engineering proceedings

MDPI

Proceedings

Voltammetric Sensors Based on the Electropolymerized Phenolic Acids or Triphenylmethane Dyes for the Antioxidant Analysis †

Guzel Ziyatdinova *, Elvira Yakupova and Anastasiya Zhupanova

Analytical Chemistry Department, Kazan Federal University, Kremleyevskaya, 18, 420008 Kazan, Russia; elviraeakupova96@mail.ru; zhupanova.nastya@mail.ru
* Correspondence: ziyatdinovag@mail.ru

† Presented at the 9th International Electronic Conference on Sensors and Applications, 1–15 November 2022. Available online: https://ecsa-9.sciforum.net/.

Abstract: Sensors with the electrochemically formed polymeric films as sensitive layer are of high interest in electroanalysis. Voltammetric sensors based on the glassy carbon electrodes (GCE) covered with carbon nanomaterials and electropolymerized phenolic acids (gallic and ellagic) or triphenylmethane dyes (thymolphthalein and aluminon) were developed. Conditions of potentiodynamic electropolymerization were optimized. The electrodes were characterized with scanning electron microscopy, cyclic voltammetry, chronoamperometry, and electrochemical impedance spectroscopy (EIS). In differential pulse mode, sensors give sensitive and selective response to different classes of the antioxidants (capsaicinoids, flavanones and flavonols). The practical applicability of the sensors was demonstrated on food and plant samples.

Keywords: electrochemical sensors; electropolymerization; phenolic acids; dyes; flavonoids; plant material; food analysis

Citation: Ziyatdinova, G.; Yakupova, E.; Zhupanova, A. Voltammetric sensors based on the electropolymerized phenolic acids or triphenylmethane dyes for the antioxidant analysis. *Eng. Proc.* **2022**, *4*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Stefano Mariani

Published: 1 November 2022

1. Introduction

Electrochemical sensors based on the electropolymerized coverages as sensitive layer are of high interest in electroanalysis. Various monomers are successfully used for the sensors creation in particular compounds with phenolic moiety. As known, their electropolymerization leads to the formation of non-conductive coverages [1,2]. Therefore, carbon nanomaterials (nanotubes, nanofibers) are successfully applied as a platform for further electropolymerization of a suitable monomer [2]. On the other hand, the presence of carbon nanomaterials increases the electrode surface roughness and electroactive area providing higher amount of the polymeric coverages electrodeposited as well as its uniform surface distribution. The presence of phenolic fragments in the polymer structure can provide the sensitive response to the compounds containing similar moieties in particular, natural phenolic antioxidants [2].

Among a wide range of phenolic monomers, natural phenolic acids (gallic and ellagic) and triphenylmethane dyes (thymolphthalein and aluminon) are of interest (Figure 1) and have been studied in current work. Their electropolymerization conditions have been optimized and properties of the electrodes created have been characterized by scanning electron microscopy (SEM), cyclic voltammetry, chronoamperometry and electrochemical impedance spectroscopy (EIS). The electrodes act as a sensitive and selective voltammetric sensors to different classes of the antioxidants (capsaicinoids, flavanones (hesperidin and naringin) and flavonols (rutin and quercetin)). Simultaneous quantification of flavanones and flavonols has been achieved using sensors based on the electropolymerized triphenylmethane dyes for the first time. The practical applicability of the sensors has been demonstrated on food and plant samples.

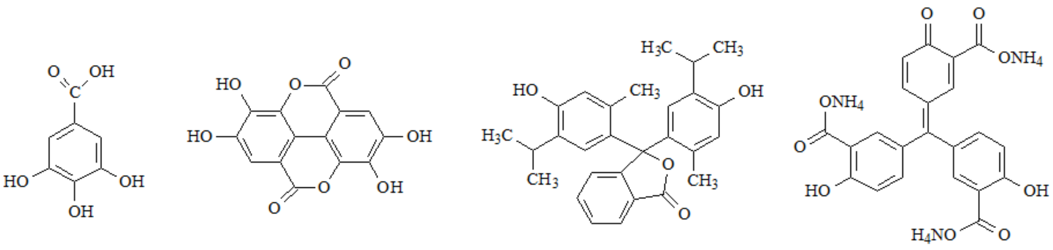

Gallic acid Ellagic acid Thymolphthalein Aluminon

Figure 1. Structure of monomers under investigation.

2. Materials and Methods

Ellagic acid (95% purity) from Sigma-Aldrich (Darmstadt, Germany), aluminon, thymolphthalein, gallic acid, naringin (95%), hesperidin (94%), and quercetin dihydrate (95%) from Sigma (Steinheim, Germany), capsaicin (95%), dihydrocapsaicin (85%), and nonivamide (97%) from Sigma (Bengaluru, India) and rutin trihydrate (97%) from Alfa Aesar (Heysham, UK) were used. Standard solutions of 0.86 mM ellagic acid, 10 mM aluminon and thymolphthalein were prepared in methanol (c.p. grade) and 10 mM gallic acid in ethanol (rectificate). Stock solutions of the analytes (10 mM for naringin, rutin and quercetin, and 0.40 mM for hesperidin in methanol (c.p. grade), 10 mM solutions of capsaicinoids in ethanol) were prepared in 5.0 mL flasks. The exact dilution was used for the preparation of less concentrated solutions.

Multi-walled carbon nanotubes (MWCNTs) (outer diameter 40–60 nm, inner diameter 5–10 nm, and 0.5–500 µm length) from Aldrich (Steinheim, Germany), single-walled carbon nanotubes functionalized with polyaminobenzene sulfonic acid (f-SWCNTs) ($d \times l$ is 1.1 nm × 0.5–1.0 µm) from Sigma-Aldrich (Steinheim, Germany), and graphitized (iron-free) carbon nanofibers (CNF) ($d \times l$ 100 nm × 20–200 µm and average diameter 130 nm) from Aldrich (St. Louis, MO, USA) were used as electrode surface modifiers. Sonication for 30 min (15 min for CNF) in an ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Korea) was applied for the preparation of carbon nanomaterials homogeneous 0.5 mg mL^{-1} of MWCNTs and 1.0 mg mL^{-1} of f-SWCNTs or CNF suspensions. 1% sodium dodecylsulfate (Panreac, Barcelona, Spain) and dimethylformamide were used as dispersive media for MWCNTs and f-SWCNTs, respectively. Other chemicals were c.p. grade and used as received.

Potentiostats/galvanostats µAutolab Type III (Eco Chemie B.V., Utrecht, The Netherlands) 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 were used for the electrochemical measurements. The glassy electrochemical cell of 10 mL volume was used. The working glassy carbon electrodes (GCE) of 3 mm diameter (CH Instruments, Inc., Bee Cave, TX, USA and BASi® Inc., West Lafayette, IN, USA) and 1 mm diameter, or modified electrodes, an Ag/AgCl reference electrode, and a platinum wire as the auxiliary electrode were used.

The pH measurements were performed at “Expert-001” pH meter (Econix-Expert Ltd., Moscow, Russia) using a glassy electrode.

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

3. Results and Discussion

3.1. Electropolymerization of Phenolic Acids and Triphenylmethane Dyes and Electrodes Characteristics

Layer-by-layer modification of GCE surface was perfomed. Firstly, bare GCE was covered with 4.0 μL of MWCNTs or 2.0 μL f-SWCNTs or CNF suspensions and the solvents were evaporated to dryness at the room temperature. Then, electropolymerization of phenolic acids and triphenylmethane dyes was performed in potentiodynamic mode. Poly(gallic acid) layer was obtained at the surface of GCE with 1 mm diameter, other polymeric coverage at GCE of 3 mm diameter. All monomers studied undergone irreversible electrooxidation with one step (two steps for the ellagic acid) on the anodic branches on the cyclic voltammograms (Table 1) that correspond to the oxidation of phenolic moiety with formation of phenoxyl radical that undergo the following reactions of dimerization and polymerization.

Table 1. Oxidation potentials of monomers under consideration.

Monomer	Electrode	Supporting Electrolyte	pH	E (V)
Gallic acid	MWCNTs/GCE	Phosphate buffer	6.0	0.46
Ellagic acid		Phosphate buffer	7.0	0.29 and 0.50
Aluminon	f-SWCNTs/GCE	0.1 M NaOH	13.0	0.50
Thymolphthalein	CNF/GCE	Phosphate buffer	7.0	0.57

The oxidation currents were gradually decreased up to full disappearance as the number of cycles increased that agreed with the reported data for the same and other phenol-containing compounds [2–6] and indicated formation of insulating polymeric coverage. The electropolymerization conditions (monomer concentration, supporting electrolyte type and pH, potential scan rate and range, number of cycles) were optimized. The corresponding data are summarized in Table 2.

Table 2. Optimized conditions of phenolic acids and triphenylmethane dyes electropolymerization.

Monomer	Electrode	$c_{monomer}$ (μM)	Supporting Electrolyte	pH	Number of Cycles	Potential Range (V)	Potential Scan Rate (V s^{-1})
Gallic acid	MWCNTs/GCE	10	Phosphate buffer	6.0	15	−0.2–1.0	0.10
Ellagic acid		10	Phosphate buffer	7.0	7	0.0–1.0	0.10
Aluminon	f-SWCNTs/GCE	100	0.1 M NaOH	13.0	10	0.1–0.8	0.10
Thymolphthalein	CNF/GCE	100	Phosphate buffer	7.0	10	0.0–1.7	0.075

The electrode surface has been characterized with SEM, cyclic voltammetry, chronoamperometry and EIS. Polymeric coverages exhibit porous structure with the shape of particles and their aggregates (folded structure with pores and channels in the case of polyaluminon) deposited on the surface of carbon nanomaterials (Figure 2). SEM data confirm high roughness of the polymer-modified electrodes and increase of their surface area.

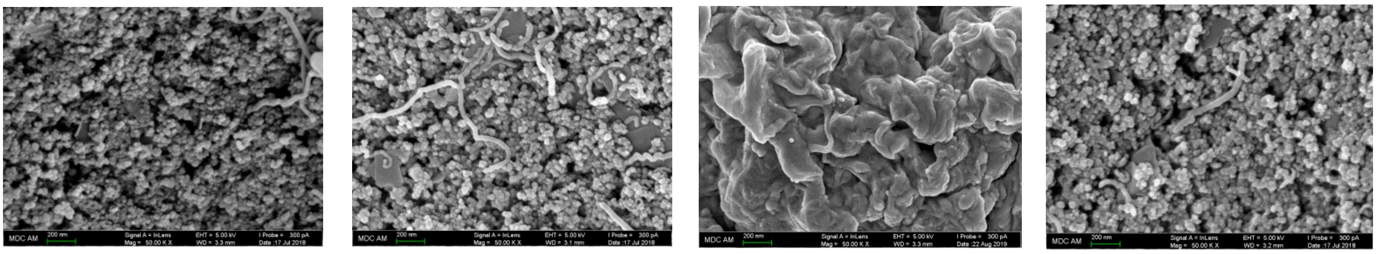
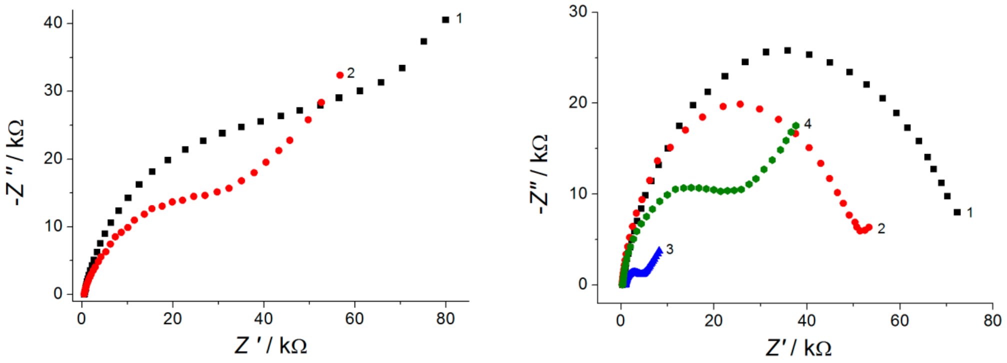

(**a**) (**b**) (**c**) (**d**)

Figure 2. SEM images of the electrode surface: (**a**) poly(gallic acid)/MWCNTs/GCE; (**b**) poly(ellagic acid)/MWCNTs/GCE; (**c**) polyaluminon/f-SWNTs/GCE; (**d**) polythymolphthalein/CNF/GCE.

Electrochemical data for $[Fe(CN)_6]^{4-}$ ions oxidation indicates also statistically significant (2.3–8.9-fold) increase of the electroactive surface area of the polymer-based electrodes vs. bare GCE. As EIS spectra show (Figure 3), a statistically significant decrease of the semicircle diameter i.e., of the charge transfer resistance for the modified electrodes confirm higher electron transfer rate. Thus, the electropolymerized coverages based on the phenolic acids and triphenylmethane dyes are perspective materials for the application in electroanalysis.



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

Figure 3. Electrochemical impedance spectra in the presence of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in neutral medium for bare GCE (1) and polymer-modified electrode: (**a**) poly(gallic acid)/MWCNTs/GCE (2); (**b**) poly(ellagic acid)/MWCNTs/GCE (2); (**c**) polyaluminon/f-SWNTs/GCE (3); (**d**) polythymolphthalein/CNF/GCE (4).

3.2. *Voltammetric Sensing of the Antioxidants*

The electrodes have shown a sensitive and selective response to different classes of the antioxidants (capsaicinoids, flavanones (hesperidin and naringin) and flavonols (rutin and quercetin)). The electrooxidation parameters of the antioxidants have been found (Table 3) on the basis of cyclic voltammetry data at various pH of the supporting electrolyte and potential scan rate. All analytes are oxidized with the participation of equal number of protons and electrons. Electrooxidation of capsaicinoids, naringin, and flavonols is a surface-controlled process. The diffusion-driven electrochemical process has been confirmed for the hesperidin and naringin at the polyaluminon/f-SWCNTs/GCE. 4-Hydroxy-3-methoxyphenyl fragments of capsaicinoids, 3′-hydroxy-4′-methoxyphenyl fragment of hesperidin, hydroxyl group and catechol fragment in the ring B of naringin and flavonols, respectively, undergo electrooxidation with formation of *o*-quinone fragments that agree to the reported data [7–10].

Table 3. Electrooxidation parameters of the capsaicinoids and phenolic antioxidants at the polymer-modified electrodes.

Antioxidant	**Electrode**	**Supporting Electrolyte**	α_a [1]	n [2]	D ($cm^2\ s^{-1}$) [3]	Γ ($pmol\ cm^{-2}$) [4]	k_s (s^{-1}) [5]
Capsaicin	Poly(gallic acid)/MWCNTs/GCE	BRB [6] pH 2.0	0.53	2		33 ± 1	$(2.4 \pm 0.3) \times 10^2$
Dihydrocapsaicin			0.49	2		22.4 ± 0.7	$(2.1 \pm 0.2) \times 10^2$
Nonivamide			0.51	2		21.5 ± 0.7	$(1.3 \pm 0.1) \times 10^2$
Naringin	Poly(ellagic acid)/MWCNTs/GCE	PB [7] pH 6.5	0.52	2		16.6 ± 0.6	$(1.66 \pm 0.08) \times 10^2$
Hesperidin	Polyaluminon/f-SWCNTs/GCE	PB pH 5.0	0.51	2	$(2.6 \pm 0.3) \times 10^{-6}$		
Naringin			0.40	2	$(1.3 \pm 0.1) \times 10^{-6}$		
Quercetin	Polythymolphthalein/CNF/GCE	PB pH 7.5	0.67	2		20.9 ± 0.8	$(1.2 \pm 0.1) \times 10^2$
Rutin			0.90	2		82 ± 3	$(3.5 \pm 0.2) \times 10^2$

[1] anodic transfer coefficient, [2] number of electrons transferred, [3] diffusion coefficient, [4] surface coverage, [5] standard heterogeneous rate constant, [6] Britton-Robinson buffer, [7] phosphate buffer.

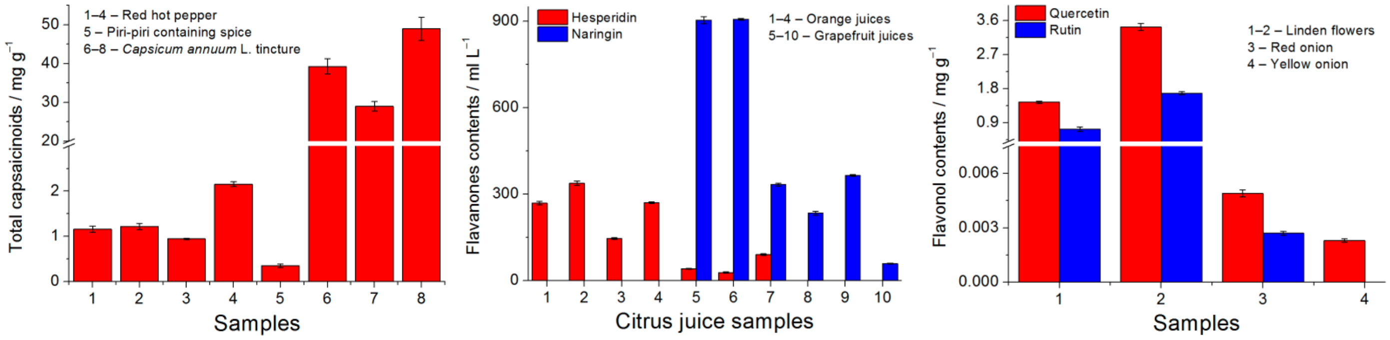
Novel voltammetric sensors based on the electropolymerized phenolic acids and triphenylmethane dyes for the quantification of antioxidants worked in differential pulse mode. The possibility of selective simultaneous quantification of flavanones (hesperidin and naringin) and flavonols (quercetin and rutin) has been achieved. The analytical characteristics of natural antioxidants (Table 4) are improved vs. reported earlier for other electrochemical sensors. Furthermore, high selectivity of the sensors response to target analytes in the presence of other natural phenolics and ascorbic acid is important advantage of the sensors developed allowing their practical application.

Table 4. Analytical characteristics of natural antioxidant determination using polymer-based sensors.

Antioxidant	**Detection Limit (nM)** [1]	**Linear Dynamic Range (μM)**
Capsaicin	2.9	0.010–1.0 and 1.0–50
Dihydrocapsaicin	5.9	0.025–0.75 and 0.75–75
Nonivamide	6.1	0.050–5.0 and 5.0–75
Naringin	14	0.050–1.0 and 1.0–100
Hesperidin	20	0.10–2.5 and 2.5–25
Naringin	62	0.10–2.5 and 2.5–25
Quercetin	7.3	0.025–1.0
Rutin	4.7	0.025–1.0

[1] detection limit is calculated as $3SD_a/b$, where SD_a is the standard deviation of the calibration graph intercept and b is the slope.

Polymer-based sensors have been successfully tested on food and plant samples analysis. Total capsaicinoids contents have been evaluated in spices of red hot pepper and *Capsicum annuum* L. tinctures (Figure 4a). Flavonols (hesperidin and naringin) have been measured in fresh and commercial orange and grapefruit juices. Samples 1–7 were analyzed using polyaluminon-based sensor for the simultaneous determination of flavanones while poly(ellagic acid)-based sensor for naringin was tested on the samples 8–10 (Figure 4b). Quantification of quercetin and rutin has been performed in red and yellow anions and linden (*Tilia* L.) flowers (Figure 4c).



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

Figure 4. Quantification of antioxidants in food and plant samples: (**a**) total capsaicinoids in spices of red hot pepper and *Capsicum annuum* L. tinctures at poly(gallic acid)/MWCNTs/GCE in Britton-Robinson buffer pH 2.0; (**b**) hesperidin and naringin in citrus juices at polyaluminon/f-SWCNTs/GCE in phosphate buffer pH 5.0 and at poly(ellagic ac-id)/MWCNTs/GCE in phosphate buffer pH 6.5; (**c**) quercetin and rutin in linden (*Tilia* L.) flowers and onion at polythymolphthalein/CNF/GCE in phosphate buffer pH 7.5.

Thus, electropolymerized phenol-containing compound/carbon nanomaterial composites can be considered as a promising sensing platform in the antioxidants electroanalysis.

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 upon 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.

References

1. Da Silva, L.V.; de Almeida, A.K.A.; Xavier, J.A.; Lopes, C.B.; dos Santos Silva, F.A.; Lima, P.R.; dos Santos, N.D.; Kubota, L.T.; Goulart, M.O.F. Phenol based redox mediators in electroanalysis. *J. Electroanal. Chem.* **2018**, *827*, 230–252.
2. Ziyatdinova, G.; Guss, E.; Yakupova, E. Electrochemical sensors based on the electropolymerized natural phenolic antioxidants and their analytical application. *Sensors* **2021**, *21*, 8385.
3. Abdel-Hamid, R.; Newair, E.F. Voltammetric determination of polyphenolic content in pomegranate juice using a poly(gallic acid)/multiwalled carbon nanotube modified electrode. *Beilstein, J. Nanotechnol.* **2016**, *7*, 1104–1112.
4. Saritha, D.; Reddy, A.V.B.; Venu, M.; Madhuri, C.; Kiranmai, S.; Anitha, K.; Madhavi, G. Fabrication, characterization and development of a modified poly(bromocresol purple/multiwalled carbon nanotubes) carbon paste electrode for the determination of sulfanilic acid. *Anal. Bioanal. Electrochem.* **2019**, *11*, 123–136.
5. Ziyatdinova, G.; Guss, E.; Budnikov, H. Selective electrochemical sensor based on the electropolymerized *p*-coumaric acid for the direct determination of L-cysteine. *Electrochim. Acta* **2018**, *270*, 369–377.
6. Ziyatdinova, G.; Zhupanova, A.; Davletshin, R. Simultaneous determination of ferulic acid and vanillin in vanilla extracts using voltammetric sensor based on electropolymerized bromocresol purple. *Sensors* **2022**, *22*, 288.
7. Wang, M.Y.; Zhang, D.; Tong, Z.; Xu, X.; Yang, X. Voltammetric behavior and the determination of quercetin at a flowerlike Co_3O_4 nanoparticles modified glassy carbon electrode. *J. Appl. Electrochem.* **2011**, *41*, 189–196.
8. Yardim, Y. Sensitive detection of capsaicin by adsorptive stripping voltammetry at a boron-doped diamond electrode in the presence of sodium dodecylsulfate. *Electroanalysis* **2011**, *23*, 2491–2497.

9. Sims, M.J.; Li, Q.; Kachoosangi, R.T.; Wildgoose, G.G.; Compton, R.G. Using multi-walled carbon nanotube modified electrodes for the adsorptive striping voltammetric determination of hesperidin. *Electrochim. Acta* **2009**, *54*, 5030–5034.
10. Masek, A.; Zaborski, M.; Chrzescijanska, E. Electrooxidation of flavonoids at platinum electrode studied by cyclic voltammetry. *Food Chem.* **2011**, *127*, 699–704.