engineering proceedings

MDPI

Proceeding Paper

Electrode Based on the Manganese Dioxide Nanorods and Hexadecylpyridinium Bromide for the Rosmarinic Acid Voltammetric Assay †

Guzel Ziyatdinova*

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

† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/.

Abstract: Nanostructures of transition metal oxides have been shown as effective sensing layers of the electrodes used in the electroanalytical chemistry. Manganese dioxide nanorods (MnO_2 NRs) are of interest and have been applied in colorant electroanalysis. An electrode modified with MnO_2 NRs prepared in hexadecylpyridinium bromide (HDPB) medium is developed for rosmarinic acid quantification. The application of HDPB as dispersive agent provides stabilization of nanomaterial suspension in water medium. The electrode developed gives an improved response to rosmarinic acid, i.e. 60 mV redox peak potential separation and 1.7-fold increased redox currents have been observed. Quasi-reversible electrooxidation controlled by surface processes has been confirmed. The analytical response of rosmarinic acid has been obtained by differential pulse voltammetry (DPV) in Britton-Robinson buffer (BRB) pH 5.0. Method makes possible rosmarinic acid determination from 2.5×10^{-8} to 1.0×10^{-6} M and from 1.0×10^{-6} to 1.0×10^{-5} M and provides detection limit equaled to 9.7×10^{-9} M. These characetristics are improved vs. reported electrochemical approaches. The selectivity of the electrode response to rosmarinic acid is shown using 1000-fold excess of inorganic ions, 100-fold excesses of saccharides, and 10-fold excesses of ascorbic and *p*-coumaric acids, eugenol, carvacrol, and thymol. Other phenolic acids (gallic, ferulic, caffeic) and flavonoids (quercetin, rutin) give an interference effect. Rosemary spices has been studied to prove practical applicability of the MnO_2 NRs-based electrode.

Keywords: modified electrodes; voltammetry; metal oxide nanomaterials; natural phenolics; rosmarinic acid

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

Copyright: © 2023 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Rosmarinic acid (Figure 1) is a natural phenolic compound produced mainly by plants of Lamiaceae family (genus *Salvia, Lavandula, Ocimum, Melissa, Origanum*, and *Thymus*) as well as by other higher plants including ferns [1].

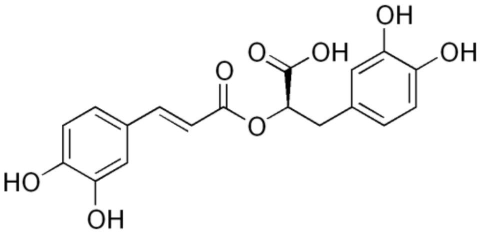


Figure 1. Rosmarinic acid.

Being bioactive compound, rosmarinic acid causes a positive health effect of medicinal and culinary herbs [1,2]. Thus, the determination of rosmarinic acid is of practical interest.

This phenolic acid contains two catechol rings which make it electroactive. This allows to use voltammetry and amperometry for analytical purposes. Various types of nanomaterials are used as electrode surface modifiers to provide sufficient sensitivity and selectivity of rosmarinic acid determination. Nevertheless, only several modified electrodes have been developed to date. Carbon paste electrode modified with carbon nanotubes dispersed in chitosan and immobilized DNA gives liner response to 4.0×10^{-8}–1.5×10^{-6} M of rosmarinic acid with the detection limit equaled to 1.4×10^{-8} M [3]. The carbon nanotube paste electrode with *n*-octyl-pyridinium hexafluorophosphate allows determination of 0.0–6.8×10^{-4} M and detection limit of 1.5×10^{-8} M [4]. Carbon paste electrode with incorporated heterodinuclear complex [Fe^{III}Zn^{II}(μ-OH)(2-[bis(2-pyridylmethyl)aminomethyl]-6-[(2-hydroxy-5-methylbenzyl)(2-pyridyl-methyl)aminomethyl]-4-methylphenol)](ClO$_4$)$_2$ shows long-term stability of rosmarinic acid determination in square-wave mode. Analytical dynamic range of 2.98×10^{-5}–3.83×10^{-4} M and the detection limit equaled to 2.3×10^{-6} M have been achieved [5]. More complex glassy carbon electrode (GCE) modification based on the layer-by-layer combination of poly(*o*-phenylenediamine) and platinum nanoparticles does not show significant improvement in rosmarinic acid analytical characteristics ($(1–55)\times10^{-6}$ M dynamic range and detection limit 5×10^{-7} M) [6]. The most sensitive response to rosmarinic acid within 1×10^{-7}–1×10^{-4} M and 1×10^{-4} –5×10^{-4} M gives carbon paste electrode based on the nanostructures of magnetic functionalized molecularly imprinted polymer in particular, $Fe_3O_4@SiO_2@NH_2$ nanoparticles. A low detection limit of 8.5×10^{-8} M have been achieved [7].

The application of metal oxide nanomaterials in combination with surfactants as electrode surface modifier could be a favourable approach in rosmarinic electroanalysis. Among them, manganese dioxide nanorods (MnO_2 NRs) are a perspective one for the application in the electroanalysis due to the improved electron transfer rate, high effective surface area, low toxicity, and low-priced [8]. The application of hexadecylpyridinium bromide (HDPB) surfactant media as a dispersive agent for MnO_2 NRs gives stable suspension of nanomaterials. On the other hand, surfactant HDPB is co-immobilized at the surface of the electrode as has been recently shown on example of synthetic colorants [9].

The aim of this study is to develop a highly sensitive rosmarinic acid voltammetric assay using electrode based on the MnO_2 NRs and HDPB.

2. Materials and Methods

Stock 10 mM ethanolic solution of rosmarinic acid (96% purity reagent from Sigma-Aldrich (Steinheim, Germany)) was used. An exact appropriate dilution was applied if necessary. Other chemicals were of c.p. grade.

MnO_2 NRs (99%, $ø \times L$ = 5–30 nm × 80–100 nm) from Sigma-Aldrich (Steinheim, Germany) were used. Their 1 mg mL^{-1} homogeneous suspension was obtained in 1.0×10^{-3} M HDPB water solution (obtained from 98% HDPB from Aldrich (Steinheim, Germany)) by 40 min sonication in the ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea).

4 μL of MnO_2 NRs suspension were drop-casted for electrode modification and evaporated to dryness of the solvent under ambient conditions was performed. Electrode surface renewal was performed after each measurement by cleaning on the alumina slurry (0.05 μm particle size).

Voltammetric measurements were performed at the potentiostat/galvanostat Autolab PGSTAT 12 (Eco Chemie B.V., Utrecht, The Netherlands) and the NOVA 1.10.1.9 software (Eco Chemie B.V., Utrecht, The Netherlands). A three-electrode system consisted of GCE (ø = 3 mm, CH Instruments, Inc., Bee Cave, TX, USA), or a MnO_2 NRs–HDPB/GCE as working electrode, Ag/AgCl reference electrode and a platinum wire as an auxiliary electrode was used.

The "Expert-001" pH meter (Econix-Expert Ltd., Moscow, Russia) with a glassy electrode was applied for the pH evaluation.

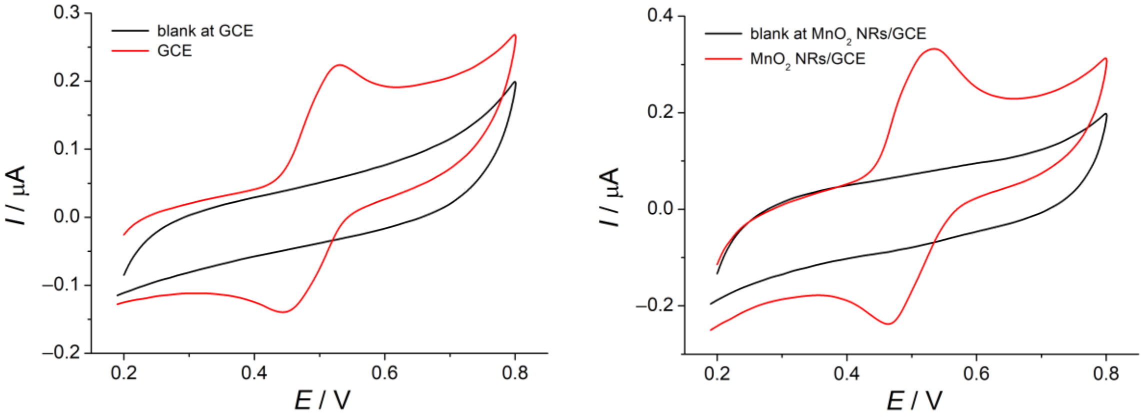
Commercially available rosemary spices were studied. Rosmarinic acid extraction was performed by single ultrasound-assisted extraction with ethanol (rectificate). Extraction was optimized using oxidation currents of the extract obtained. The best extraction yield was got at 1:30 plant material/extragent ratio for 10 min of extraction time.

Statistical treatment was performed for five replications at *P* = 0.95. The results were shown as an average value ± coverage interval.

3. Results and Discussion

3.1. Voltammetric Characteristics of Rosmarinic Acid

The oxidation characteristics of rosmarinic acid in Britton-Robinson buffer (BRB) pH 2.0 were studied by cyclic voltammetry. Reversible redox pare was observed at bare GCE (Figure 1a).



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

Figure 2. Cyclic voltammograms of 5.0×10^{-6} M rosmarinic acid in BRB pH 2.0 at the: (**a**) bare GCE; (**b**) MnO_2 NRs–HDPB/GCE. Potential scan rate is 0.10 V s^{-1}.

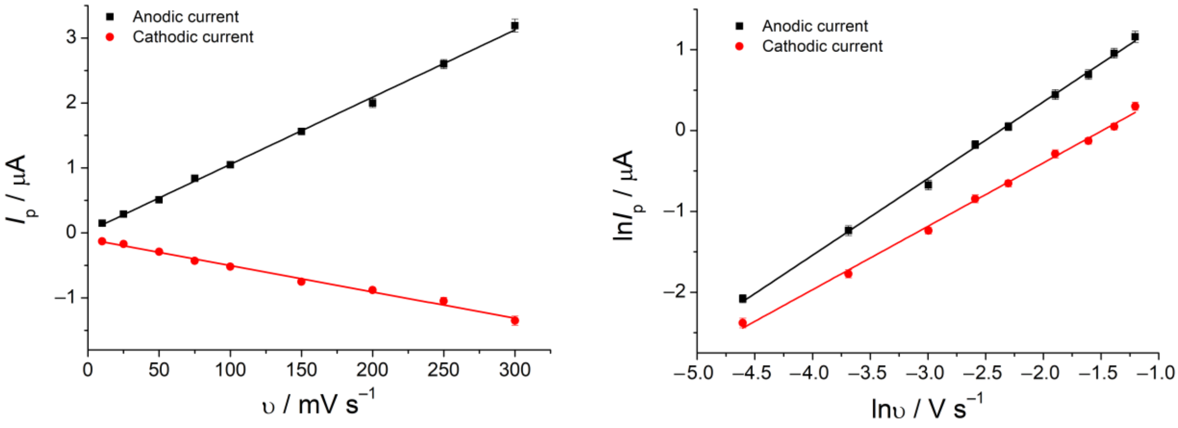
In the case of MnO_2 NRs–HDPB/GCE, the redox peak potential separation was kept the same although 10 mV anodic shift of both peaks was observed (Figure 1b) that is negligible taking into account the accuracy of the potential measurement. The redox peak currents are 1.6–1.7-fold increased compared to those ones at bare GCE (Table 1) that confirms improved response to rosmarinic acid of the modified electrode.

Table 1. Voltammetric characteristics of rosmarinic acid in BRB pH 2.0 at various electrodes (*n* = 5; *P* = 0.95).

Electrode	E_a (mV)	I_a (μA)	E_c (mV)	I_c (μA)	I_c/I_a
Bare GCE	522	0.11 ± 0.01	462	0.10 ± 0.005	0.91
MnO_2 NRs–HDPB/GCE	532	0.180 ± 0.005	472	0.170 ± 0.004	0.94

Varying BRB pH, the shift of rosmarinic acid redox peaks potentials to less positive values was observed. This fact proves protons involvement in the redox process occurred. Oxidation currents gradually increased in strong acidic medium. The maximal currents were obtained in BRB pH 5.0. Further pH increase showed decrease of the redox currents which was more pronounces at pH 7.0 and higher pH values due to the rosmarinic acid oxidation by air oxygen. This behavior usually takes place for natural phenolic compounds [10]. BRB pH 5.0 was used in subsequent investigations.

The investigation of the potential scan rate effect on the redox behavior of rosmarinic acid showed that electrooxidation proceeded quasi-reversible as a redox peak potential separation and currents ratio indicated. The redox peak currents were proportional to potential scan rate (Figure 3a) and the slopes (0.97 and 0.81 for the anodic and cathodic peaks, respectively) for the plots ln*I* vs. lnυ ((Figure 3b) confirm surface-controlled electrooxidation of rosmarinic acid.



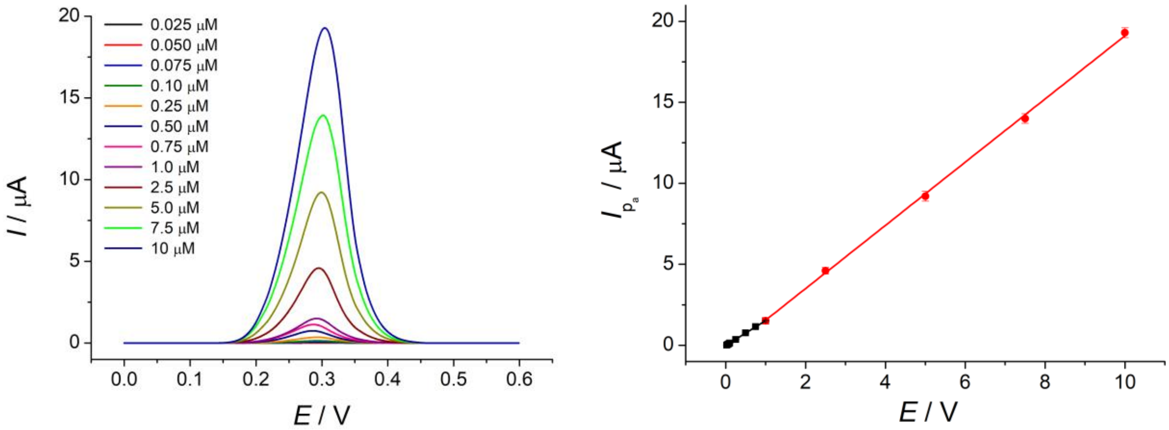
(**a**) (**b**)

Figure 3. Dependence of the 1.0×10^{-5} M rosmarinic acid redox currents at the MnO_2 NRs–HDPB/GCE in BRB pH 5.0 on potential scan rate: (**a**) $I = f(\upsilon)$; (**b**) $\ln I = f(\ln\upsilon)$.

3.2. Determination of Rosmarinic Acid using MnO_2 NRs–HDPB/GCE

The analytical response of rosmarinic acid has been obtained by differential pulse voltammetry (DPV) in BRB pH 5.0. The effect of pulse parameters on the oxidation currents was evaluated and the maximum currents were obtained using pulse amplitude of 0.075 V and pulse time of 0.025 s.

Rosmarinic acid oxidation peak was observed at 290 mV which height linearly increased with concentration growth from 2.5×10^{-8} to 1.0×10^{-6} M and from 1.0×10^{-6} to 1.0×10^{-5} M (Figure 4) with a detection limit of 9.7 nM that were significantly improved compared to other electrochemical methods using modified electrodes [3–7] (Table S1). The method developed showed high accuracy as confirmed by the recovery of 99–100% in the model solutions of rosmarinic acid.



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

Figure 4. (**a**) Baseline-corrected DPVs of rosmarinic acid at the MnO_2 NRs–HDPB/GCE in BRB pH 5.0. Pulse amplitude is 0.075 V, pulse time is 0.025 s, potential scan rate is 0.010 V s^{-1}; (**b**) Calibration plots of rosmarinic acid.

The selectivity test was performed using standard components contained in plant materials. Inorganic ions (K^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^-, and SO_4^{2-}) up to 1.0×10^{-3} M and saccharides (fructose, rhamnose, glucose, sucrose) up to 1.0×10^{-4} M did not affect response of 1.0×10^{-6} M rosmarinic acid. Ascorbic, tannic, and phenolic acids, flavonoids, eugenol, and isopropylmethylphenols are oxidized at the MnO_2 NRs–HDPB/GCE. Peak potential separation for rosmarinic and ascorbic acids equaled to 120 mV and there was no oxidation peak overlap up to 1.0×10^{-5} M of ascorbic acid in the mixture. Eugenol, *p*-coumaric acid, carvacrol, and thymol oxidized at more positive potentials (520, 670, 680, and 680 mV, respectively) and their 10-fold excess did not interfere rosmarinic acid determination. Other phenolic acids (gallic, ferulic, caffeic), flavonoids (quercetin, rutin), and tannic acid gave an interference effect.

Summarizing the selectivity study, the total response of easy oxidizable phenolics will be registered on the MnO_2 NRs–HDPB/GCE for plant samples containing usually several classes of natural phenolic compounds.

3.2.1. Rosemary Spices Analysis

The rosemary ethanolic extracts exhibited oxidation peaks at 300 and 550 mV (Figure 5), which are fully resolved. The stretched shape of the first peak descending part with a weakly pronounced shoulder at 330-380 mV that indicated the impact of other compounds to the oxidation peak and was confirmed by standard addition method. The recovery of 85–87% was obtained and agreed well with the chemical composition of rosemary, in particular, phenolic acids and flavonoids [11–13] that interfered rosmarinic acid determination.

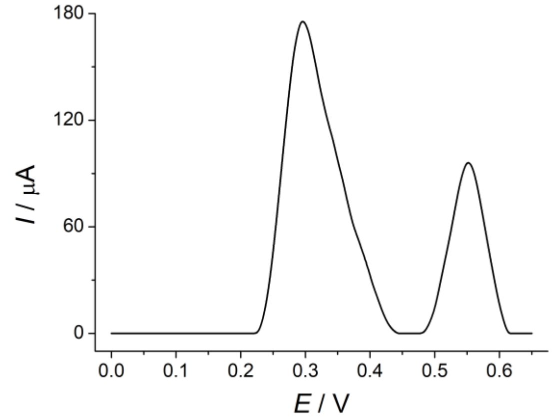


Figure 5. Baseline-corrected DPVs of 50 µL rosemary extract at the MnO_2 NRs–HDPB/GCE in BRB pH 5.0. Conditions of DPV are similar to Figure 4.

Therefore, the first oxidation peak of rosemary extract could be applied for the spice antioxidant capacity assay using rosmarinic acid equivalents. Corresponding data for rosemary of different trademarks are presented in Table 2.

Table 2. Antioxidant capacity of rosemary in rosmarinic acid equivalents based on the voltammetric determination using MnO_2 NRs–HDPB/GCE in BRB pH 5.0 ($n = 5$; $P = 0.95$).

Rosemary sample	**Antioxidant capacity (µg of rosmarinic acid g^{-1})**	**RSD (%)**
1	219 ± 7	2.5
2	737 ± 27	3.0
3	186 ± 5	2.0

Thus, highly sensitive rosmarinic acid voltammetric assay was developed using MnO_2 NRs–HDPB/GCE. The practical applicability was demonstrated on the rosemary spices antioxidant capacity measuring.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Comparison of rosmarinic acid analytical characteristics on various electrodes.

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.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Petersen, M.; Simmonds, M.S.J. Rosmarinic acid. *Phytochem.* **2003**, *62*, 121–125.
2. Fachel, F.N.S.; Pra, M.D.; Azambuja, J.H.; Endres, M.; Bassani, V.L.; Koester, L.S.; Henriques, A.T.; Barschak, A.G.; Teixeira, H.F.; Braganhol, E. Glioprotective effect of chitosan-coated rosmarinic acid nanoemulsions against lipopolysaccharide-induced inflammation and oxidative stress in rat astrocyte primary cultures. *Cell. Mol. Neurobiol.* **2020**, *40*, 123–139.
3. Mohamadi, M.; Mostafavi, A.; Torkzadeh-Mahani, M. Voltammetric determination of rosmarinic acid on chitosan/carbon nanotube composite-modified carbon paste electrode covered with DNA. *J. Electrochem. Soc.* **2015**, *162*, B344–B349.
4. Wang, K.; Cui, X.; Zheng, Y.; Liu, B.; Sang, H.; Dong, R. Electrochemical determination of rosmarinic acid in edible flowers using ionic liquid modified electrode. *Int. J. Electrochem. Sci.* **2022**, *17*, 221284.
5. Santhiago, M.; Peralta, R.A.; Neves, A.; Micke, G.A.; Vieira, I.C. Rosmarinic acid determination using biomimetic sensor based on purple acid phosphatase mimetic. *Anal. Chim. Acta* **2008**, *613*, 91–97.
6. Özdokur, K.V.; Koçak, Ç.C. Simultaneous determination of rosmarinic acid and protocatechuic acid at poly(*o*-phenylenediamine)/Pt nanoparticles modified glassy carbon electrode. *Electroanalysis* **2019**, *31*, 2359–2367.
7. Alipour, S.; Azar, P.A.; Husain, S.W.; Rajabi, H.R. Determination of rosmarinic acid in plant extracts using a modified sensor based on magnetic imprinted polymeric nanostructures. *Sens. Actuat. B* **2020**, *323*, 1–11.
8. Tehseen, B.; Rehman, A.; Rahmat, M.; Bhatti, H.N.; Wu, A.; Butt, F.K.; Naz, G.; Khan, W.S.; Bajwa, S.Z. Solution growth of 3D MnO_2 mesh comprising 1D nanofibres as a novel sensor for selective and sensitive detection of biomolecules. *Biosens. Bioelectron.* **2018**, *117*, 852–859.
9. Gimadutdinova, L.; Ziyatdinova, G.; Davletshin, R. Selective voltammetric sensor for the simultaneous quantification of tartrazine and brilliant blue FCF. *Sensors* **2023**, *23*, 1094.
10. Ziyatdinova, G.; Budnikov, H. Natural phenolic antioxidants in bioanalytical chemistry: State of the art and prospects of development. *Russ. Chem. Rev.* **2015**, *84*, 194–224.
11. Mena, P.; Cirlini, M.; Tassotti, M.; Herrlinger, K.A.; Dall'Asta, C.; Del Rio, D. Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (*Rosmarinus officinalis* L.) extract. *Molecules* **2020**, *21*, 1576.
12. Hcini, K.; Sotomayor, J.A.; Jordan, M.J.; Bouzid, S. Identification and quantification of phenolic compounds of tunisian *Rosmarinus officinalis* L. *Asian J. Chem.* **2013**, *25*, 9299–9301.
13. Borrás-Linares, I.; Stojanović, Z.; Quirantes-Piné, R.; Arráez-Román, D.; Švarc-Gajić, J.; Fernández-Gutiérrez, A.; Segura-Carretero, A. *Rosmarinus officinalis* leaves as a natural source of bioactive compounds. *Int. J. Mol. Sci.* **2014**, *15*, 20585–20606.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.