherols by thin-layer chromatography, and their subsequent determination by gas-liquid chromatographic or colorimetric methods. Recently several methods for the determination of tocopherols by high-pressure liquid chromatography (HPLC) have been proposed [2–4].

Parallel with the evolution of HPLC analysis, electrochemical methods have been developed. Tocopherols can be made available for reduction on the hanging mercury drop electrode by prior oxidation with Ce (IV) to tocophenylquinones [5–7]. Direct oxidation at different carbon electrodes were reported [8–12].

A further refinement and possibility for a rapid routine method led to this development using an electrochemical micro flow-through detector, as described by many authors, e.g. Gilgen and Rach [13].

Experimental Procedures

a) Reagents

Ethanol puriss Ph. Helv. was obtained from Fluka (Buchs SG, Switzerland), toluene and sulphuric acid ($d = 1.84$) p.a. from E. Merck (Darmstadt, Germany) and α- and β-tocopherol from Supelco (Bellfonte, PA, USA). A mixture of 50% α-, 30% γ-, 10% β- and 10% δ-tocopherol in 50% vegetable oil was obtained from Eastman Kodak (Rochester, N.Y., USA). The basal electrolyte was ethanol/toluene (2+1) containing 0.01 *M* sulfuric acid.

b) Equipment

The jet flow detector assembly (Fig. 1) was composed of a:

double-head (in series) peristaltic pump, H. J. Guldener (Zürich, Switzerland) Vario II, using Iso-Versinik/1.33 mm flexible tubing W. Meier (Lucerne, Switzerland);

high-pressure liquid chromatography syringe-loading loop injector Rheodyn 7120 with 10-µl fixed sample loop, Rheodyn (Berkeley, CA, USA);

micro flow-through detector EA 1096, Metrohm (Herisau, Switzerland);

polarographic unit Metrohm E 506 or VA-Detector E 611 with a Hewlett-Packard XYT plotter HP 7004 B (Palo Alto CA, USA);

0044-3026/80/0170/0413/$1.00

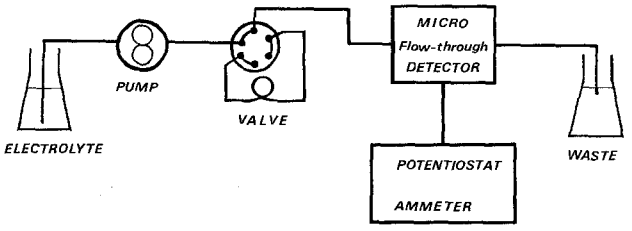

Fig. 1. Scheme for micro flow-through detector assembly

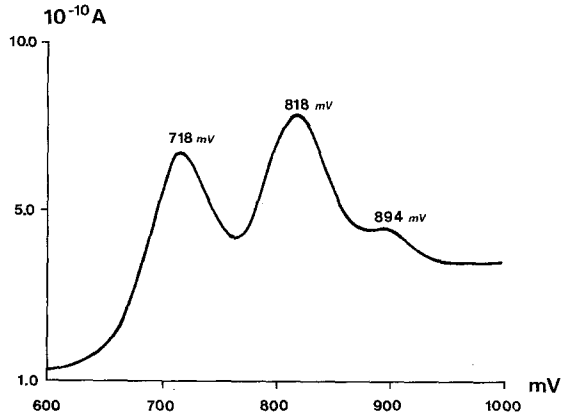

Fig. 2. DPP potential/current curve for mixture of tocopherols determined by the micro flow-through detector

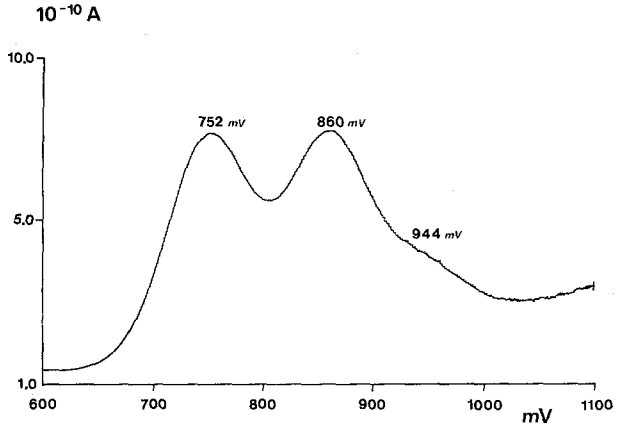

Fig. 3. DPP potential/current curve for mixture of tocopherols determined by batch measurement using a rotating disc glassy carbon working electrode

glassy carbon working and auxiliary electrodes (EA 286/1, Metrohm), the reference electrode was silver/silver chloride in ethanol saturated with lithium chloride (EA 442, Metrohm), using a G 3 sintered glass diaphragm having an open diameter of 3 mm;

the micro flow-through detector and injection valve were connected with $^1/_{16}''$ i.d. flexible teflon tubing;

before any analysis the electrolyte mixture was prepared and degassed with use of a water pump whilst vigorously stirred during the evacuation of the reagent flask (approx. 15 min).

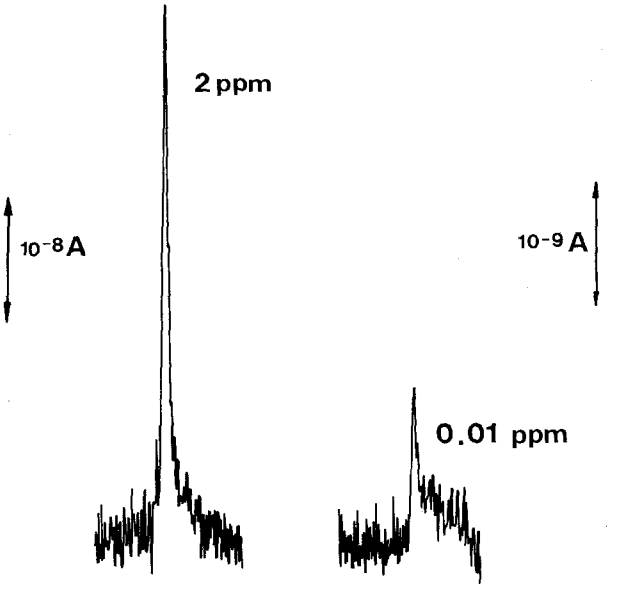

Fig. 4. Typical time–current curves for micro flow-through detector with 10-µl sample volume containing 4×10^{-11} and 0.6×10^{-11} mole of DL-α-tocopherol (concentration 2 and 0.01 ppm)

Procedure for Determination

The degassed electrolyte was drawn from the container to the peristaltic pump through the 6-port injector. The flow-through detector was only closed when all the trapped air had been pushed through the small sections in the detector out of the tubing to the waste container. The steady flow was maintained during approx. one hour, during which time 6–10 potential sweeps (0–1.2 V) were applied. After this procedure an unlimited number of determinations could be done with an analysis interval of approximately two minutes.

Results and Discussion

The diagram (Fig. 1) illustrates the experimental set-up of the micro flow-through detector.

In order to establish precise peak potentials, the solvent tank was filled with a mixture of α-, β-, γ- and δ-tocopherols dissolved in the standard electrolyte. After carefully degassing, the solvent flow was adjusted to 1 ml/min. A potential sweep was applied from 600 to 1000 mV *vs* saturated silver chloride in ethanol. Figure 2 shows the polarogram so obtained (pulse amplitude: +5 mV, pulse time: 60 ms, measuring time: last 20 ms of pulse, scan rate: 6.6 mV/*s*). The peak potentials for the tocopherols are: α-tocopherol 718 mV, β/γ-tocopherol 818 mV and δ-tocopherol 894 mV. At this tocopherol concentration ratio the peak potentials could be exactly determined. For comparison, Figure 3 reports the potential current curves obtained by batch measurement using a glassy carbon rotating disc electrode as working electrode and a stationary platinum wire auxiliary electrode (the reference electrode remains silver/silver chloride in ethanol saturated with lithium chloride). There are slight differences in the

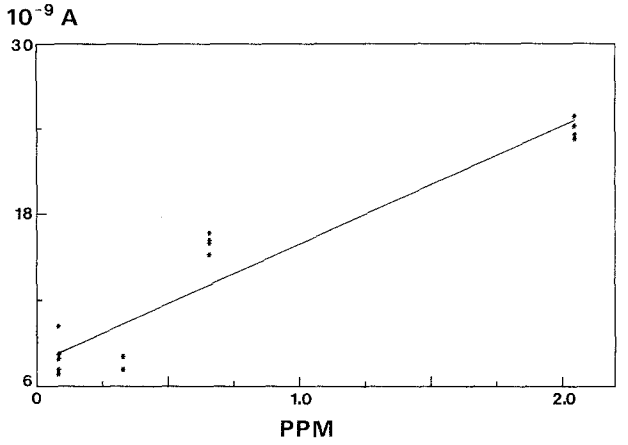

Fig. 5. Standard graph for DL-α-tocopherol in micro flow-through detector using peak hights at medium to low concentration range (detection limit 0.01 ppm)

peak potentials compared with the above reported potential current curves. They are due to many factors which are not discussed here.

Quantitative determinations with the micro flow-through detector were performed at the peak potential predetermined by injection of a standard sample solution by the injector. Each measurement of the sample was preceded and followed by a measurement of the standard. Each measurement was repeated several times. Figure 4 represents typical measurements for tocopherol solutions with 2 or 0.01 ppm DL-α-tocopherol (corresponding to 4.6×10^{-6} mole/l or 2.3×10^{-8} mole/l). The figure represents typical results obtained from this type of equipment. The dection limit can be set to 0.01 ppm (2×10^{-8} mole/l) of any tocopherols. Typical results are 2×10^{-7} mole/l.

Figure 5 represents the results of 16 separate measurements on the medium-to-low scale range for the determination of the tocopherols (coefficient of correlation $r = 0.893$). The results of the determination of tocopherols in commercially available oils and fats are included in Table 1. They were dissolved in the electrolyte mixture, not containing sulfuric acid, and were subsequently injected into the micro flow-through detector. The values are average of at least 5 determinations.

The determination of the tocopherols by the classical methods, e.g. by HPLC [1] or electrochemical oxidation was made by Podlaka et al. [12]. Both methods gave significant results. The advantage of the electrochemical micro flow-through detector is its high sensitivity, short analysis time and extremely small sample quantities.

1 The most critical part of these determinations is the stability of the reference potential. The reference potential can be stabilized by using the silver/silver chloride electrode and a G3 sintered glass diaphragm

Table 1. Concentration of tocopherols in representative samples of fat and fat-containing foods (average values of a least 5 measurements)

	Tocopherol concentration in ppm	Literature values [14] in ppm
Fish oil	59 α	–
Cacao butter	415 β/γ	–
Grape seed oil	303 β/γ	–
Maize oil	270 α	20 –300 α
Olive oil	140 α	8 –240 α
Carrots[a]	70 α	1 – 5.7 α
	101 β/γ	1 – 2 β
Milk powder[a]	7 α	3 – 7.3 α
Whole wheat flour[a]	9 α	3 – 11 α
	30 β/γ	5.7– 24 β
Potato flakes[a]	3 α	1.2– 3 α
	3 β/γ	

[a] The lipids were extracted according the A.O.C.S. official method Ba 3-38 using methanol/chloroform (1:2) as solvent

Interfering Substances

Peak potentials of some other phenolic antioxidants that are found in oils and fats normally do not interfere with the electrochemical detection. From Fig. 1 it can easily be seen that the most serious interference is from the tocopherols present. In mixtures containing ratios of the four tocopherols above 1:10, the low concentration tocopherol can only be determined with limited precision.

Reproducibility and Detection Limits

The reproducibility of this method is ±5% at the 0.1–600-ppm level for α, β/γ and δ-tocopherols. The detection limit is 0.01 ppm for all three tocopherols (the peak being at least double the size of the baseline noise). Reproducibility and detection limits are determined for the tocopherols in lipids or the lipids extracted from complex foods.

Conclusion

The electrochemical detector presented here is a valuable analytical tool for the evaluation of tocopherol concentration in oils, fats and foods. In comparison with the conventional electrochemical methods and other detection systems, the detection limit is reduced to 0.01 ppm in fat (2×10^{-8} mole/l), which represents a considerable gain in sensitivity.

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Note added in proof

A great number of tocopherol determinations was performed since the manuscript was submitted for publication. In one type of fat/foods only interferences with other oxidizable substances caused problems for the tocopherol determination. Tocopherol concentration in coffee oil cannot be determined directly by the proposed method as not yet determined substances are oxidized in the tocopherol oxidation potential region.