

# Possibilities of Simultaneous Voltammetric Detection of Tocopherols in Non-Aqueous Media

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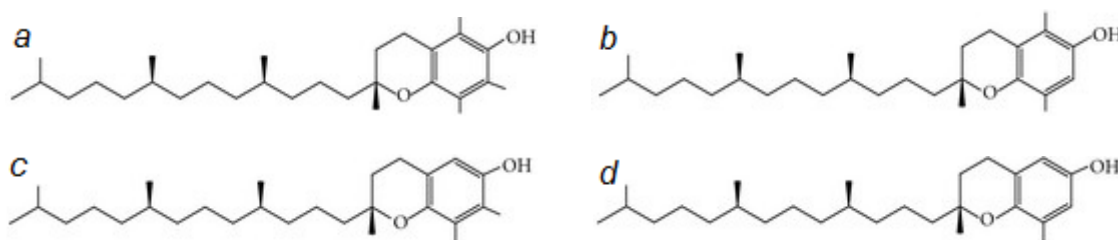
## Abstract

An electrochemical study on possibilities of simultaneous detection of tocopherols based on their anodic oxidation at various electrode materials in non-aqueous supporting electrolytes was performed. Several working conditions, such as selection of working electrode, supporting electrolyte, effect of surfactant, water content and parameters of square-wave voltammetry, were optimized. An overlapping of corresponding peaks of alpha-, gamma- and delta- form. The separation of oxidation signals was improved at optimum working conditions, but the signals were not completely resolved. However, it was found that this phenomenon did not cause significant changes in peak currents for various concentration ratios of tocopherols, which could be possibly utilized in analytical applications.

**Key words:** Tocopherols; Glassy carbon electrode; Square wave voltammetry; Food analysis.

## Introduction

Tocopherols (vitamin E; VE) are lipophilic phenolic antioxidants, naturally occurring in vegetable oils, which are widely used in pharmaceutical and cosmetic industries<sup>1,2</sup>. These organic compounds are known as vitamins that organism needs in small quantities for the proper functioning of its metabolism. Hence, it is necessary to control their intake for dietary purposes and to determine their content in foodstuffs. VE protect human body against cancer, cataract and cardiovascular disease. Avitaminosis may cause circulatory disorders and affects the metabolism pathway in muscle<sup>2-4</sup>. From chemical point of view, tocopherols are classified according to number of methyl groups on chromanol ring containing an isoprenoid side chain without double bonds (see Fig. 1) which is responsible for their lipophilic character<sup>1,5</sup>.



**Fig.1.** Chemical structure of  $\alpha$ -tocopherol (a),  $\beta$ -tocopherol (b),  $\gamma$ -tocopherol (c) and  $\delta$ -tocopherol (d).

Their simultaneous voltammetric determination have been reported only in few papers. In all cases, an overlapping of the corresponding anodic peaks was observed<sup>5,7-9</sup>. In 1973, McBride and Evans used linear sweep voltammetry for the determination of the individual tocopherols in vegetable oils dissolved in the mixture of ethanol and benzene<sup>7</sup>. Clough utilized square wave voltammetry only for their simultaneous electrochemical detection in 1992. Unfortunately, voltammetric method for simultaneous quantification of all forms of

tocopherols was not proposed. In 2001, Coatanea used differential pulse voltammetry of tocopherols mixtures at platinum microelectrode. However, only one broad oxidation peak was obtained due to presence of sample matrix (edible oils) <sup>9</sup>. Diaz continued in the previously mentioned works and he used a chemometric method to resolve overlapping anodic peaks of tocopherols for the first time <sup>5</sup>. It is necessary to mention that effect of used organic solvent, content of water or presence of surfactant on overlapping signals have not been studied in detail yet. Such factors were studied thoroughly and obtained results are presented in this contribution.

## Experimental

Surfactants (Triton, sodium dodecyl sulfate, cetylpyridinium chloride, cetyltrimethylammonium bromide), organic solvents and analytical standards of (+)- $\alpha$ -tocopherol, (+)- $\gamma$ -tocopherol and (+)- $\delta$ -tocopherol were purchased from Sigma-Aldrich (Prague, Czech Republic). Tested supporting electrolytes were different organic solvents (99.9% acetonitrile, 99.8% acetone, 96% ethanol, 99.8% dimethylformamide and 99.9% propylene carbonate) containing always 0.1 mol L<sup>-1</sup> LiClO<sub>4</sub>. Ultrapure water with resistivity >18.3 M $\Omega$  cm was obtained by Milli-Q system (Millipore, USA) and was used for preparation of surfactant solutions.

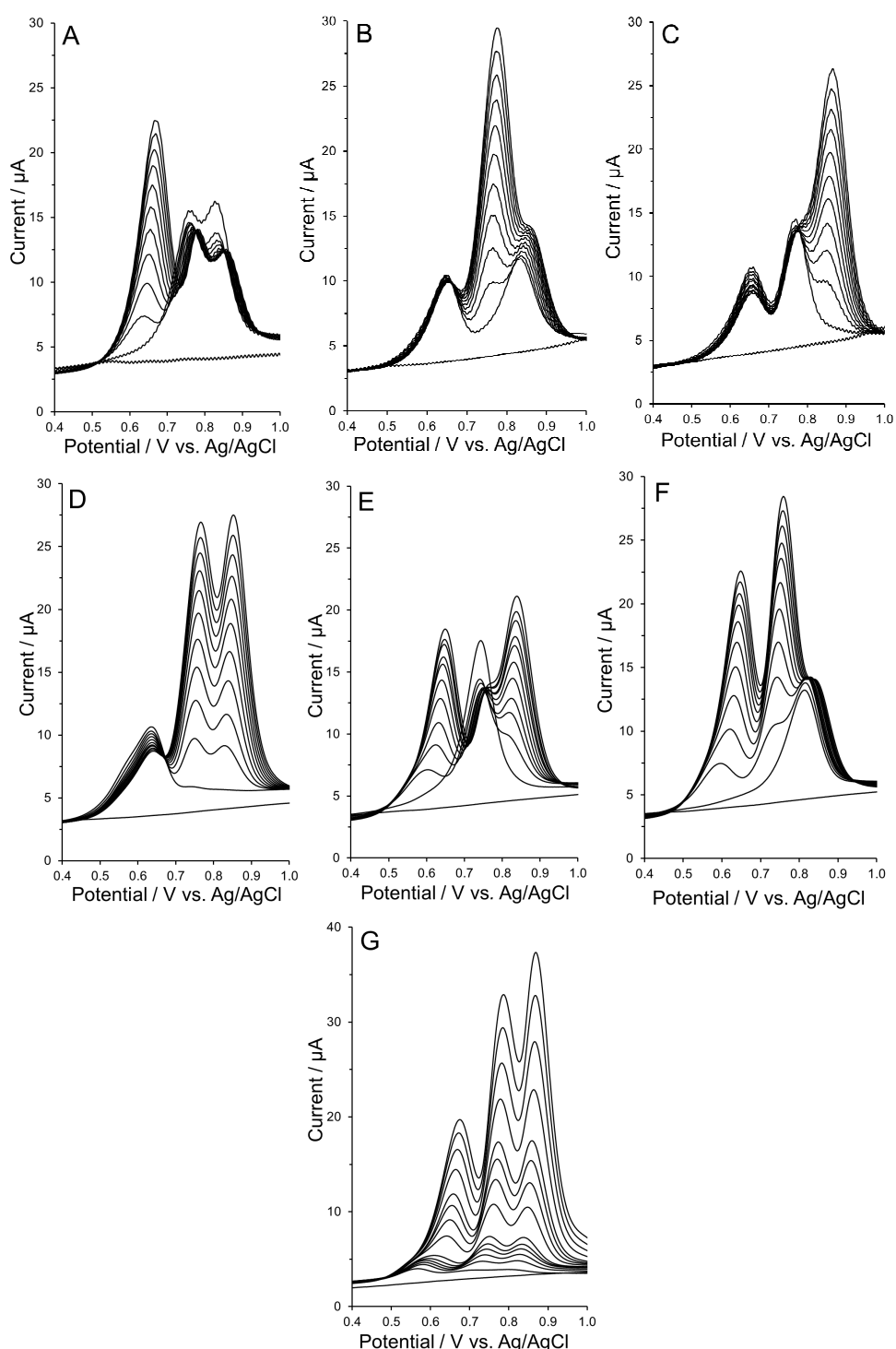
All electrochemical measurements were carried out at glassy carbon electrode (GCE) with 3 mm diameter from Metrohm (Prague, Czech Republic), together with the calomel electrode Hg|Hg<sub>2</sub>Cl<sub>2</sub> with 0.1 M LiClO<sub>4</sub> as salt bridge (reference), and Pt-wire (auxiliary electrode). Above mentioned electrochemical setup was connected to a potentiostat PGSTAT101 from Metrohm (Prague, Czech Republic) operating with Nova 1.11 software. Surface of working electrode was renewed on a polishing pad with water suspension of Al<sub>2</sub>O<sub>3</sub> powder (particle size 1.0  $\mu$ m) for 30 s, washed with ultrapure water and dried using pulp paper.

## Results and discussion

At the beginning, it must be mentioned that  $\beta$ -tocopherol was not investigated in this study because this form of vitamin E is not present in foodstuffs from plant origin which are target samples. During optimization studies, it was necessary to select optimum supporting electrolyte and working electrode providing the highest current response for oxidation of tocopherols and sufficient separation of anodic peaks. Dimethylformamide (DMF), propylene carbonate (PC), acetone (AC), ethanol (EtOH) and acetonitrile (ACN) were tested as optimum organic solvent. No current response of tocopherols was recorded when DMF and PC were used. Better results were obtained using AC and EtOH. Three overlapping peaks with low current responses were observed in case of EtOH. Glassy carbon electrode (GCE), Pt disk electrode (PtE), pyrolytic graphite electrode (PGE), boron-doped diamond electrode (BDDE) and GCE modified with thin layer of multi-wall carbon nanotubes (GCE/MWCNTs) were investigated in simultaneous voltammetric detection of tocopherols. The optimum peak separation of  $\alpha$ -tocopherol ( $\alpha$ -TOH),  $\gamma$ -tocopherol ( $\gamma$ -TOH) and  $\delta$ -tocopherol ( $\delta$ -TOH) was obtained at GCE in pure ACN containing 0.1 mol L<sup>-1</sup> LiClO<sub>4</sub>. Presence of surfactants did not improve significantly the peak separation. Peaks were only shifted to more negative potentials in the presence of water (90% ACN).

As a second step, optimum working parameters of square wave voltammetry (SWV) were found varying the potential step ( $E_{\text{step}}$ ), frequency ( $f$ ) and amplitude, which influence resulting voltammetric response. The best peak separation of tocopherols was achieved at  $E_{\text{step}} = 1 \text{ mV s}^{-1}$ ,  $f = 25 \text{ Hz}$  and potential amplitude 25 mV. Peak potentials in these conditions were +0.641 V for  $\alpha$ -TOH, +0.767 V for  $\gamma$ -TOH and +0.852 V for  $\delta$ -TOH. These conditions were used to measure calibration curves for the mixture of tocopherols. Figs. 2A, 2B and 2C show calibration voltammograms within concentration range 20-200  $\mu\text{mol L}^{-1}$  always of one

selected form when two others had a constant concentration of  $50 \mu\text{mol L}^{-1}$ . Figs. 2D, 2E and 2F were recorded when concentration of two forms was simultaneously increased. The last Fig. 2G show voltammograms of each form in concentration range  $3\text{-}300 \mu\text{mol L}^{-1}$ .



**Fig 2.** Voltammograms of  $20\text{-}200 \mu\text{mol L}^{-1}$  of one tocopherol when two others had a constant concentration of  $50 \mu\text{mol L}^{-1}$  (A, B and C). Voltammograms of  $20\text{-}200 \mu\text{mol L}^{-1}$  of two tocopherols when one had a constant concentration of  $50 \mu\text{mol L}^{-1}$  (D, E and F). Simultaneous detection of  $3\text{-}300 \mu\text{mol L}^{-1}$  of all tocopherol forms. SWV at GCE in 99.9% ACN containing  $0.1 \text{ mol L}^{-1} \text{ LiClO}_4$ ,  $E_{\text{step}} = 1 \text{ mV}$ ,  $E_{\text{ampl}} = 25 \text{ mV}$  and  $f = 25 \text{ Hz}$ .

Finally, it can be concluded that signals between  $\gamma$ -tocopherol and  $\delta$ -tocopherol were so close when one of these forms were in higher concentration than other, whereas, the separation of  $\alpha$ -tocopherol and  $\delta$ -tocopherol was larger, as is observed in voltammograms. However, it was found that this phenomenon did not cause significant changes of peak currents for defined concentration range (20-80  $\mu\text{mol L}^{-1}$ ) which could be utilized for analytical applications.

Recovery of proposed protocol was investigated for chosen concentrations of tocopherols, namely 40  $\mu\text{mol L}^{-1}$   $\alpha$ -TOH, 30  $\mu\text{mol L}^{-1}$   $\gamma$ -TOH and 50  $\mu\text{mol L}^{-1}$   $\delta$ -TOH. Results showed that if the surface of GCE is polished after each measurement, recoveries of 116.3, 93.0 and 93.7 %, respectively, could be achieved. In contrast, recoveries of 127, 148.2 and 136.5 %, respectively, were obtained at unpolished GCE.

### Conclusion

Various working parameters were investigated to develop the voltammetric method suitable for simultaneous detection of  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and  $\delta$ -tocopherol and their possible quantification. Square wave voltammetry (potential step 1  $\text{mV s}^{-1}$ , frequency 25 Hz and potential amplitude 25 mV) of these forms of vitamin E at GCE in pure ACN containing 0.1  $\text{mol L}^{-1}$  lithium perchlorate was chosen as optimum electroanalytical method. Unfortunately, evident overlapping of anodic peaks has not yet been fully resolved. However, using of appropriate data processing, such as deconvolution of signals or application of elimination voltammetry, could help to further improve the resolution of signals and to enable precise analytical quantification of different tocopherols. Such investigations are currently in the progress.

### Acknowledgments

Financial support from the Faculty of Chemical Technology, University of Pardubice (project No. SGS-2019-003) is gratefully acknowledged.

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