IOCN 2022

Proceedings Paper

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

Guzel Ziyatdinova *, Elvira Yakupova and Anastasiya Zhupanova

Analytical Chemistry Department, Kazan Federal University, Kremleyevskaya, 18, Kazan 420008, Russia; elviraeakupova96@mail.ru (E.Y.); zhupanova.nastya@mail.ru (A.Z.)
* Correspondence: Ziyatdinovag@mail.ru

† Presented at the 3rd International Online-Conference on Nanomaterials, 25 April–10 May 2022; Available online: https://iocn2022.sciforum.net/.

Abstract: Chemically modified electrodes based on polymer nanocoatings as sensitive layers are one 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. Conditions of monomers' potentiodynamic electropolymerization were optimized. Electrodes surface was 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 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' response to flavanones in the presence of typical interferences and natural phenolics was confirmed. The approaches were successfully applied to citrus juices.

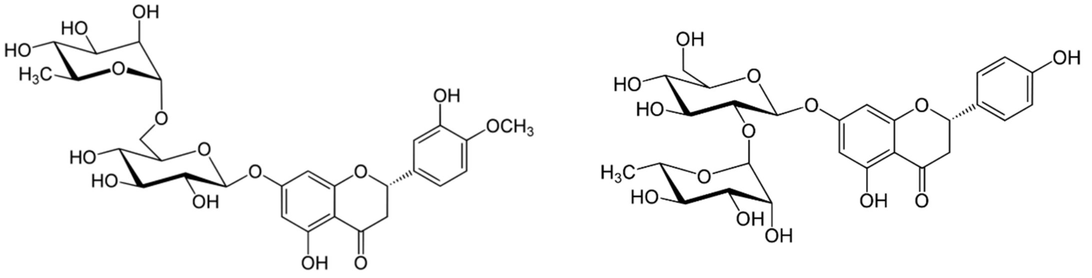
Keywords: electropolymerized nanocoatings; natural phenolics; carbon nanotubes; flavanones; electroanalysis; citrus juices

Citation: Ziyatdinova, G.; Yakupova, E.; Zhupanova, A. Novel Electrodes Based on the Electropolymerized Nanocoatings for the Selective Voltammetric Quantification of Flavanones. **2022**, *2*, x. https://doi.org/10.3390/xxxxx

1. Introduction

Chemically modified electrodes are one 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 the coverage with the electropolymerized films. The non-conductive polymers based on phenolic compounds are of interest as far as giving a highly sensitive and selective response to the 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 a high loading and more uniform coverage of the electrode surface [1].

Among a 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. As far as their antioxidant effect is caused by electron transfer reactions, the 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 the antioxidant one [6]. Nevertheless, like other natural phenolic antioxidants [7], they exhibit prooxidant properties when presented in high concentrations. Therefore, rigorous control of their contents in the real samples is required.



(**a**) (**b**)

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

A limited number of chemically modified electrodes have 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], 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 the modified electrodes. The analytical characteristics are typical and linear dynamic ranges are mainly cover 10^{-7}–10^{-5} M concentrations. In many cases, the linear dynamic ranges are narrow enough that limits 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 simultaneous determination of hesperidin and naringin with high sensitivity and selectivity has been achieved.

2. Materials and Methods

Ellagic acid (95% purity) from Aldrich (Steinheim, Germany) and aluminon from Sigma (Steinheim, 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 (Steinheim, Germany). The stock solution 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 a 30 min sonication using the ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Korea).

Chlorogenic (95%) and ferulic (99%) acids from Aldrich (Steinheim, Germany), ascorbic (99%), gallic (99%), caffeic (98%) and *p*-coumaric (98%) acids, quercetin dihydrate (95%), catechin hydrate (98%) from Sigma (Steinheim, Germany), rutin trihydrate (97%) from Alfa Aesar (Heysham, United Kingdom), sinapic acid (97%) and tannin (Ph. Eur.) from Fluka (Seelze, Germany) have been 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 the potentiostat/galvanostat μAutolab Type III (Eco Chemie B.V., Utrecht, The Netherlands) 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 the "Expert-001" pH meter (Econix-Expert Ltd., Moscow, Russian Federation) with a glassy electrode.

A high-resolution field emission scanning electron microscope MerlinTM (Carl Zeiss, Oberkochen, Germany) 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

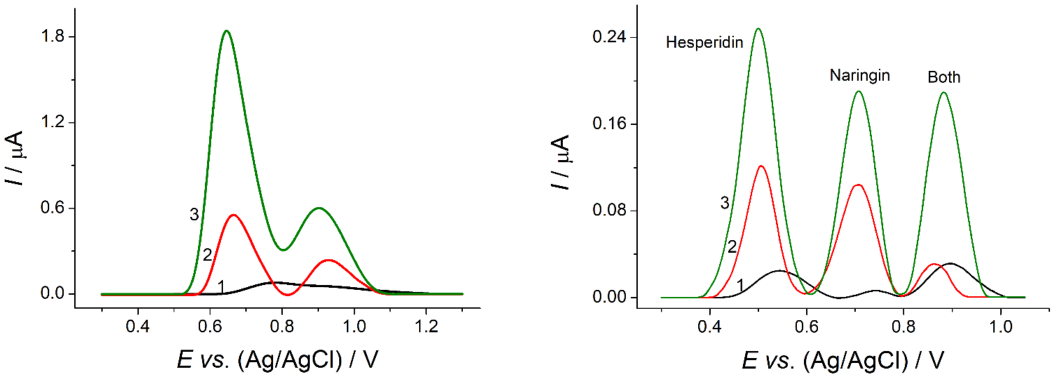
3.1. Polymer-Modified Electrodes Preparation and Characterization

Electropolymerization of ellagic acid and aluminon has been carried out on the surface of GCE modified preliminary with MWCNTs or f-SWCNTs by drop-casting technology (4.0 μL of MWCNTs or 2.0 μL f-SWCNTs suspensions have been applied). This approach provides sufficient conductivity of the electrode as well as a high surface area. Polymeric coatings have been electrodeposited in potentiodynamic mode. Both monomers are 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. The decrease of the oxidation steps has been observed on the following cycles which means the formation of non-conducting polymer and typical for the phenolic compounds [1]. The oxidation peaks of monomers are 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, the number of cycles) have been optimized based on the response of target analytes (naringin on poly(ellagic acid)/MWCNTs/GCE and 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 provides significant improvement of the voltammetric response of flavanones under consideration. The shifts of oxidation potentials to less positive values (Figure 2) confirm the increase of the electron transfer rate at the modified electrodes that is proved by charge transfer resistance data obtained by electrochemical impedance spectroscopy in the presence of 1.0 mM equimolar mixture of hexacyanoferrate(II)/(III) ions (Table 2). A statistically significant increase of the flavanones oxidation currents (Figure 2) is 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).



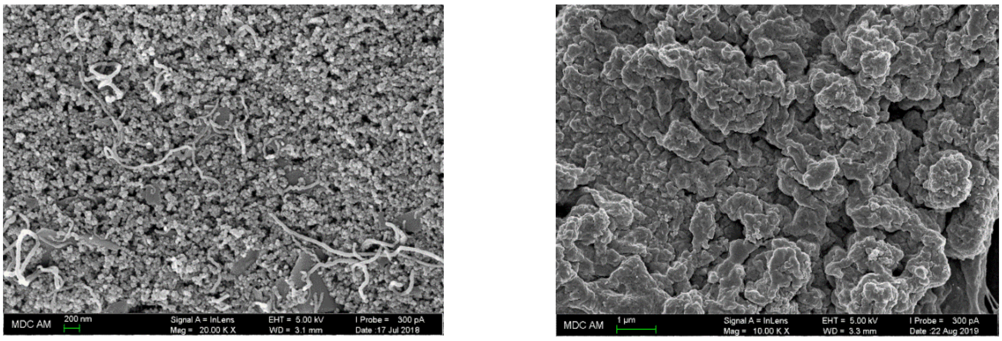
(**a**) (**b**)

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 (cm²)
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

Electrode surface morphology has been studied by scanning electron microscopy (Figure 3). The polymeric coatings exhibit porous structure with the shape of spherical particles of 30–50 nm diameter for poly(ellagic acid) (Figure 3a) and 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 the reported for other phenol-based polymeric coatings [1,20,21].

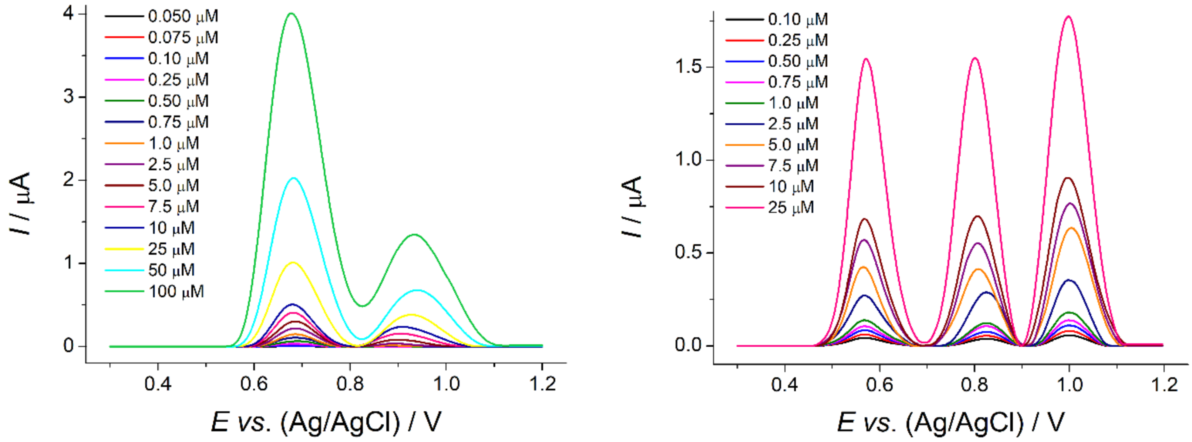


(**a**) (**b**)

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

3.2. Analytical Application of the Electrodes

The electrodes created have been used for analytical purposes in the differential pulse mode. The best responses of flavanones have been observed in a 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 are well-pronounced oxidation peaks on the voltammograms (Figure 3).



(**a**) (**b**)

Figure 3. 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 are linearly dependent on their concentration. The analytical characteristics obtained (Table 3) are significantly improved or comparable with the reported to other modified electrodes. Simultaneous determination of hesperidin and naringin has been performed for the first time.

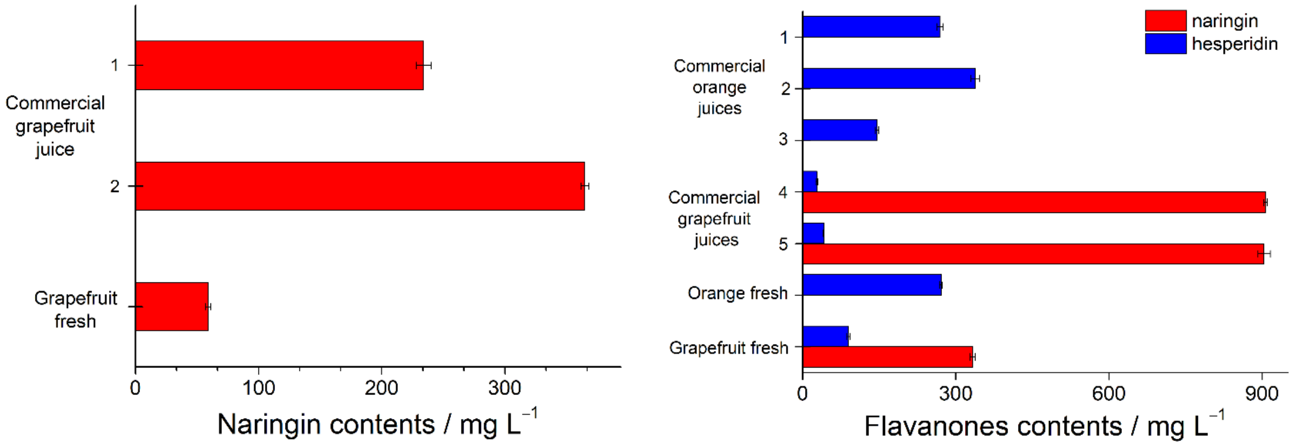
Table 3. Analytical characteristics of flavanones at the polymer-modified electrodes.

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 are characterized by high accuracy of flavanones determination (recovery of 99.3–100.3%) as shown on the model systems. The relative standard deviation of 0.55–3.1% confirms 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 has been renewed before each measurement.

The interference study has shown an 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) don't show interference effect. Structurally related natural phenolics are the major potential interferences as far as widely distributed in citrus fruits. Poly(ellagic acid)/MWCNTs/GCE shows 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 do not interfere with hesperidin and naringin response. Sample dilution can be used for the masking of the interferences signals while the target flavanones' response is still sufficient.

The electrodes developed have been successfully applied to citrus (fresh and commercial) juices analysis. The standard addition method has been used for the confirmation of the signal-forming compounds. The absence of matrix effects in the determination of flavanones is confirmed by recoveries of 98–101%. Grapefruit and orange juices analysis results are presented in Figure 4. The data obtained agree well with the results of the independent methods (*F*-test confirms similar accuracy of the methods).



(**a**) (**b**)

Figure 4. Flavanones contents in citrus juices: (**a**) naringin contents in grapefruit juices based on the electrooxidation at the poly(ellagic acid)/MWCNTs/GCE in phosphate buffer pH 7.5; (**b**) hesperidin and naringin contents in orange and grapefruit juices based on the electrooxidation at the polyaluminon/f-SWNTs/GCE in phosphate buffer pH 5.0.

4. Conclusions

Novel modified electrodes based on the 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.

Author Contributions: Conceptualization, G.Z.; methodology, G.Z.; validation, G.Z. and E.Y.; investigation, E.Y. and A.Z.; writing—original draft preparation, G.Z.; writing—review and editing, G.Z.; visualization, G.Z., E.Y. and A.Z.; supervision, G.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors thank Aleksei Rogov (Laboratory of Scanning Electron Microscopy, Interdisciplinary Center for Analytical Microscopy, Kazan Federal University) for the scanning electron microscopy measurements.

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

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