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Highly Sensitive Determination of Hesperidin Using Electrode Modified with Poly (Ferulic Acid) †

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Abstract: Hesperidin is a major phenolic antioxidant of orange fruits and responsible for their positive health effect. It is used as part of therapy of blood vessel conditions. Methods for hesperidin quantification are of practical interest. Recently, several voltammetric approaches have been developed for hesperidin quantification. Nevertheless, the analytical characteristics could be improved. To solve this problem, glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes (MWCNTs) and poly(ferulic acid) has been developed. Polymeric coverage has been obtained electrochemically under potentiodynamic conditions. Their optimization based on the hesperidin voltammetric response has been performed. The poly(ferulic acid) layer has to be obtained from 250 μM monomer solution in 0.1 M NaOH by fifteen potential scans from −0.2 to 1.0 V with the scan rate of 100 mV s^{-1}. Hesperidin oxidation currents are 2.8-fold increased at the polymer-modified electrode vs. carbon nanotube-based electrode at the same oxidation potential. Differential pulse voltammetry in phosphate buffer pH 5.5 has been used for the quantification of hesperidin. Linear dynamic ranges of 0.025–1.0 μM and 1.0–10 μM has been achieved with the limits of detection and quantification of 7.0 and 23.4 nM, respectively. The analytical characteristics obtained are the best ones reported to date.

Keywords: voltammetry; modified electrodes; electropolymerization; phenolic acids; flavanones

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1. Introduction

Hesperidin (hesperetin-7-*O*-rutinoside) is a major phenolic antioxidant of the orange fruits and responsible for their positive health effect [1]. It is used as part of therapy of blood vessel conditions [2,3]. Nevertheless, overdose of hesperidin can lead to harmful effect. On the other hand, the hesperidin content in orange fruits and juices is one of the parameters characterizing their nutritional value. Therefore, control of hesperidin in oranges is of interest and simple methods for hesperidin quantification are required.

Hesperidin is an electroactive compound that stipulated development of voltammetric approaches for hesperidin quantification. Recently, boron-doped diamond [4], disposable pencil graphite (bare [5] and electroactivated [6]) electrodes have been developed for the determination of hesperidin. To improve the sensitivity and selectivity of hesperidin response, several chemically modified electrodes have been fabricated using carbon nanomaterials [7–9], SnO_2 [10], mesoporous SiO_2 [11], and Au [12] nanoparticles, polymeric films based of polyaluminon [13] and molecularly imprinted poly-*o*-aminothiophenol [14]

as a sensitive layer. The analytical characteristics of hesperidin at these electrodes are not impressive (linear dynamic ranges of n × 10^{-8}–n × 10^{-5} M or even worse with the limits of detection n × 10^{-8}–n × 10^{-6} M) and can be further improved.

The current work deals with the development of a novel modified electrode based on the combination of MWCNTs and electropolymerized ferulic acid. Such type of modification has shown effectivity in the electroanalysis of natural phenolic antioxidants [15] and structurally related naringin [16]. This type of modifier is novel for hesperidin. The conditions of ferulic acid electropolymerization at the GCE covered with MWCNTs have been optimized. A highly sensitive voltammetric approach for hesperidin quantification has been developed.

2. Materials and Methods

The 0.40 mM stock solution of hesperidin was prepared in methanol (c.p. grade) using 94% hesperidin from Sigma (Steinheim, Germany). Less concentrated solutions were obtained by exact dilution.

MWCNTs (outer diameter 40–60 nm, inner diameter 5–10 nm and 0.5–500 μm length) from Aldrich (Steinheim, Germany) were used as a platform for further electrodeposition of poly(ferulic acid). Homogeneous 0.5 mg mL^{-1} suspension of MWCNTs was prepared in 1% sodium dodecylsulfate (Panreac, Barcelona, Spain) by 30 min of sonication in an ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea). GCE surface was polished on 0.05 μm alumina slurry, rinced with acetone and distilled water. Then, 5 μL of MWCNTs suspension was drop casted on the electrode surface.

All reagents were c.p. grade. Distilled water was used for the measurements. The laboratory temperature was (25 ± 2 °C).

Electrochemical measurements were conducted on the potentiostat/galvanostat Autolab PGSTAT 12 (Eco Chemie B.V., Utrecht, The Netherlands) with the NOVA 1.10.1.9 software (Eco Chemie B.V., Utrecht, The Netherlands). The glassy electrochemical cell of 10 mL volume was used. The tree-electrode system consisted of the working GCE of 3 mm diameter (BASi® Inc., West Lafayette, IN, USA), or a modified electrode, an Ag/AgCl reference electrode, and a platinum wire as the auxiliary electrode.

The pH measurements were carried out using the "Expert-001" pH meter (Econix-Expert Ltd., Moscow, Russia) with a glassy electrode.

3. Results and Discussion

Electrodeposition of poly(ferulic acid) was performed in a potentiodynamic mode in a basic medium providing easier oxidation of the monomer. There is clear irreversible oxidation step at 0.31 V of the cyclic voltammograms of ferulic acid (Figure 1) corresponding to the one electron detachment from the phenolate ion with the formation of phenoxyl radical (Scheme 1) which undergoes further reactions dimerization and polymerization similar to other hydroxycinnamic acids [15]. Oxidation currents are significantly decreased on the following scans that is caused by the formation of insulating coverage and agree well with reported for other phenolic acids [15].

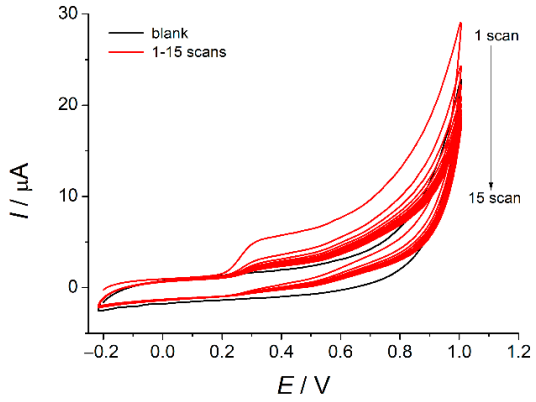
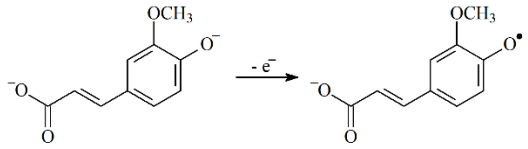


Figure 1. Electropolymerization of 250 μM ferulic acid at MWCNTs/GCE in 0.1 M NaOH. Potential scan rate is 100 mV s^{-1}.



Scheme 1. One electron oxidation of ferulic acid in 0.1 M NaOH.

Since the electropolymerization conditions affect the properties of the polymeric coverage obtained, the optimization of poly(ferulic acid) deposition has been performed on the basis of the hesperidin response. The poly(ferulic acid) layer has to be obtained from the 250 μM monomer solution in 0.1 M NaOH by fifteen potential scans from −0.2 to 1.0 V with a scan rate of 100 mV s^{-1}.

Polymer-modified electrode provides a statistically significant increase in hesperidin oxidation currents in comparison with an electrode based on MWCNTs and an unmodified electrode (Table 1). These data confirm the effectivity of the modified electrode developed in hesperidin sensing.

Table 1. Voltammetric characteristics of 10 μM hesperidin at various electrodes in phosphate buffer pH 7.0 on the basis of differential pulse voltammetry data.

Electrode	E_{ox_1} (V)	I_{ox_1} (μA)	E_{ox_2} (V)	I_{ox_2} (μA)
GCE	0.563	0.056 ± 0.002	0.935	0.045 ± 0.002
MWCNTs/GCE	0.504	0.18 ± 0.01	0.886	0.23 ± 0.01
Poly (ferulic acid)/MWCNTs/GCE	0.509	0.50 ± 0.02	0.904	0.42 ± 0.02

Differential pulse voltammetry has been applied for hesperidin quantification. The variation of phosphate buffer pH has shown that the best hesperidin response occurs at pH 5.5. A further increase of pH leads to a decrease of the oxidation currents that is caused by the partial oxidation of hesperidin by air oxygen that is typical for flavonoids [17].

Effect of the pulse parameters on the hesperidin response has been evaluated. The oxidation potential is insignificantly decreased as pulse amplitude and time are increased. The oxidation currents are changed statistically significant. The increase of pulse amplitude provides growth of the hesperidin oxidation currents, which achieves maximum at pulse amplitude of 100 mV. An increase in the pulse time from 25 to 100 ms leads to a significant decrease of the oxidation currents. Thus, pulse amplitude of 100 mV and pulse time 25 ms have been chosen for further measurements.

There are two well-resolved oxidation peaks of hesperidin on the voltammograms (Figure 2).

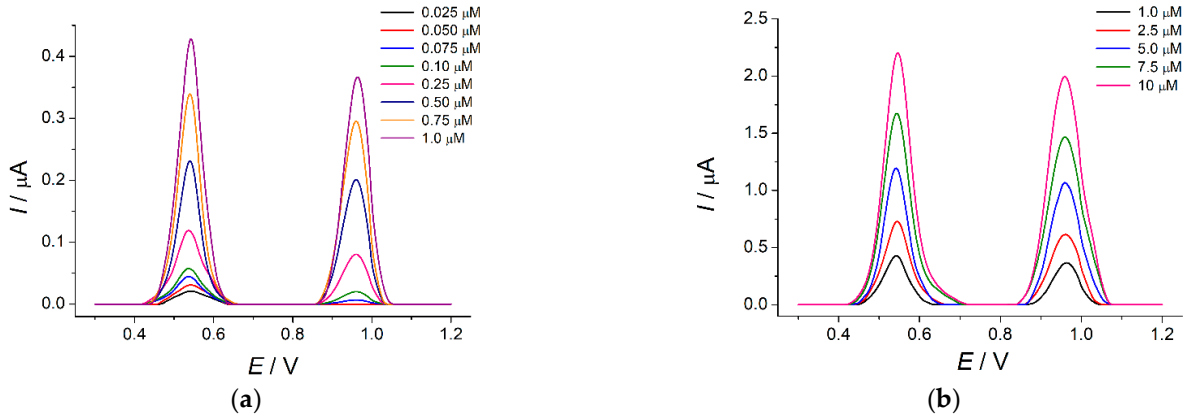


Figure 2. Baseline-corrected differential pulse voltammograms of hesperidin at the poly(ferulic acid)/MWCNTs/GCE in phosphate buffer pH 5.5: (**a**) concentration range of 0.025–1.0 μM; (**b**) concentration range of 1.0–10 μM. ΔE_{pulse} = 100 mV, t_{pulse} = 25 ms, υ = 20 mV s^{-1}.

The first oxidation step has been used as an analytical signal. Linear dynamic ranges of 0.025–1.0 μM and 1.0–10 μM has been achieved with the limits of detection and quantification of 7.0 and 23.4 nM, respectively. The analytical characteristics achieved are the best one among reported to date.

The accuracy of the method developed has been tested using the added-found method using model solutions of hesperidin (Table 2). The recovery of 99.6–100% indicated a high accuracy of hesperidin determination. The relative standard deviation of 0.79–3.2% confirms the absence of random errors in the determination and the good reproducibility of the electrode response since the electrode surface has been renewed after each measurement.

Table 2. Determination of hesperidin in model solutions at the poly(ferulic acid)/MWCNTs/GCE in phosphate buffer pH 5.5 (n = 5; p = 0.95).

Added Amount (μg)	Found Amount (μg)	RSD (%)	*R* (%)
0.0610	0.061 ± 0.002	3.2	100
0.610	0.61 ± 0.01	1.6	100
2.44	2.43 ± 0.04	1.4	99.6
12.2	12.2 ± 0.1	0.79	100
24.4	24.3 ± 0.3	1.0	99.6

4. Conclusions

A sensitive voltammetric method for the determination of hesperidin has been developed using electrode modified with MWCNTs and poly(ferulic acid). The obtained analytical characteristics are significantly improved compared to those described earlier for other electrodes including chemically modified ones. The method is characterized by rapidity, simplicity, and reliability of the results obtained.

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Conflicts of Interest: The authors declare no conflict of interest.

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