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Novel approach for the voltammetric evaluation of antioxidant activity using DPPH•-modified electrode

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ABSTRACT

The electrochemical behavior of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) immobilized on the electrode surface has been studied. Bare glassy carbon electrode (GCE) and modified with dispersions of CeO_2 nanoparticles in water (CeO_2-H_2O/GCE) and cationic surfactant cetylpyridinium bromide medium (CeO_2-CPB/GCE) has been investigated as a platform for the DPPH• immobilization. The best voltammetric characteristics (peak potential separation of 70 mV, system reversibility with currents ratio of 0.98 and the highest peaks currents) have been observed on CeO_2-CPB/GCE. The effect of CeO_2 nanoparticles concentration has been evaluated. Scanning electron microscopy and electrochemical impedance spectroscopy have been applied for the electrode characterization. DPPH•/CeO_2-CPB/GCE has been used for the estimation of the antioxidants activity of natural phenolic antioxidants (quercetin, tannin, catechin and ferulic acid) expressed as the EC_{50} parameter according to differential pulse voltammetric (DPV) data. The EC_{50} decreased in the following order: quercetin (29 ± 1 μM), tannin (29 ± 4 μM), catechin (117 ± 4 μM) and ferulic acid (731 ± 17 μM). These data are in a good agreement with the results of standard spectrophotometric determination. The developed approach has been successfully applied for the antioxidant activity evaluation of medicinal herbs tinctures, infusions and decoctions.

1. Introduction

Free radical oxidation in living systems promoted by reactive oxygen and nitrogen species is considered as one of the reasons of aging and a wide range of pathological states like atherosclerosis, cancer, heart and neurodegenerative diseases and etc. [1–3]. The harmful effect of these processes is prevented or balanced by the antioxidant defense system consisting of the endogenous and exogenous antioxidants [4]. The last ones represented by a wide range of compounds of different classes contained in foodstuffs, bioactive additives and pharmaceuticals including medicinal herbs. The majority of low-molecular weight antioxidants act as the free radical traps breaking the propagation of the chain radical process [5]. Therefore, the evaluation of the antioxidant power of individual compounds and real samples of complex antioxidant composition is of interest.

One of the common parameters for the antioxidant properties characterization is the antioxidant activity based on the reactions with stable free radicals. Among then, DPPH• is the most frequently used standard radical. In this case, the antioxidant activity is expressed as a portion of the reduced radicals via the reaction with antioxidants of the sample or in the equivalents of the individual antioxidants, for instance, Trolox (commercial water-soluble vitamin E) [6,7]. Antioxidant activity for the individual compounds is usually expressed as the efficient concentration (EC_{50}), that is the amount of antioxidant necessary to decrease by 50% the initial DPPH• concentration [8].

Various methods of monitoring the amount of DPPH• in the antioxidant test systems have been reported including electron spin resonance spectroscopy [9], nuclear magnetic resonance [10] and spectrophotometry [8,11]. The last one became the most widely and commonly used approach due to its simplicity and cost-efficiency.

The reactions of the antioxidants with DPPH• is based on the electron transfer that allows the use of electrochemical methods for monitoring of this process. The number of the electroanalytical methods advantages like simplicity, high sensitivity, cost-efficiency and possibility of miniaturization and automatization makes them very attractive in antioxidant analysis [12,13] and could be applied for the DPPH-based antioxidant activity evaluation.

Several electrochemical approaches have been developed for the evaluation of the antioxidant capacity. All of them are based on the DPPH• reaction with antioxidants in solution with different

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types of detection including cyclic voltammetry (CV) and DPV, amperometry and biamperometry. Their characteristics are summarized in Table 1. The quantitative evaluation is based on the decrease of DPPH• reduction currents after reaction with the antioxidants.

The application of flow injection systems allows to significantly improve the sample throughput (up to 180 samples per hour) as well as provides a strict control of reaction conditions in both space and time that are essential for determination of species sensitive to environmental conditions (light, temperature, presence of O_2 and etc.) [25]. It should be noted, that the water-organic media with high content of organic solvents are used, that is caused by the instability of DPPH• in water medium. In order to solve this problem, the immobilization of DPPH• on the electrode surface can be applied. The electroreduction mechanism of the solid DPPH• microparticles mechanically immobilized on the graphite electrode has been investigated by cyclic voltammetry [26]. The one reversible redox couple with a formal potential of 0.340 V versus Ag|AgCl (pH 7.0) has been observed followed by further chemical reactions. The electrochemical reactions are obviously confined to a rather small volume of the DPPH microcrystals, as no exhaustive conversion of the compound could be achieved.

The present work is focused on the development of novel voltammetric approach for the antioxidant activity evaluation based on the reactions of the antioxidants with DPPH• immobilized on the electrode surface and its application to medicinal herbs extract analysis.

2. Experimental

2.1. Reagents

10.0 and 2.50 mM stock solutions of DPPH• (Aldrich, Germany) were prepared by dissolving a definite amount in methanol (HPLC grade purity).

Quercetin (95% purity) and catechin hydrate (98%) from Sigma (Germany), ferulic acid (99%) from Aldrich (Germany) and tannin of Ph.Eur. purity from Fluka (Germany) were used. 1.00–10.0 mM surfactant stock solutions were prepared daily dissolving a definite amount of the substance in 5.0 mL of ethanol (rectificate). More dilute solutions were prepared before measurements in 10.0 mL volumetric flasks by dilution of the stock solution with ethanol.

Cerium dioxide nanoparticles dispersion in water (10%) with the particle size <25 nm was obtained from Sigma-Aldrich (USA). The working dispersions (0.5–1.5 mg mL^{-1}) were prepared by appropriate dilution with water or CPB (Aldrich, Germany) solution. The final CeO_2 dispersion contained 0.45 mM of CPB.

All other chemicals were of analytical reagent grade purity and used as received. Double distilled water was used for the measurements. The experiments were carried out at laboratory temperature (25 °C).

2.2. Apparatus

Voltammetric measurements were performed on potentiostat/galvanostat μAutolab type III with the software GPES, version 4.9.005 (Eco Chemie B.V., Netherlands). Electrochemical impedance spectroscopy (EIS) measurements were carried out on potentiostat/galvanostat Autolab PGSTAT 302N with FRA module and software Nova 1.10 (Eco Chemie B.V., Netherlands). The electrochemical cell consisted of the working GCE (DPPH•/GCE, DPPH•/CeO_2-H_2O/GCE or DPPH•/CeO_2-CPB/GCE) with 3.14 mm^2 geometric surface area, silver-silver chloride saturated KCl reference electrode and counter electrode (platinum wire).

Scanning electron microscopy (SEM) of the electrode surfaces was performed using tabletop scanning electron microscope TM-1000 (Hitachi, Japan).

"Expert-001" pH meter (Econix-Expert Ltd., Russia) equipped with the glassy electrode was used for pH measurements.

Spectrophotometric measurements were performed on spectrophotometer PE-5300 (NPO Ecros, Russia).

2.3. Preparation of the modified electrodes

The GCE was carefully polished with alumina (0.05 μm) on polishing cloth. Then, it was rinsed with acetone and double distilled water before use. Layer-by-layer drop casting method was been applied for the electrode surface modification. 2 μL of CeO_2 dispersion in water (CeO_2-H_2O) or CPB (CeO_2-CPB) were dropped on and the solvent was evaporated to dryness. Then, 2 μL of

Table 1
The electrochemical methods for the evaluation of antioxidant activity using DPPH•.

Method	Electrode	Supporting electrolyte	Detection potential/V	Quantification units	Real samples	Ref.
CV	Pt	0.5 mM Bu_4NBF_4 in methanol	0.350	Inhibition %	Ferrocene derivatives	[14]
					Metalloporphyrins	[15]
	Pt	0.1 M Bu_4NClO_4 in dymethyl sulfoxide	0.043	Inhibition %	Flavones	[16]
DPV	Pt SPE	0.033 M KCl in methanol	0.160	α-Tocopherol equivalents	Olive oil	[17]
Amperometry	GCE	0.033 M phosphate buffer pH = 7.4–40% ethanol	0.140	Trolox equivalents	Tea, wine and other beverages	[18]
Automated amperometry	Pencil Pb	Ethanolic phosphate buffer (pH 7.4, 0.1 M KCl),	0.100	Trolox equivalents	Tea, fruits, and thai herbal plant	[19]
Biamperometry	GCE	Ethanol-phosphate buffer solution, pH 7.40	ΔE = 0.200	Trolox equivalents	Tea, coffee, wine and juices	[20]
	Pt	Ethanol-phosphate buffer solution, pH 7.40	ΔE = 0.200	Trolox equivalents	Natural juices and soft drinks	[21]
Amperometry in flow injection mode	Au SPE	Mixture of phosphate buffer pH 6 and ethanol (1:1)	−0.100	Trolox equivalents	Red wine	[22]
	MWNT/ CGE	Ethanolic phosphate buffer (pH 7.0, 0.3 M KCl),	0.050	Trolox equivalents	Thai indigenous vegetables	[23]
Batch-injection analysis with amperometric detection	GCE	0.2 M acetate buffer (pH 5.5) in ethanol (40:60, v/v)	0.050	Inhibition %	Tea and plant extracts	[24]

SPE – screen-printed electrode; MWNT – multi-walled carbon nanotubes.