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A novel method for flow injection analysis of total antioxidant capacity using enzymatically produced ABTS$^{\bullet+}$ and biamperometric detector containing interdigitated electrode

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Abstract

Application of interdigitated array microelectrodes as electrochemical sensors for determination of antioxidant capacity is reported. Electrochemical measurements with interdigitated electrodes (IDE) were studied in both stationary solutions and the flow system. The method is based on biamperometric measurements using ABTS$^{\bullet+}$|ABTS redox couple in phosphate buffer solution, pH 7.40. During analysis, the ABTS radical cation was enzymatically produced by peroxidase in a tubular flow-through reactor. The performance of bioreactor was tested at different concentrations of immobilized enzyme, ABTS and hydrogen peroxide. The influence of flow rate on proper operation of the bioreactor was also studied. The results of antioxidant activity were determined using Trolox as a standard. The applied IDE detector accomplished good sensitivity of 0.3 nA/μM of Trolox and offered linear range between 20 to 500 μM of Trolox.

The comparison of results ($R^2 = 0.9915$) for antioxidant activity between spectroscopic and FIA biamperometric measurements by interdigitated electrodes confirmed the applicability of the proposed method for determination of antioxidant capacity.

Keywords: Interdigitated electrodes; Antioxidant; ABTS$^{\bullet+}$|ABTS redox couple; Biamperometry; Trolox

1. Introduction

Interdigitated electrodes (IDE) have various advantages concerning their ability to enhance the current response, and offer higher sensitivity than the macro-electrode. Therefore interdigitated electrode has been used in a variety of electrochemical applications including development of sensors, electrochemical measurements in highly resistant media, detection of intermediate species, and trace determination of easily oxidizable organic and inorganic species.

Sanderson and Anderson described the electrochemical behavior of IDE almost 20 years ago [1]. Morita and coauthors studied electrochemical measurements with carbon-based interdigitated array electrodes in batch mode and in flow system [2], while Kudera et al. presented application of multi microelectrode array of eight different sizes in biological electrochemistry [3]. Application of interdigitated array electrodes for determination of enzyme activity was also described [4]. Xie et al. used a chip with four separated parallel arrays of iridium-made ultramicroelectrodes and miniaturized flow device for trace heavy metal measurements in water [5]. The measuring system consisting of four pairs of thin gold interdigitated electrodes and two auxiliary electrodes fixed on micro fluidic platform was described as a chip-based detector for rapid detection and quantification of nucleic acids [6]. Interdigitated ultramicroelectrode arrays (IDUAs) as possible transducers in a portable microfluidic-based biosensor were designed with the aim to maximize signal-to-noise ratio [7], while palladium nanoscaled interdigitated array electrode was made with deep UV lithography and applied for the detection of binding affinity of biomolecular structures by impedimetric measurements [8].

Biamperometric measurements involve electrochemical detection by two identical working electrodes, polarized with a small voltage difference, and applied in the solution contain-

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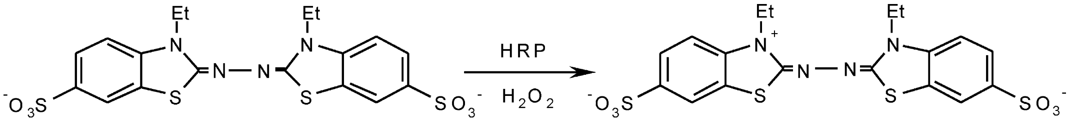
ing indicating reversible redox couple. Indirect biamperometric method of measurements is based on homogeneous reaction between an analyte and an indicating reversible redox couple. The most considered indicating systems for biamperometric determination include $Fe^{3+}|Fe^{2+}$, $I_2|I^-$, $Br_2|Br^-$, $VO_3{}^-|VO^{2+}$, Ce (IV)|Ce (III) and $Fe(CN)_6{}^{3-}|Fe\ (CN)_6{}^{4-}$.

Tougas and coauthors explained the basis for flow injection analysis by biamperometric technique [9]. Determination of various analytes as components of complex biological samples (urine, blood) was possible due to high selectivity of biamperometric method [10,11].

In recent years, a considerable interest has been focused on analytical methods for evaluation of antioxidant activity of food [12,13] and beverage samples [14,15]. One of the most widely used method for antioxidant activity evaluation is based on decolorization assay based on the scavenging of stable ABTS radical cation (2,2′-azino-bis(3-ethylbenzo thiazoline-6-sulfonic-acid)) [16,17]. Iveković et al. explained electrochemical generation of ABTS radical cation and its application for flow injection analysis of antioxidant activity by spectrophotometric detection [18]. Determination of antioxidant activity by amperometric and biamperometric method using DPPH|DPPH$^{\bullet}$ redox couple and classic glassy carbon disc electrode in the batch mode of measurements has recently been presented [19,20]. Kadnikova and Kostić described biocatalytical oxidation of ABTS by hydrogen peroxide using horseradish peroxidase encapsulated in the sol–gel glass [21]. Campanella and coworkers have recently presented an electrochemical method for determination of antioxidant capacity using a biosensor [22].

The aim of this study was the development of an electrochemical method for the flow injection analysis of antioxidant capacity, based on continuous enzymatic production of ABTS$^{\bullet+}$ and biamperometric detection by interdigitated electrode (IDE). In this study ABTS$^{\bullet+}$|ABTS redox couple was used as indicating redox pair. ABTS$^{\bullet+}$ radical cation represents an oxidized form while ABTS is a reduced form of the redox pair. During the analysis, continuous one electron oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) into corresponding radical cation ABTS$^{\bullet+}$ (Eq. (1)) was achieved by peroxidase-catalyzed reaction in the flow-through tubular reactor.



(1)

Enzymatically produced ABTS$^{\bullet+}$ react in the mixing coil with different compounds of antioxidant AH (analyte) producing ABTS according to reaction (2).

$$\mathrm{ABTS}^{\bullet+} + \mathrm{AH} \rightarrow \mathrm{ABTS} + \mathrm{A}^{\bullet} + \mathrm{H}^{+} \qquad (2)$$

After reaction, ABTS$^{\bullet+}$ concentration was reduced. In the proposed biamperometric measurement by IDE, current intensity is proportional to the residual reduced concentration of ABTS$^{\bullet+}$ after reaction with an antioxidant. When analyzed solution contains a suitable ratio of reduced/oxidized forms of indicating redox pair, the interferences from the analytically undesirable oxidative or reductive species presented in the solution are minimized. Consequently, in the case of the studied method, possible interferences caused by oxidative form of antioxidants designated as A$^{\bullet}$ in Eq. (2) would be avoided.

The applied interdigitated electrodes contributed to biamperometric measurements of antioxidant capacity with many advantages including high selectivity, short response time, high rate of steady state current, high sensitivity, and also enabled application of the flow injection analysis.

2. Experimental

2.1. Reagents and solutions

Commercially available chemicals of the highest purity were used. L-Ascorbic acid, and uric acid, L-glutathione (reduced form), 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), ABTS (2,2-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt, 98%), *N*, *N*′-methylenebisacrylamide (99%), 2,2-dimethoxy-2-phenylacetophenone (99%), and peroxidase (147 units/mg) from horseradish (type 1) were obtained from Sigma–Aldrich (St. Louis, USA). Acrylamide was purchased from Fluka (Buchs, Switzerland). Sodium dihydrogen phosphate, sodium hydrogen phosphate, potassium chloride, hydrogen peroxide (30%) were from Kemika (Zagreb, Croatia). Potassium peroxodisulfate ($K_2S_2O_8$) was from Merck (Germany). Ten milliliter of 10 mM solutions of antioxidants were prepared daily. The solutions of water-soluble antioxidants (L-ascorbic acid, L-glutathione, Trolox, uric acid (5 mM), gallic acid and *N*-acetyl-L-cysteine) were prepared using double deionized water from Millipore-MilliQ system (USA). The ABTS$^{\bullet+}$ solution (50 ml) was prepared 24 h before the spectrophotometric analysis by mixing 0.2 ml 65 mM $K_2S_2O_8$ and 10 ml 5 mM ABTS using phosphate buffer, pH 7.4.

2.2. Apparatus and instrumentation

Electrochemical measurements were carried out on Potentiostat 273 A (Princeton Applied Research, USA) connected to a computer for data collection and analysis. Cyclic voltammetry was performed in a standard three-electrode electrochemical cell for preconditioning of IDE. Interdigitated electrodes (IDE) IME 1525.3 FD Au P (ABTCH, Richmond, USA) were employed as working electrodes while a disc glassy carbon electrode was used as an auxiliary electrodes and $Hg_2Cl_2|3$ M KCl was used as a reference electrode. Interdigitated array electrodes was microlitographically fabricated sensor chip, formed from magneton sputtered gold on borosilicate glass substrate and consist two separated working electrode arrays on chip (6.4 mm × 5.5 mm, digit length $W_a = 2.985$ mm, digit width $(W_g) = 15\ \mu$m, interdigit space $(W) = 15\ \mu$m and the number of digit pairs = 25),