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Analytical Methods

Electrochemical assay of the antioxidant ascorbyl palmitate in mixed medium

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ABSTRACT

Electrooxidation of ascorbyl palmitate (AP) over gold screen-printed electrode (AuSPE) and gold nanoparticles modified graphite (AuNPs/gr) was examined in mixed water-alcohol medium. Voltammetric and amperometric studies showed that: (i) AP oxidation on the AuSPE proceeds at higher potential than on AuNPs/gr; (ii) the current density on AuNPs/gr was 2.4 times higher than on AuSPE; (iii) the linear dynamic range for AuNPs/gr doubled that for AuSPE. At the optimal for AuNPs/gr operating potential (250 mV) the following operational parameters were determined: sensitivity 1.627 ± 0.138 μA mM^{-1} mm^{-2}; linearity up to 500 μM; LOD = 5.8 μM. Quantification of the AP content in a real sample – stabilised flaxseed oil, was performed.

1. Introduction

Edible vegetable oils are very susceptible to oxidation – a process which is widely known as oil spoiling. A common routine to delay the rancidification process is to add antioxidants to them (also known as oils' stabilisers), such as the derivatives of ascorbic acid. Ascorbic acid is widespread in nature but sparingly associated with fats of oils because of its hydrophilic nature (Niki, 1987) that restricts its solubility in vegetable oils. That is why the most frequently used synthetic derivative of ascorbic acid is ascorbyl palmitate, an ester with lipophilic properties (Galesso, Gatta, & Galiano, 1993) which is practically water-insoluble (Benedini et al., 2011). Ascorbic palmitate (AP) – a white powder with a soapy taste and citrus-like odour, rather soluble in ethanol, which makes it miscible with lipid-containing foods because of its relatively good hydrophobicity (Coppen, 1989), is commonly used for protection of edible oils (e.g. flaxseed oil) as the best and the most appropriate antioxidant against lipid peroxidation. AP prevents oxidative rancidity by quenching singlet oxygen, thus turning into an oxidised form, which has no antioxidant activity, according to the equation (Shahidi & Wanasundara, 2011):

The most widely used techniques for assaying the total amount of ascorbyl palmitate are based on chromatography. Both HPLC (Austria, Semenzato, & Bettero, 1997; Sottofattori, Anzaldi, Balbi, & Tonello, 1998; Vaupotic & Krbavcic, 2000) and UHPLC (Pedjie, 2010) were successfully applied to determine its overall content in vegetable oils, cosmetic formulations or pharmaceutical products. These methods, however, do not give information about the oxidative state of the analyte, i.e. what is the concentration of its reduced form that is still active as an antioxidant. Another widely used method for quantitative analysis of AP, and specifically of its remaining antioxidant activity, is iodometric titration (Vaupotic & Krbavcic, 2000) although it often gives overestimated analyte levels due to the fact that iodine usually reacts with peroxides, phenols and other interfering compounds normally present in the real samples.

In this context, the electrochemical methods for the quantitative assay of the AP remaining antioxidant activity appear as the most reliable ones (Buratti, Cosio, Benedetti, & Mannino, 2001; Chang & Chang, 2005) since the choice of the appropriate working conditions (electrode material, electrolyte composition and working potential) allows for practically interference-free determination of the analyte. Despite the above advantage of these methods, the number of publications in the current literature reporting such a development is limited to the above mentioned two works and no interest in their further upgrade was established.

The present study deals with the characterisation of the catalytic activity of Au SPE and gold nanoparticles modified graphite

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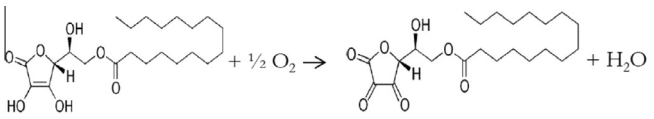

Scheme 1. Oxidation of ascorbyl palmitate by molecular oxygen in acidic medium.

(Au NPs/gr) at the electrooxidation of AP in water–ethanol medium. A vast increase of the AP oxidation rate on Au NPs/gr as compared to other electrode types and especially the glassy carbon proposed earlier by Buratti et al. (2001) for electrochemically sensing AP, will be demonstrated. The analytical performance of the Au NPs/gr electrode will be characterised over the potential range from 150 to 350 mV. Finally, the applicability of the electrochemical sensor under study will be illustrated with the assay of the AP content in real samples (stabilised with AP flaxseed oil).

The Au NPs/gr electrode material was originally developed by our group aiming at biosensor applications (Dimcheva, Horozova, & Dodevska, 2011; Dimcheva, Horozova, Ivanov, & Godjevargova, 2013), since the Au NPs can be easily functionalised with proteins or enzymes through chemisorption of sulphur-containing moieties (Dimcheva, Dodevska, & Horozova, 2013; Dimcheva, Horozova, et al., 2013; Dimcheva et al., 2011; Dodevska, Horozova, & Dimcheva, 2013). The analytical studies were inspired by our recent research experience in the development of amperometric enzymatic biosensors for L-ascorbic acid (Dimcheva, Dodevska, et al., 2013; Dodevska et al., 2013).

2. Experimental

2.1. Materials

Ascorbyl palmitate (AP, formula weight 414.5 g mol^{-1}; F. Hoffman-*La Roche* and Co. Ltd., Basel, Switzerland), Na_2HPO_4 and NaH_2PO_4 (Sigma–Aldrich); $HAuCl_4 \cdot H_2O$ (Fisher), used as 50 mM solution in 0.1 M HCl; anhydrous ethanol (Valerus, Bulgaria); anhydrous methanol (Merk); tetraethyl ammonium bromide, TEAB (Acros) were of analytical grade and used as received.

The working electrodes were: (i) screen printed gold electrodes (Au SPE, d = 1.0 mm, BVT, Czech Republic, visible surface area of *ca.* 0.79 mm^2); (ii) rods of spectroscopic graphite, type RWO (Ringsdorf Werke, Bohn, Germany) with a diameter d = 3.0 mm, visible surface area of 7.07 mm^2); or (iii) glassy carbon electrode (d = 3.0 mm, Sigradur, HTW, Germany, visible surface area of 7.07 mm^2).

Buffer solutions (0.1 M) were made of sodium phosphates (monobasic and dibasic) dissolved in double distilled water with pH 5.6 or 7.0. Double-distilled water was used in all water and buffer solutions.

The concentration of L-ascorbyl palmitate was determined in a real sample: stabilised flaxseed oil containing 0.05 g L-ascorbyl palmitate per 100 g oil.

2.2. Apparatus

All electrochemical experiments were performed in a conventional three-electrode cell (working volume 25 ml). A Ag/AgCl (3 M KCl) was used as a reference electrode, working electrode (Au SPE, graphite, glassy carbon or Au-Pd), and a platinum wire as an auxiliary electrode, connected to a computer-controlled electrochemical workstation PalmSens (Palm Instruments BV, The Nederlands). All the potentials given in the paper were reported against Ag/AgCl, 3 M KCl.

Cyclic voltammograms (CVs) were registered at scan rate 50 mV s^{-1} in quiescent solutions. During the chronoamperometric measurements the solution was stirred at 460 rpm with a magnetic stirrer (IkaMag RCT, Ika, Germany). The pH of the buffer solutions was adjusted with a pH metre pH 211 (Hanna Instruments, USA). Centrifuge BOECO S-8 (BOECKEL Co., Germany) and rotary evaporator Rotavapor – R (Büchi, Switzerland) were used in preparing the real samples.

2.3. Preparation of the modified graphite electrodes

The electrochemical deposition of gold on spectroscopic graphite was carried out according to a previously developed protocol (Dimcheva, Horozova, et al., 2013; Dimcheva et al., 2011), in brief: prior to modification, the graphite electrodes were mechanically cleaned by polishing on a fine wet sand-paper, with a gradual decrease of the size of the abrasive particles (P800, P1200, P1500 and P2000) and followed by ultrasonication in distilled water for 3 min each. Gold nanoparticles were then deposited by a brief electrolysis (10 s) at a constant potential of −155 mV (vs. Ag/AgCl, 3 M KCl) from 50 mM solution of $HAuCl_4$ dissolved in 0.1 M HCl. After the deposition, the surface of the electrode was washed with bi-distilled water and cleaned electrochemically in 0.5 M H_2SO_4 by continuous cycling over the potential range from 0 to 1.7 V for at least 20 cycles until three consecutive voltammograms overlap.

After measurements, the electrodes were water rinsed and stored in double distilled water at ambient temperature until next measurements. The electrodes with lost activity were regenerated by mechanical polishing on wet sand paper, then ultrasonicated in bi-distilled water, followed by electrodeposition of Au NPs.

2.4. Measurement procedure

Ascorbyl palmitate electrooxidation was examined by cyclic voltamperometry (CV) and chronoamperometry at a constant potential. CVs were registered at a scan rate of 50 mV s^{-1} in the following media: methanol or ethanol containing 0.1 M TEAB as inert supporting electrolyte; or ethanol mixed with phosphate buffer (pH = 5.6 or 7.0) in 50:50 (v/v) ratio.

The amperometric detection of the ascorbyl palmitate was performed by successive addition of aliquots of the stock solution (0.01 M dissolved in ethanol, prepared daily) to 20 ml buffer (pH = 5.6) – ethanol (1:1) mixture with simultaneous registration of the current at a constant potential. The concentration dependencies of the current were measured on the working electrode, as follows: the electrode was poised at a constant potential, E (typically 200 or 250 mV vs. Ag/AgCl, 3 M KCl) in the ethanol – aqueous buffer, pH = 5.6 as a background electrolyte and a steady-state current response, I_0 (μA) was awaited to be established under constant stirring. Afterwards, an aliquot (20; 50; 200; 500 or 1000 μl) of the analyte stock solution (0.01 M AP in ethanol) was added and the new value of the steady-state electrode response I_S (μA) was read. The time for reaching a steady-state value of the current under the given conditions did not exceed 0.5 min. All the information needed for plotting the calibration graphs was obtained from chronoamperometric records. All experimental results represent the mean value of at least three measurements (n = 3–6) with the regression analysis performed using MS EXCEL and Origin 7.5.

2.5. Preparation of the real sample

To examine the opportunity to assay AP in vegetable oils by chronoamperometry, a fresh real sample was prepared shortly before its electrochemical quantification by adding 0.050 g AP (dissolved in 500 μL of ethanol) to 100 g flaxseed oil, thus producing a standard 0.05% ascorbyl palmitate solution (further denoted in the text as stabilised flaxseed oil). The analyte AP was extracted from it through alcoholic extraction, followed by the separation of the two