

# Simultaneous Adsorptive Stripping Square-Wave Voltammetric Determination of Vitamin E and Vitamin K in Food Supplements

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## Abstract

The new voltammetric method has been developed for the simultaneous determination of  $\alpha$ -tocopherol and phylloquinone. Adsorptive stripping square wave voltammetry is based on the accumulation of these biologically active compounds onto the freshly polished surface of solid glassy carbon electrode from an aqueous-organic mixture (50 % acetonitrile) and a subsequent voltammetric detection of both vitamins in 0.01 mol L<sup>-1</sup> HNO<sub>3</sub> containing 0.1 mol L<sup>-1</sup> KCl of pH 2.08. Obtained results have shown high sensitivity and short linear range due to self-interaction of vitamins during of adsorption onto surface of the electrode. This electroanalytical method shows that is simple and low-cost in quality control of food supplements.

**Keywords:** vitamin E; vitamin K; adsorptive stripping voltammetry; glassy carbon electrode; food supplements

## Introduction

Vitamin E and K are fat-soluble vitamins<sup>1-4</sup>, known as non-polar organic compounds<sup>5</sup>. Vitamin E as one of the most popular food ingredients<sup>6</sup>, part of vegetable oil and fats<sup>7,8</sup>, comprises various tocopherols and tocotrienols isomers<sup>3-4,9-10</sup>. On the other side, vitamin K mostly found in green plants and formed by bacteria<sup>11</sup>, it is widely used in the diets for its anti-hemorrhagic properties<sup>12</sup>, made up of a group of three organic compounds: phylloquinone (VK1), menaquinone (VK2) and menadione (VK3)<sup>13</sup>. Vitamin E is an antioxidant in the immune system, especially important for the protection of cytoplasmic membranes from peroxidation by the reduction of fatty acids<sup>3,4,8</sup> and damage of oxidative DNA<sup>14</sup>, where its deficiency may cause fertility disorder<sup>15,16</sup>, circulatory disorder<sup>1</sup>, fast-aging by free radicals, Alzheimer<sup>4,6,7</sup>, kidney disease<sup>12</sup>, cancer, tumor, arthritis<sup>4</sup>; while vitamin K is an oxidizing agent that essential in the carboxylation of glutamic acid that is known as the precursor of blood-clotting factors<sup>11,17,18</sup>, where the deficiency affects deactivation of prothrombin, hemorrhage<sup>11</sup>, prosthetic valve failure<sup>19</sup>, the disorder in bone formation<sup>17</sup>.

The studies show the highest biologically active form are  $\alpha$ -tocopherol (one of the eight isoforms of vitamin E) and phylloquinone (vitamin K1)<sup>3,20</sup>, thus they were selected to provide information about the simultaneous determination by using adsorptive stripping voltammetry (AdSV). So, we propose a simple, fast and inexpensive method of simultaneous detection of

tocopherols and vitamin K that is applicable to quality control analysis in pharmaceutical products by application of square wave voltammetry (SWV) at a solid glassy carbon electrode (GCE).

## Experimental

$\alpha$ -Tocopherol and phylloquinone, acetonitrile (ACN) of HPLC purity, were purchased from Sigma-Aldrich, while hexane used for cleaning surface of the electrode, 65 % nitric acid and KCl for preparation of detection aqueous medium from Lach-Ner (Neratovice, Czech Republic). Ultrapure water with resistivity 18.3 M $\Omega$  cm was obtained by Milli-Q system (Darmstadt, Germany) and used for the preparation of all solution.

### *Pretreatment of glassy carbon electrode*

The solid glassy carbon electrode (GCE) (type 6.1204.300, Metrohm, Herrisau, Switzerland) with 3 mm diameter, was polished for 30 sec with a suspension of Al<sub>2</sub>O<sub>3</sub> powder (0.3  $\mu$ m) before each measurement, then immersed in 95 % hexane in an ultrasonic bath for 5 min. All this procedure was necessary since it was known that targeted analytes remain in the surface of the electrode, because of their abilities to be adsorbed<sup>1,2</sup>. After this treatment, the electrode was dried and ready for the physical binding of vitamin E and vitamin K in the surface of the glassy carbon electrode, as a pre-concentration stage and then to perform electrochemistry. Before each electrochemical detection, baseline measurement was checked after all conditions of pretreatment were done.

### *Instrumentation*

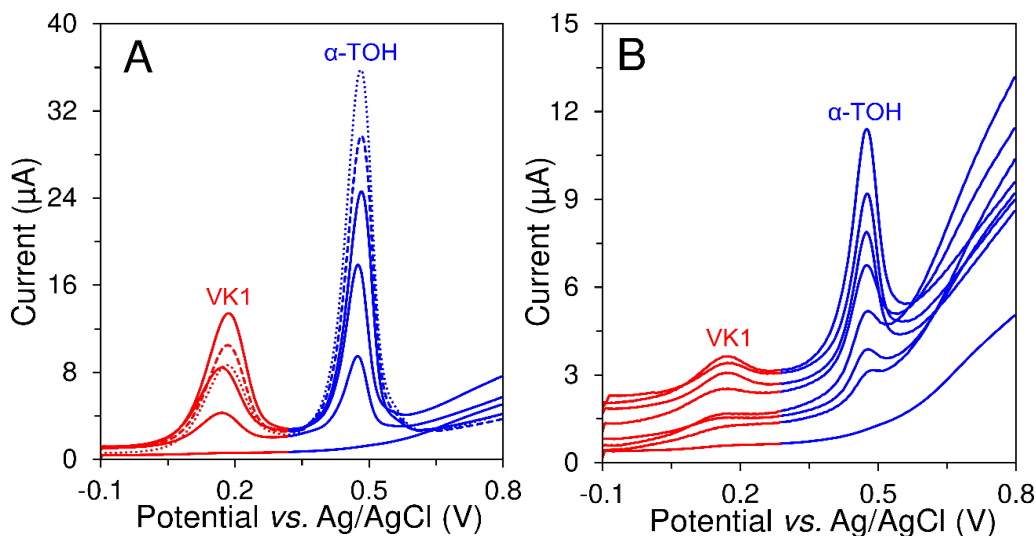
Measurements carried in a glass 10 mL at 25 °C, were conducted in potentiostat/galvanostat (Autolab/PGSTAT101) from Metrohm (Herrisau, Switzerland) controlled by Nova 1.11 as a software, at a system of three-electrodes consisting GCE (working), Pt wire (auxiliary) and Ag/AgCl with 3.0 M KCl salt bridge (reference).

## Results and Discussion

Pre-concentration of analytes was placed onto the surface of GCE at open-circuit, by immersing electrode into a continuously stirred (400 rpm) 10 mL of 50% ACN for 300 s. As the analytical technique, square wave voltammetry was employed to determine the optimal conditions where anodic mode with following parameters shows the most satisfying results: ( $E_{\text{begin}}$ ) -0.1V, ( $E_{\text{end}}$ ) +0.8V, ( $E_{\text{deposition}}$ ) -0.1V, ( $t_{\text{deposition}}$ ) the 60 sec, ( $E_{\text{step}}$ ) 5 mV, frequency (f) 80 Hz and amplitude ( $E_{\text{ampl}}$ ) 30 mV and all the datas were recorded in supporting electrolyte containing 0.01M HNO<sub>3</sub> and 0.1M KCl, pH 2.08. Characteristic oxidation peaks were provided for  $\alpha$ -tocopherol (-0.45 V) and phylloquinone (-0.15 V) after they were accumulated onto the surface of GCE and the best indicator for that is current, where the latter was increased with the addition of the targeted analytes.

### Analytical performance of developed voltammetric method

VK1 and  $\alpha$ -TOH provide almost the same peak current responses ( $I_p$ ) for equal concentration ( $c$ ) even if they are determined separately. However, when they were determined simultaneously, they showed different behavior, as it is shown in Figure 1. It seems that  $\alpha$ -TOH provided significantly higher peak current response than VK1. This fact suggested that the TOH is adsorbed preferably onto the surface of GCE from 50% ACN.



**Figure 1.** SWAdSV voltammograms of calibration curves of VK1 and  $\alpha$ -TOH at optimized working conditions: (A) 0 (blank), 2, 4, 6, 8 (dashed), and 10 (dotted line); (B) 0 (blank), 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0  $\mu\text{mol L}^{-1}$  VK1 and  $\alpha$ -TOH.

### Conclusions

The study shows that the simultaneous determination of vitamin E and K in food supplements is possible. Through this voltammetric method is able only determination of targeted vitamins sums, expressed as the concentration equivalents of the most biologically active forms ( $\alpha$ -tocopherol and phylloquinone), because of similar electrochemical properties of their individual forms. Even so, short linear calibration ranges were achieved due to the limited size of the working electrode surface.

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