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Electropolymerized Dyes as Sensing Layer for Natural Phenolic Antioxidants of Essential Oils †

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Abstract: Essential oils are widely used in aromatherapy, food, and pharmaceutical industries. They contain a range of electroactive natural phenolic antioxidants like eugenol, *trans*-anethole, thymol, carvacrol, and vanillin. Therefore, the sensitive voltammetric determination of these compounds is of practical interest. Voltammetric sensors based on the layer-by-layer combination of carbon nanotubes and electropolymerized dyes were developed. Pyrogallol red, mixture of phenol red and *p*-coumaric acid, thymolphthalein, bromocresol purple were used as monomers. The created sensors were used in the quantification of target analytes using differential pulse voltammetry in Britton-Robinson buffer. The detection limits in the range of 3.7×10^{-8}–7.3×10^{-7} M were achieved.

Keywords: electrochemical sensor; electropolymerization; dyes; phenolic antioxidants; plant materials

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1. Introduction

Essential oils have a wide application in aromatherapy as part of alternative medicine, in the food, pharmaceutical, and cosmetic industry as fragrance and flavor additives [1–3]. The antibacterial, antimicrobial, antiviral, and antioxidant properties of essential oils are caused by the presence of bioactive compounds including natural phenolic antioxidants such as eugenol, *trans*-anethole, thymol, carvacrol, vanillin, etc. [4]. The noticeable prooxidant effect is typical for low-molecular antioxidants such as phenolic compounds when presented in high concentration [5]. Thus, quantification of these marker compounds in essential oils is of practical necessity. The presence of electroactive fragments in the phenolic antioxidants structure makes it possible to use voltammetry for their determination. However, the number of voltammetric sensors for the determination of individual antioxidants in essential oils is quite limited [6–14]. Almost all of them are based on the application of electrode surface modifiers, among which the polymeric coverages are out of consideration.

Recently, the effectivity of electropolymerized triphenylmethane dyes as sensing layer for antioxidants including phenolic compounds has been shown [15–21]. Thus, the current work deals with the development of novel voltammetric sensors for the quantification of natural phenolic antioxidants of essential oils using electropolymerized dyes as a sensing layer. Triphenylmethane dyes (pyrogallol red, thymolphthalein, bromocresol purple, and phenol red) have been used as monomers and *p*-coumaric acid as a co-monomer. Layer-by-layer combination with carbon nanomaterials (single- (SWCNTs) or multi-walled nanotubes (MWCNTs)) has been applied to provide sufficient electroconductivity of the electrodes. The surface and electron transfer parameters of the developed sensors have been characterized by scanning electron microscopy (SEM), voltammetry, and electrochemical impedance spectroscopy.

2. Materials and Methods

Thymol (99.5% purity) from Sigma (Steinheim, Germany), 98% carvacrol, 99% vanillin, and 99% eugenol from Aldrich (Steinheim, Germany), 98% *trans*-anethole from TCI (Tokyo, Japan) were used as standards. Their 1.0×10^{-2} M stock solutions were prepared in ethanol (rectificate) or methanol (c.p.) and stored at +4 °C. Thymolphthalein (95% purity) and 98% *p*-coumaric acid from Sigma (Steinheim, Germany), phenol red from Sigma-Aldrich (St. Louis, MO, USA), pyrogallol red and 90% bromocresol purple from Sigma-Aldrich (Steinheim, Germany) were used as monomers. Their 1.0×10^{-2} M (1.0×10^{-3} M for pyrogallol red) stock solutions were prepared in ethanol (methanol for thymolphthalein). The exact dilution was used for the preparation of less concentrated solutions.

Other reagents were of c.p. grade and were used as was.

MWCNTs (outer Ø 40–60 nm, inner Ø 5–10 nm, l = 0.5–500 μm), carboxylated MWCNTs (inner Ø 9.5 nm, l = 1.5 μm, carboxylation degree > 8%), polyaminobenzene sulfonic acid functionalized single-walled carbon nanotubes (SWCNTs-f) (Ø 1.1 nm, l = 0.5–1.0 nm) from Aldrich (Steinheim, Germany) were used as a platform for further polymeric coating immobilization. Homogeneous suspensions of MWCNTs (0.5 mg mL^{-1} in 1% sodium dodecylsulfate (Panreac, Barcelona, Spain)), carboxylated MWCNTs (1.0 mg mL^{-1} in 1% sodium dodecylsulfate), and SWCNTs-f (1.0 mg mL^{-1} in dimethylformamide) were prepared by sonication for 30 (for MWCNTs and SWCNTs-f) or 15 (for carboxylated MWCNTs) min in an ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea).

Bare GCE were mechanically polished on the alumina slurry (0.05 μm grain), thoroughly rinsed with acetone and distilled water. Then, 2 μL of carbon nanomaterial suspension was drop casted and evaporated to dryness for 7 (for MWCNTs) or 10 min.

Electropolymerization under conditions of cyclic voltammetry was used for the formation of the polymeric coverage. The working conditions of the process were found for each monomer individually depending on the voltammetric response of the target analyte.

Potentiostats/galvanostats Autolab PGSTAT 302N with the FRA 32M module (Metrohm B.V., Utrecht, The Netherlands) and μAutolab Type III (Eco Chemie B.V., Utrecht, The Netherlands) with NOVA 1.10.1.9 and Nova 1.7.8 software, respectively, were used for the electrochemical measurements. GCE (3 mm diameter) from CH Instruments, Inc. (Bee Cave, TX, USA) or from BASi® Inc. (West Lafayette, IN, USA) and modified electrodes, a reference Ag/AgCl electrode, and auxiliary electrode (platinum wire) were placed in the electrochemical glass cell containing supporting electrolyte and cyclic or differential pulse voltammograms were recorded.

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

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

3. Results and Discussion

3.1. Polymer-Based Sensors Fabrication and Charcaterization

All dyes under study and *p*-coumaric acid (Figure 1) contain phenolic fragments in their structure and are electrochemically active at the GCE modified with carbon nanomaterials. Taking into account that electron detachment proceeds easier from the phenolate ion, the basic medium could be preferable for electropolymerization. However, the partial oxidation of monomers by air oxygen occurs under basic conditions. Therefore, dyes' electropolymerization was performed at neutral pH.

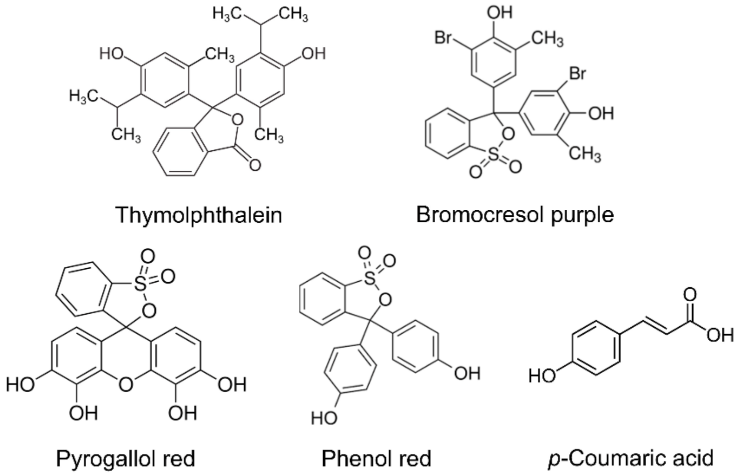
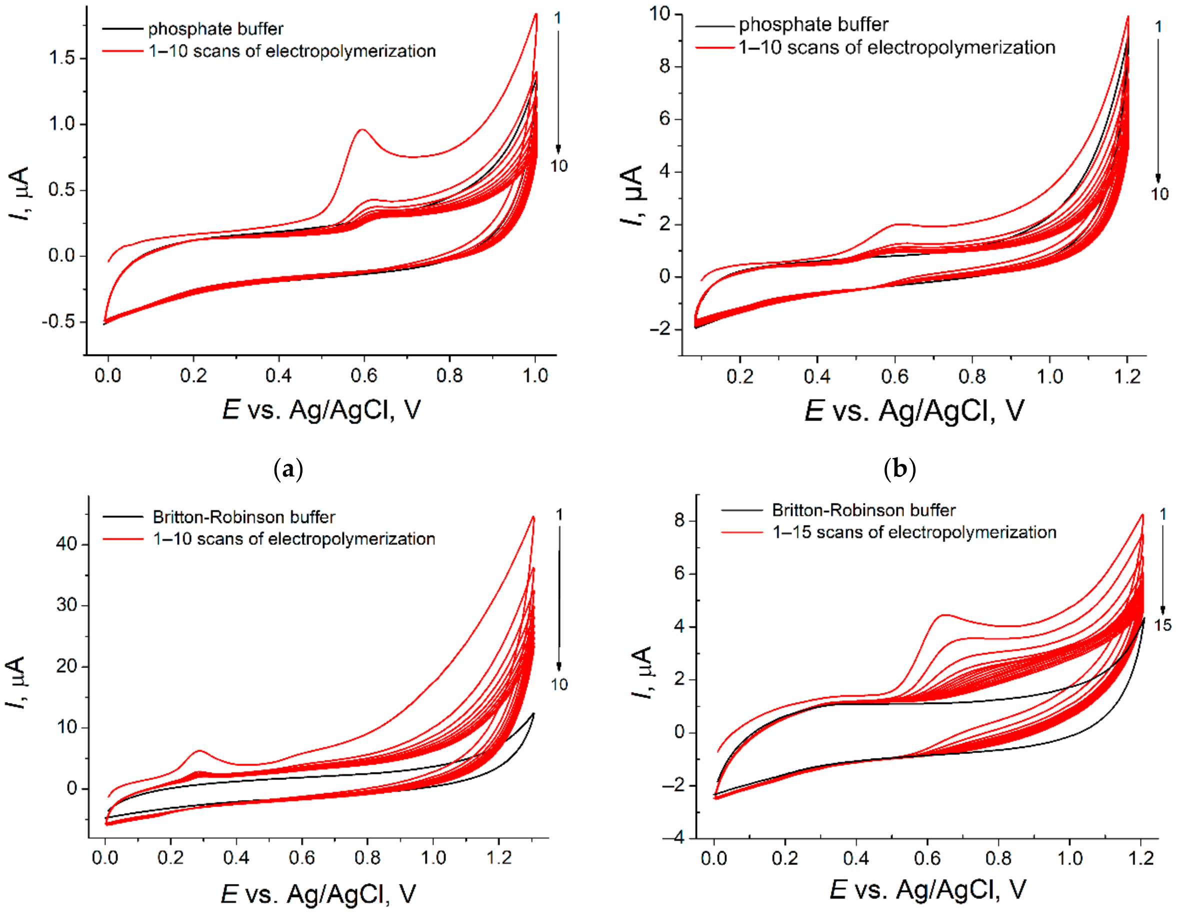


Figure 1. Structure of monomers under study.

There are well-defined oxidation peaks on the cyclic voltammograms (Figure 2, curve 1) which are shifted a bit in the anodic direction on the following scans. The oxidation currents are significantly decreased with the growth of scans number indicating formation of non-conductive coverages.



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

(**c**) (**d**)

Figure 2. Electropolymerization of triphenylmethane dyes: (**a**) 1.0×10^{-5} M thymolphthalein at the MWCNTs/GCE in phosphate buffer pH 7.0; (**b**) 2.5×10^{-5} M bromocresol purple at the SWCNTs–f/GCE in phosphate buffer pH 7.0; (**c**) 1.0×10^{-4} M pyrogallol red at the carboxylated

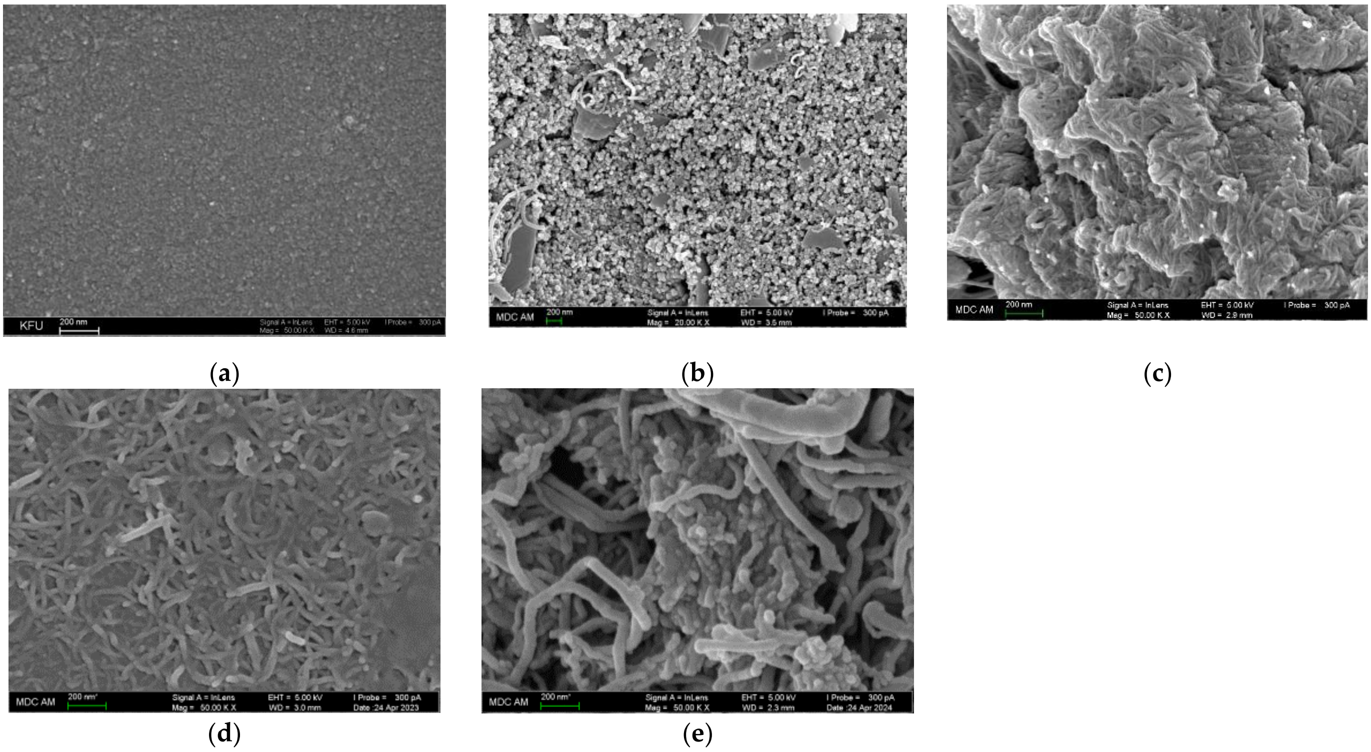
MWCNTs/GCE in Britton-Robinson buffer pH 7.0; (**d**) 1.0×10^{-4} M mixture of phenol red and *p*–coumaric acid at the MWCNTs/GCE in Britton-Robinson buffer pH 7.0.

Electropolymerization conditions (supporting electrolyte, monomer concentration, number of scans, potential range and scan rate) providing the best voltammetric response of the target analyte were found (Table 1). The following analytes were tested: thymol, vanillin, eugenol, and *trans*-anethole at the poly(thymolphthalein)-, poly(bromocresol purple)-, poly(pyrogallol red)-, and poly(phenol red–co–*p*-coumaric acid)-modified electrodes, respectively.

Table 1. Electropolymerization conditions of triphenylmethane dyes ($n = 5$; $p = 0.95$).

Sensing Layer	*c*, M	Number of Scans	Potential Range, V	υ, mV s^{-1}	Supporting Electrolyte
Poly(thymolphthalein)/MWCNTs	1.0×10^{-5}	10	0.0–1.0	100	0.1 M phosphate buffer pH 7.0
Poly(bromocresol purple)/SWCNTs-f	2.5×10^{-5}	10	0.0–1.2	100	
Poly(pyrogallol red)/Carboxylated MWCNTs	1.0×10^{-4}	10	0.0–1.3	75	Britton-Robinson buffer pH 7.0
Poly(phenol red–co–*p*-coumaric acid)/MWCNTs	1.0×10^{-4}	15	0.0–1.2	50	

The surface morphology of the electrodes was checked by SEM (Figure 3). The successful immobilization of the coverages at the modified electrodes was clearly seen vs. bare GCE (Figure 3a).


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

(**d**) (**e**)

Figure 3. SEM images of the sensor surface: (**a**) bare GCE; (**b**) poly(thymolphthalein)/MWCNTs/GCE; (**c**) poly(bromocresol purple)/SWCNTs-f/GCE; (**d**) poly(pyrogallol red)/Carboxylated MWCNTs/GCE; (**e**) poly(phenol red–co–*p*-coumaric acid)/MWCNTs/GCE.

The electrochemical properties of the polymer-modified electrodes were studied using ferro/ferricyanide ions in 0.1 V KCl. The electroactive surface is significantly increased compared to bare GCE (88 ± 5 mm^2 for poly(thymolphthalein)/MWCNTs/GCE, 42 ± 1 mm^2 for poly(bromocresol purple)/SWCNTs-f/GCE, 96 ± 2 mm^2 for poly(pyrogallol red)/Carboxylated MWCNTs/GCE, and 11.4 ± 0.6 mm^2 for poly(phenol red–co–*p*-coumaric acid)/MWCNTs/GCE vs. 8.9 ± 0.3 mm^2 for bare GCE). The heterogeneous electron transfer rate constant calculated from the electrochemical impedance spectroscopy data is in the range from 4.14×10^{-5} to 9.12×10^{-5} cm s^{-1} which confirms the improvement of the electron transfer at the polymer-modified electrodes.

3.2. Sensing of Phenolic Antioxidants

The created electrodes were applied for sensing of natural phenolic antioxidants—marker of essential oils (thymol, vanillin, eugenol, and *trans*-anethole).

The effect of Britton-Robinson buffer pH on voltammetric response parameters was tested for each antioxidant. The highest oxidation currents were observed at pH 2.0 for all studied analytes that agreed well with reported earlier data [5]. The increase in pH lead to slow decease in the oxidation peak current of the antioxidants due to the chemical oxidation with air oxygen.

Under differential pulse voltammetry conditions, the linear response of the sensors toward target phenolic antioxidants was obtained in a wide range of concentrations. The corresponding analytical characteristics are presented in Table 2. The analytical characteristics of the sensors are comparable or improved vs. existing ones [6–14].

Table 2. Figures of merit for voltammetric sensors for natural phenolic antioxidants of essential oils.

Sensor	Analyte	Method	E_{ox}, V	Linear Dynamic Range, M	Detection Limit, M
Poly(thymolphthalein)/MWCNTs/GCE	Thymol	DPV [1]	0.81	5.0×10^{-8}–2.5×10^{-5} 2.5×10^{-5}–1.0×10^{-4}	3.7×10^{-8}
	Carvacrol		0.83	1.0×10^{-7}–1.0×10^{-5} 1.0×10^{-5}–1.0×10^{-4}	6.3×10^{-8}
Poly(bromocresol purple)/SWCNTs-f/GCE	Vanillin	DPV	0.86	1.0×10^{-7}–5.0×10^{-6} 5.0×10^{-6}–2.5×10^{-5}	6.4×10^{-8}
Poly(pyrogallol red)/Carboxylated MWCNTs/GCE	Eugenol	DPV	0.57	7.5×10^{-7}–1.0×10^{-4}	7.3×10^{-7}
Poly(phenol red–co–*p*-coumaric acid)/MWCNTs/GCE	*trans*-Anethole	AdDPV [2]	0.95	1.0×10^{-7}–7.5×10^{-6} 7.5×10^{-6}–7.5×10^{-5}	9.5×10^{-8}

[1] Differential pulse voltammetry, [2] Adsorptive differential pulse voltammetry.

Sensing of natural phenolic antioxidants of essential oils was highly accurate as proved by recovery values (98–102%). The main advantage of the developed sensors is the high selectivity of response in the presence of typical interfering substances and other natural phenolic antioxidants (Table 3).

Table 3. Tolerance limits of interferences for the determination of natural phenolic antioxidants of essential oils using poly(triphenylmethane dye)-modified electrodes.

Interference	Tolerance Limit, M			
	1.0×10^{-6} M Thymol or Carvacrol	**1.0×10^{-6} M Vanillin**	**5.0×10^{-6} M Eugenol**	**1.0×10^{-6} M *trans*-Anethole**
K^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^-, SO_4^{2-}	1.0×10^{-3}	1.0×10^{-3}	5.0×10^{-3}	1.0×10^{-3}
Glucose, rhamnose, sucrose	1.0×10^{-4}	1.0×10^{-4}	5.0×10^{-4}	1.0×10^{-4}
Thymol	—	0	2.5×10^{-5}	2.5×10^{-7}
Cavacrol	—	0	5.0×10^{-5}	2.5×10^{-7}
Vanillin	0	—	5.0×10^{-4}	0

trans-Anethole	1.0×10^{-7}	0	5.0×10^{-4}	—
Eugenol	5.0×10^{-6}	1.0×10^{-4}	—	1.5×10^{-5}
α-Pinene	1.0×10^{-4}	5.0×10^{-5}	5.0×10^{-4}	2.5×10^{-7}
Limonene	1.0×10^{-4}	5.0×10^{-5}	5.0×10^{-4}	5.0×10^{-7}

4. Conclusions

Electropolymerized thriphenylmethane dyes have been shown to be an effective sensing layer for the potential application in electroanalysis of major phenolic antioxidants-markers of essential oils. The sensing system is easy to fabricate, highly reproducible, and provides a sensitive, selective, and reliable response to target analytes. Future development of the topic under study to be focused on the application of the sensors in real samples analysis for their standardization and quality control. Furthermore, the fabrication of screen-printed electrodes as a basis for sensing layer immobilization can significantly simplify the measurements, reduce it cost, and make more attractive for the use in practice.

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

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