Proceeding Paper

Novel Electrodes Based on the Electropolymerized Nanocoatings for the Selective Voltammetric Quantification of Flavanones †

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Abstract: Chemically modified electrodes based on polymer nanocoatings as sensitive layers are some of the intensively developed areas in modern electroanalysis. Electropolymerization of compounds containing phenolic fragments is a promising approach for electrode surface modification. Novel electrodes based on a combination of carbon nanotubes and electropolymerized ellagic acid or aluminon were developed for the direct quantification of flavanones (naringin and hesperidin)—the major flavonoids of *Citrus* fruits. The conditions of monomers' potentiodynamic electropolymerization were optimized. Electrode surfaces were characterized by scanning electron microscopy and electrochemical methods. A glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes (MWCNTs) and poly(ellagic acid) allowed for the quantification of naringin in the ranges of 0.050–1.0 and 1.0–100 μM with the detection and quantification limits of 14 and 47 nM, respectively. Simultaneous voltammetric quantification of naringin and hesperidin in the ranges of 0.10–2.5 and 2.5–25 μM for both analytes with the detection limits of 20 nM and 29 nM for naringin and hesperidin, respectively, was achieved on GCE modified with polyaminobenzene sulfonic acid functionalized single-walled carbon nanotubes (f-SWCNTs) and polyaluminon. High selectivity of the electrodes' responses to flavanones in the presence of typical interferences and natural phenolics was confirmed. The approaches were successfully applied to *Citrus* juices.

Keywords: electropolymerized nanocoatings; natural phenolics; carbon nanotubes; flavanones; electroanalysis; *Citrus* juices

1. Introduction

Chemically modified electrodes are some of the most intensively developed areas in modern electroanalysis. This trend is mainly caused by the appearance of a wide range of nanomaterials (different types of nanoscale carbon, metal, and metal oxide nanoparticles, nanostructured polymers, other nanosized compounds, and composites) that are used as effective electrode surface modifiers. One of the approaches for electrode surface modification is coverage with electropolymerized films. The non-conductive polymers based on phenolic compounds are of interest insofar as they give a highly sensitive and selective response to low-molecular-weight substances, including antioxidants [1–3]. Further development in this field using a combination of such electropolymerized coatings with carbon nanomaterials provides conductivity of the electrode as well as high loading and more uniform coverage of the electrode surface [1].

Among the wide range of analytes, natural phenolic antioxidants, being a part of the daily human diet and medicinal therapy, are of great interest and widely investigated in life sciences. Given that their antioxidant effects are caused by electron transfer reactions, electrochemical methods are often used for their determination [4,5]. Flavanones—flavonoids

of *Citrus* fruits [6]—are less investigated and almost out of consideration in electroanalysis in comparison to other natural phenolics. The major natural flavanones are naringin and hesperidin (Figure 1), possessing a wide spectrum of biological activity, including antioxidant activity [6]. Nevertheless, like other natural phenolic antioxidants [7], they exhibit prooxidant properties when presented in high concentrations. Therefore, rigorous control of their contents in real samples is required.
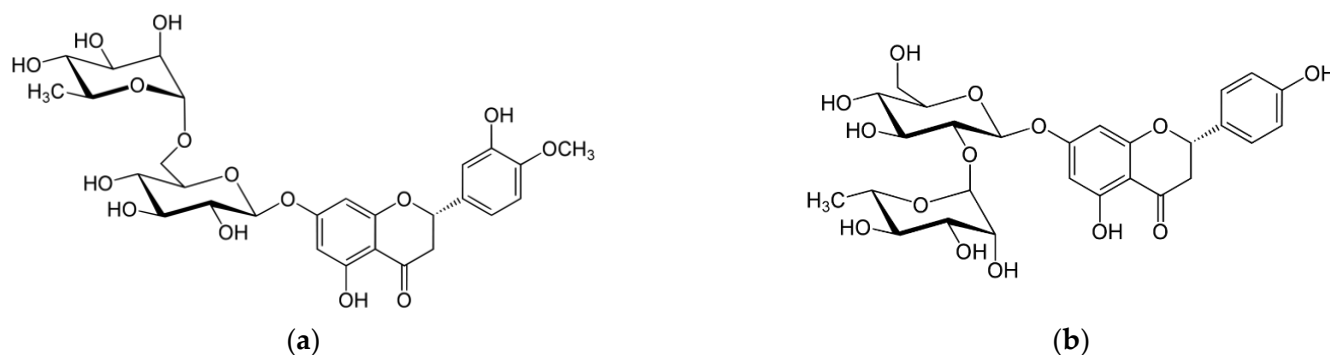

(a)

(b)

Figure 1. Structure of flavanones: (**a**) hesperidin; (**b**) naringin.

A limited number of chemically modified electrodes has been developed for the voltammetric quantification of hesperidin and naringin. Carbon nanomaterials [8,9], metal-based nanomaterials [10,11], tin dioxide [12] and silica [13] nanoparticles, amberlite-IRA 400 [14,15], and DNA [16,17], as well as poly-*o*-aminophenol [18]- and poly-*o*-aminothiophenol [19]-based molecularly imprinted polymers are used as a sensitive layers of modified electrodes. The analytical characteristics are typical, and the linear dynamic ranges mainly cover 10^{-7}–10^{-5} M concentrations. In many cases, the linear dynamic ranges are narrow enough to limit the applicability of the electrodes. Another disadvantage of the electrodes is the selectivity, which is insufficient or fully out of consideration.

Therefore, further improvement of the flavanones' analytical characteristics, as well as their simultaneous determination, is of practical interest. The current work is focused on the creation of novel modified electrodes based on a layer-by-layer combination of carbon nanotubes and electropolymerized coatings for the direct quantification of naringin and hesperidin. Ellagic acid or aluminon-containing phenolic fragments in their structure have been used as monomers. Conditions of their potentiodynamic electropolymerization (the monomer concentration, supporting electrolyte pH, potential scan rate and range, the number of cycles) have been found. The electrodes created have been studied by scanning electron microscopy and electrochemical methods. The individual quantification of naringin and the simultaneous determination of hesperidin and naringin with high sensitivity and selectivity have been achieved.

2. Materials and Methods

Ellagic acid (95% purity) from Sigma-Aldrich (Darmstadt, Germany) and aluminon from Sigma-Aldrich (Germany) were used. Their standard solutions (0.86 mM for ellagic acid and 10 mM for aluminon) were prepared in methanol (c.p. grade). Analytes (hesperidin of 94% purity and naringin (95%)) were purchased from Sigma-Aldrich (Germany). Stock solutions of 10 or 0.40 mM for naringin and 0.40 mM for hesperidin were prepared in methanol (c.p. grade) in 5.0 mL flasks. Less concentrated solutions were obtained by the exact dilution.

Multi-walled carbon nanotubes (MWCNTs) (outer diameter 40–60 nm, inner diameter 5–10 nm, and 0.5–500 μm length) from Aldrich and polyaminobenzene sulfonic acid functionalized single-walled carbon nanotubes (f-SWCNTs) (d × l is 1.1 nm × 0.5–1.0 μm) from Sigma-Aldrich (Steinheim, Germany) were used as a platform for the electrodeposition of polymeric coverages. Homogeneous suspensions of carbon nanomaterials (0.5 mg mL^{-1}

of MWCNTs in 1% sodium dodecylsulfate (Panreac, Barcelona, Spain) and 1.0 mg mL^{-1} of f-SWCNTs in dimethylformamide) were obtained by 30 min sonication using an ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Korea).

Chlorogenic (95%) and ferulic (99%) acids from Sigma-Aldrich (Germany); ascorbic (99%), gallic (99%), caffeic (98%), and *p*-coumaric (98%) acids and quercetin dihydrate (95%) and catechin hydrate (98%) from Sigma-Aldrich (Germany); rutin trihydrate (97%) from Alfa Aesar (United Kingdom); sinapic acid (97%) and tannin (Ph. Eur.) from Fluka (Germany) were used in the interference study. Their 10 mmol L^{-1} stock solutions in methanol were prepared in 5.0 mL flasks.

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 a μAutolab Type III (Eco Chemie B.V., Utrecht, The Netherlands) potentiostat/galvanostat supplied with GPES 4.9.005 software and Autolab PGSTAT 302N with the FRA 32M module (Eco Chemie B.V., Utrecht, The Netherlands) and NOVA 1.10.1.9 software. The glassy electrochemical cell of 10 mL volume was used. The tree-electrode system consisted of the working GCE of 3 mm diameter (CH Instruments, Inc., Bee Cave, TX, 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 an "Expert-001" pH meter (Econix-Expert Ltd., Moscow, Russian Federation) with a glassy electrode.

A MerlinTM (Carl Zeiss, Oberkochen, Germany) high-resolution field emission scanning electron microscope was applied for the electrode surface morphology characterization and operated at 5 kV accelerating voltage and a 300 pA emission current.

3. Results and Discussion

3.1. Polymer-Modified Electrodes Preparation and Characterization

Electropolymerization of ellagic acid and aluminon was carried out on the surface of GCE modified preliminarily with MWCNTs or f-SWCNTs by drop-casting technology (4.0 μL of MWCNTs or 2.0 μL f-SWCNTs suspensions were applied). This approach provided sufficient conductivity of the electrode as well as a high surface area. Polymeric coatings were electrodeposited in potentiodynamic mode. Both monomers were irreversibly oxidized at the electrode surface at 0.287 and 0.497 V for the ellagic acid at MWCNTs/GCE in phosphate buffer pH 7.0 and 0.50 V for aluminon at f-SWCNTs/GCE in 0.1 M NaOH. A decrease of the oxidation steps was observed for the following cycles, which means the formation of non-conducting polymer and typical for the phenolic compounds [1]. The oxidation peaks of monomers almost disappeared after the seventh cycle for ellagic acid and the tenth cycle for aluminon. The conditions of electropolymerization (the monomer concentration, supporting electrolyte pH, potential scan rate and range, and the number of cycles) were optimized based on the response of target analytes (naringin on poly(ellagic acid)/MWCNTs/GCE and the hesperidin and naringin mixture on polyaluminon/f-SWCNTs/GCE). Electropolymerization conditions providing the best voltammetric characteristics of the analytes are presented in Table 1.

Table 1. Ellagic acid and aluminon electropolymerization conditions.

Parameter	Poly (Ellagic Acid)	Polyaluminon
Supporting electrolyte	Phosphate buffer	0.1 M NaOH
pH	7.0	13
Monomer concentration (μM)	10	100
Number of cycles	7	10
Polarization window (V)	0.0–1.0	0.1–0.8
Potential scan rate (mV s^{-1})	100	100

The suggested modification of the electrode surface provided significant improvement of the voltammetric response of the flavanones under consideration. The shifts of oxidation

potentials to less positive values (Figure 2) confirmed the increase of the electron transfer rate at the modified electrodes, which was proved by charge transfer resistance data obtained by electrochemical impedance spectroscopy in the presence of a 1.0 mM equimolar mixture of hexacyanoferrate(II)/(III) ions (Table 2). A statistically significant increase of the flavanones' oxidation currents (Figure 2) was caused by the increase of the electroactive surface area of polymer-modified electrodes, as confirmed by electrochemical data based on the electrooxidation of hexacyanoferrate(II) ions (Table 2).
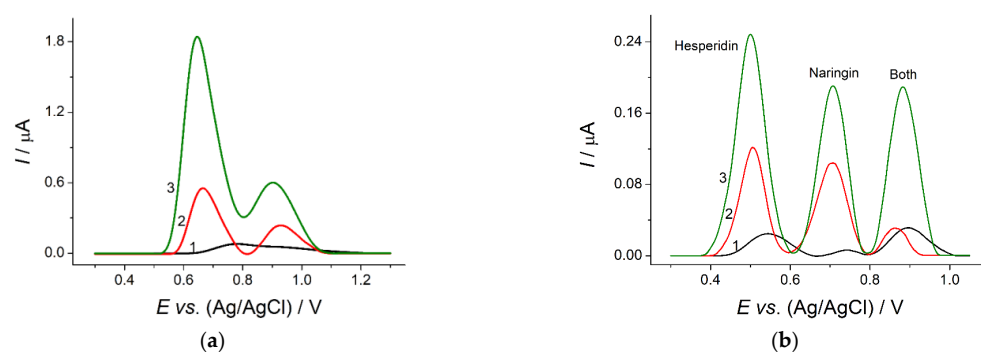

Figure 2. Baseline-corrected differential pulse voltammograms of flavanones: (**a**) 100 µM naringin at the GCE (1), MWCNTs/GCE (2), and poly(ellagic acid)/MWCNTs/GCE (3); (**b**) 5.0 µM mixture of hesperidin and naringin at the GCE (1), f-SWNTs/GCE (2), and polyaluminon/f-SWNTs/GCE (3). Supporting electrolyte is phosphate buffer pH 7.0. Modulation amplitude is 50 mV, modulation time is 50 ms, and potential scan rate is 10 mV s^{-1}.

Table 2. Charge transfer resistance (R_{ct}) and electroactive surface area (A) of the electrodes ($n = 5$; $p = 0.95$).

Electrode	R_{ct} (kΩ)	A (cm^2)
GCE	72 ± 3	0.089 ± 0.002
MWCNTs/GCE	12.1 ± 0.9	0.75 ± 0.02
Poly(ellagic acid)/MWCNTs/GCE	50 ± 2	0.79 ± 0.01
f-SWNTs/GCE	8.0 ± 0.1	0.136 ± 0.002
Polyaluminon/f-SWNTs/GCE	3.8 ± 0.2	0.206 ± 0.001

The electrode surface morphology was studied by scanning electron microscopy (Figure 3). The polymeric coatings exhibited a porous structure with the shape of spherical particles of 30–50 nm in diameter for poly(ellagic acid) (Figure 3a) and a folded structure with channels and cavities for polyaluminon (Figure 3b), confirming successful electropolymerization as well as a high roughness of the electrode surface. These results agree well with those reported for other phenol-based polymeric coatings [1,20,21].
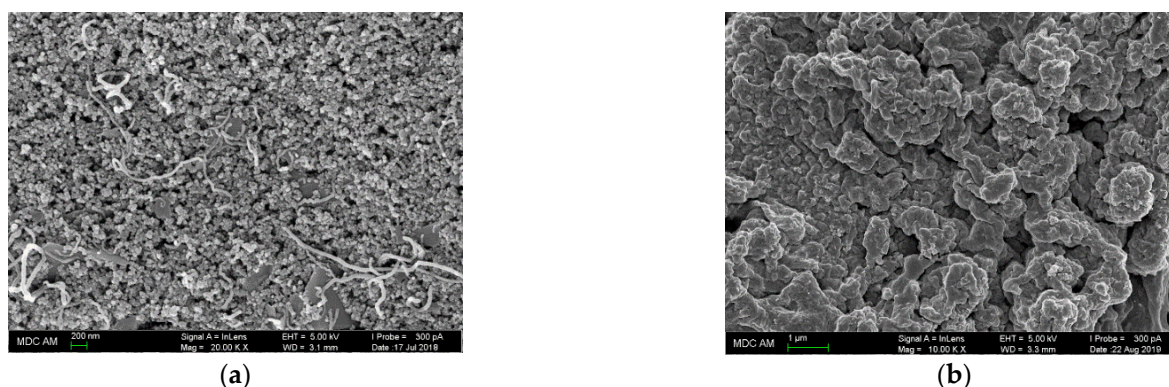

Figure 3. Electrode surface morphology obtained by scanning electron microscopy: (**a**) poly(ellagic acid)/MWCNTs/GCE; (**b**) polyaluminon/f-SWNTs/GCE.

3.2. Analytical Application of the Electrodes

The electrodes created were used for analytical purposes in the differential pulse mode. The best responses of flavanones were observed in phosphate buffer of pH 6.5 for naringin at the poly(ellagic acid)/MWCNTs/GCE and of pH 5.0 for hesperidin and naringin at the polyaluminon/f-SWNTs/GCE. There were well-pronounced oxidation peaks on the voltammograms (Figure 4).
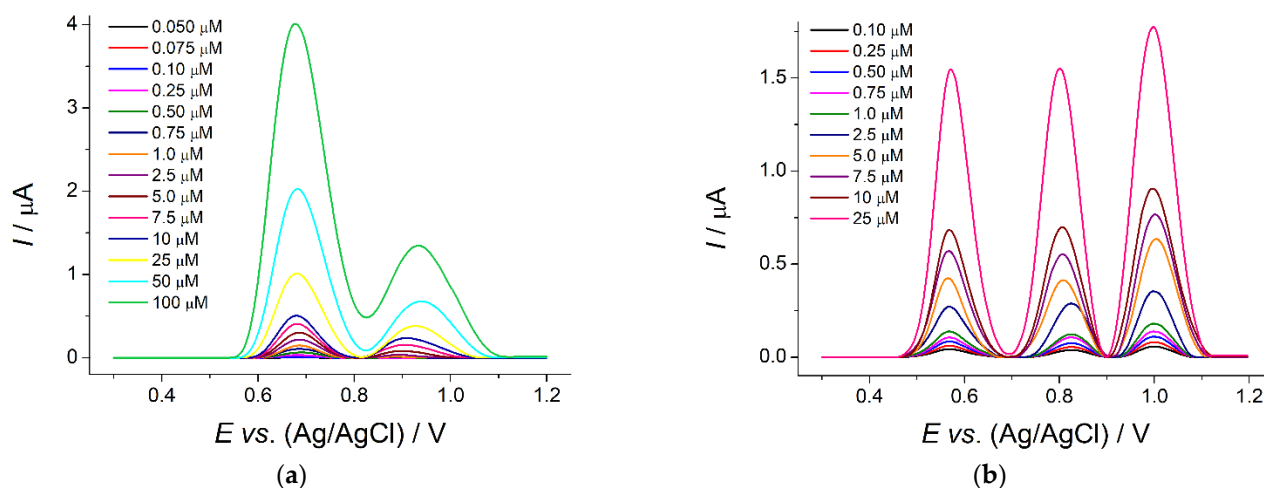

Figure 4. Baseline-corrected differential pulse voltammograms of flavanones: (**a**) 0.050–100 μM naringin at the poly(ellagic acid)/MWCNTs/GCE in phosphate buffer pH 7.5, modulation amplitude is 100 mV, modulation time is 50 ms, and potential scan rate is 10 mV s^{-1}; (**b**) 0.10–25 μM equimolar mixtures of hesperidin and naringin at the polyaluminon/f-SWNTs/GCE in phosphate buffer pH 5.0, modulation amplitude is 100 mV, modulation time is 25 ms, and potential scan rate is 10 mV s^{-1}.

The oxidation currents of flavanones were linearly dependent on their concentration. The analytical characteristics obtained (Table 3) were significantly improved or comparable with those reported with other modified electrodes. Simultaneous determination of hesperidin and naringin was performed for the first time.

Table 3. Analytical characteristics of flavanones and the polymer-modified electrodes.

Electrode	Flavanone	Detection Limit (μM)	Quantification Limit (μM)	Linear Dynamic Range (μM)
Poly(ellagic acid)/MWCNTs/GCE	Naringin	0.014	0.047	0.050–1.0 and 1.0–100
Polyaluminon/f-SWNTs/GCE	Hesperidin	0.029	0.096	0.10–2.5 and 2.5–25
	Naringin	0.020	0.062	0.10–2.5 and 2.5–25

The electrodes developed were characterized by high accuracy of flavanone determination (recovery of 99.3–100.3%), as shown with the model systems. The relative standard deviation of 0.55–3.1% confirmed the absence of random errors of determination, as well as the high reproducibility of the analytical signal of flavanones, since the surface of the electrodes was renewed before each measurement.

The interference study showed excellent selectivity of polymer-based electrodes towards flavanones. Typical interferences (1000-fold excesses of K^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^-, and SO_4^{2-}, and 100-fold excesses of glucose, rhamnose, sucrose, and ascorbic acid) did not show interference effects. Structurally related natural phenolics were the major potential interferences and were widely distributed in *Citrus* fruits. Poly(ellagic acid)/MWCNTs/GCE showed a selective response towards naringin in the presence of 10-fold excesses of phenolic acids (gallic, ferulic, caffeic, and chlorogenic acids) and hesperidin. In the case of polyaluminon/f-SWNTs/GCE, 10-fold excesses of gallic, caffeic, and chlorogenic acids, as well as tannin, 1.0 μM of ferulic, sinapic and *p*-coumaric acids, catechin, quercetin, and

rutin did not interfere with hesperidin and naringin response. Sample dilution could be used for the masking of the interference signals, while the target flavanones' response was still sufficient.

The electrodes developed were successfully applied to *Citrus* (fresh and commercial) juice analysis. The standard addition method was used for the confirmation of the signal-forming compounds. The absence of matrix effects in the determination of flavanones was confirmed by recoveries of 98–101%. Grapefruit and orange juice analysis results are presented in Figure 5. The data obtained agreed well with the results of the independent methods (*F*-test confirms similar accuracy of the methods).
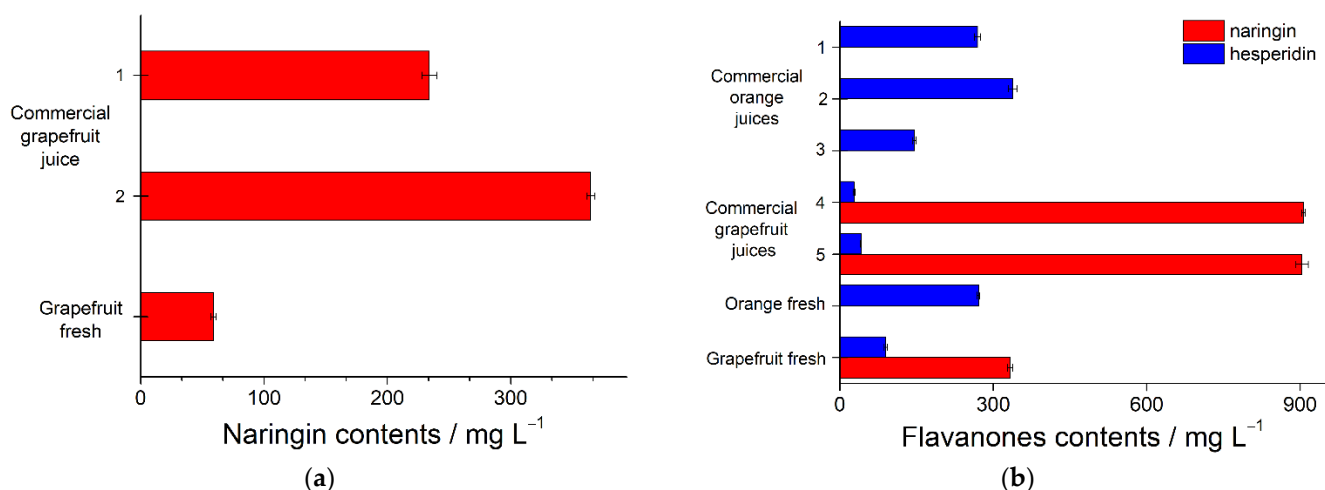

Figure 5. Flavanone contents in *Citrus* juices: (**a**) naringin contents in grapefruit juices based on electrooxidation of the poly(ellagic acid)/MWCNTs/GCE in phosphate buffer pH 7.5; (**b**) hesperidin and naringin contents in orange and grapefruit juices based on electrooxidation of the polyaluminon/f-SWNTs/GCE in phosphate buffer pH 5.0.

4. Conclusions

Novel modified electrodes based on electropolymerized ellagic acid and aluminon are highly sensitive and selective to flavanones, allowing their direct quantification. The simplicity of electrode fabrication, reliability, and cost-efficiency are important advantages of the electrodes developed. Real samples analysis data confirm the applicability of the electrodes in routine practice as an alternative to chromatographic methods.

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