Electrode Modified with Carboxylated Multi-Walled Carbon Nanotubes and Electropolymerized Pyrogallol Red for the Determination of Eugenol †

Alena Kalmykova * and Guzel Ziyatdinova

Analytical Chemistry Department, Kazan Federal University, Kremlevskaya, 18, 420008 Kazan, Russia
* Correspondence: alena.kalmykova.pnb.2000@mail.ru
† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/

Abstract: Eugenol is the major component of clove and clove-based products which is widely used in food and pharmaceutical industry, and medicine. Its content can be considered as a marker of the sample quality. Therefore, simple, sensitive, and reliable methods for eugenol quantification are required. Glassy carbon electrode (GCE) modified with carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) and electropolymerized pyrogallol red has been developed for the determination of eugenol in essential oils. The working conditions (supporting electrolyte, pH, monomer concentration and electrolysis parameters) of pyrogallol red have been found using voltammetric characteristics of eugenol. Electrode developed has been studied using scanning electron microscopy, voltammetry and electrochemical impedance spectroscopy. The effectivity of the electrode surface modification has been proved. Eugenol determination has been performed in differential pulse mode which parameters have been optimized. The linear response of the electrode towards eugenol has been obtained in the range of 0.75–100 µM with the detection limit of 0.73 µM. High selectivity of eugenol determination has been observed in the presence of typical inorganic and organic compounds including essential oils components. The approach developed has been tested on the commercial clove, cinnamon, and nutmeg essential oils. Validation with the independent method has shown similar accuracy and the absence of systematic errors of eugenol determination.

Keywords: eugenol; modified electrodes; electropolymerization; pyrogallol red; voltammetry; essential oils

1. Introduction

Eugenol (4-allyl-2-methoxyphenol) is a natural aroma compound and the major component of clove and clove-based products, various essential oils [1]. It is widely used in food and pharmaceutical industry, and medicine. Its content can be considered as a marker of the sample quality. Therefore, simple, sensitive, and reliable methods for eugenol quantification are required.

Eugenol is electroactive compound due to the presence of phenolic fragment in the structure. Therefore, a wide range of electrochemical approaches are known to date for eugenol quantification. Most of them are based on the application of chemically modified electrodes due to the high sensitivity and selectivity of the response. Among a great variety of electrode surface modifiers, polymers are of interest, although limited number of polymer-modified electrodes for eugenol has been reported.

Highly water-stable composite of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate decorated with polyvinylpyrrolidone and carbon nanotubes has been used as electrode surface modifier for sensitive determination of eugenol in differential pulse mode.
[2]. The linear dynamic range of 0.15–122.4 μM with the detection limit of 48 nM has been achieved. The practical applicability of the approach developed has been shown on curry powder samples. Electrodes based on molecularly imprinted polymers give a more selective response to eugenol in the presence of structurally related compounds such as phenol [3] or vanillin, methyl eugenol, paeonol, and methyl isoeugenol [4]. Glassy carbon electrode (GCE) with 3D porous 1-aminopropyl-3-methylimidazolium bromide functionalized graphene-carbon nanotubes composite and three-dimensional molecularly imprinted poly(p-aminobenzenethiol-co-p-aminobenzoic acid) gives a linear response to eugenol from 0.50 to 20 μM with a detection limit of 0.10 μM [4] and can be used in the curry powder, perfume, and capsule samples analysis. The most impressive analytical characteristics of eugenol (linearity of response is 0.0005–1, 1–50 and 50–160 μM with the detection limit of 0.4 nM) has been achieved using polyacrylonitrile molecularly imprinted polymer embedded graphite electrode [3].

There are no data for the voltammetric determination of eugenol using electrodes modified with electrochemically obtained polymeric coverages that are simple in preparation and eco-friendly. The current work deals with the development of GCE modified with carboxylated multi-walled carbon nanotubes (MWCNTs-COOH) and electropolymerized pyrogallol red for the determination of eugenol.

2. Materials and Methods

Eugenol (99% purity) from Aldrich (Steinheim, Germany), and pyrogallol red (Sigma-Aldrich, Steinheim, Germany) were used. Their 10 mM (1.0 mM for pyrogallol red) standard solutions were prepared in ethanol (rectificate). Reagents of 96–100% purity from Aldrich (Steinheim, Germany), Sigma-Aldrich (Steinheim, Germany), Acros Organics (Geel, Belgium) and TCI (Fukaya, Japan) were used in the interference study. Other reagents were of c.p. grade and used as received.

MWCNTs-COOH (diameter 9.5 nm, length 1.5 μm and carboxylation degree >8%) from Aldrich (Steinheim, Germany) were used as electrode surface modifier. Their homogeneous 1.0 mg mL⁻¹ suspension was prepared in 1% sodium dodecyl sulfate (Panreac, Barcelona, Spain) water solution by sonication for 15 min in an ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea).

Commercially available essential oils from clove, cinnamon and nutmeg of various trademarks were used as a real samples.

Poly(pyrogallol red) (polyPGR) coverage was obtained at the surface of MWCNTs-COOH/GCE by potentiodynamic electrolysis. Electropolymerization conditions were optimized on the basis of eugenol response at the polymer-modified electrode.

Electrochemical measurements were performed on the potentiostats/galvanostats μAutolab Type III (Eco Chemie B.V., Utrecht, The Netherlands) with Nova 1.7.8 software and Autolab PGSTAT 302N with the FRA 32M module (Metrohm Autolab B.V., Utrecht, The Netherlands) and NOVA 1.10.1.9 software. A glassy electrochemical cell of 10 mL with a three-electrode system (working GCE of ø = 3 mm (BASi® Inc., West Lafayette, IN, USA) or a modified GCE, an Ag/AgCl reference electrode, and a platinum wire as the auxiliary electrode) was used.

The “Expert-001” pH meter (Econix-Expert Ltd., Moscow, Russian Federation) with a glassy electrode was applied for the pH measurements.

Spectrophotometric measurements were performed on the spectrophotometer PE-5300 (NPO Ecros, Saint Petersburg, Russia).

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

Statistical treatment was performed for five replications (three replications in spectrophotometry) at P = 0.95.

3. Results and Discussion
3.1. Pyrogallol Red Electropolymerization

Prior to electodeposition of polymeric coverage, 2 μL of MWCNTs-COOH suspension were drop casted at the GCE and evaporated to dryness at ambient conditions. The use of MWCNTs-COOH provides a high load of polymer as well as sufficient conductivity of the electrode as far as electropolymerized triphenylmethane dyes form insulating coverages [5–7]. PolyPGR coverage was obtained in potentiodynamic mode. The irreversible two-step oxidation of pyrogallol red occurred on the cyclic voltammogram (Figure 1) which gradually disappeared with increasing scan number that was typical for the electropolymerization of compounds containing phenolic fragments [8].

![Figure 1](image)

**Figure 1.** Electropolymerization of 100 μM pyrogallol red at the MWCNTs-COOH/GCE in Britton-Robinson buffer pH 7.0. υ = 100 mV s⁻¹.

Oxidation of PGR occurs via formation of phenoxy radical which undergo further polymerization (Scheme).

![Scheme](image)

**Scheme.** Electrooxidation of PRG.

The working conditions (supporting electrolyte, pH, monomer concentration and electrolysis parameters) of pyrogallol red have been found using voltammetric characteristics of eugenol. The highest oxidation currents of eugenol were registered at the polyPGR-modified electrode obtained from 100 μM monomer in the Britton-Robinson buffer pH 7.0 by 10-fold potential cycling from 0.0 to 1.3 V with a scan rate of 75 mV s⁻¹.

Oxidation potential of eugenol is cathodically shifted on 140 mV vs. bare GCE (0.78 and 0.64 V, respectively) due to the presence of MWCNTs-COOH and their electrocatalytic properties. The oxidation peak currents were 2.8-fold increased vs. bare GCE. 10-mV anodic shift of eugenol oxidation potential to 0.65 V and two-fold increase of oxidation currents were observed at polyPGR/MWCNTs-COOH/GCE vs. MWCNTs-COOH/GCE that means the synergetic effect of both modifying layers. Moreover, polyPGR-modified electrode demonstrated lower capacitive currents compared to MWCNTs-COOH/GCE that improved the eugenol oxidation peak shape.

3.2. Electrodes Surface and Electrochemical Properties Characterization

Scanning electron microscopy showed a successful immobilization of MWCNTs-COOH and polyPGR layers on the GCE surface. MWCNTs-COOH formed a net structure from strongly intertwined tubes. Polymeric coverage was presented by thin film layer.
The electroactive surface area of the electrodes was calculated on the basis of cyclic voltammetry and chronoamperometry data for the electrooxidation of 1.0 mM $[\text{Fe(CN)}_6]^{4-}$ ions in 0.1 M KCl. Significant increase in the electroactive surface area (96±3 mm$^2$ for polyPGR/MWCNTs-COOH/GCE, 58±4 mm$^2$ for MWCNTs-COOH/GCE, and 8.9±0.3 mm$^2$ for GCE) was obtained that explained improvement of eugenol response at the modified electrodes.

Electrochemical impedance spectroscopy data fitted using Randles’ equivalent circuits ($R_s(R_{ct}Q)$ for bare GCE and $R_s(Q[R_{ct}W])$ for modified electrodes) clearly confirmed a dramatic decrease in the charge transfer resistance for modified electrodes (3.04 ± 0.09 kΩ for polyPGR/MWCNTs-COOH/GCE, 4.0 ± 0.1 kΩ for MWCNTs-COOH/GCE vs. 72.5 ± 0.9 kΩ for GCE) and an increase in the electron transfer rate. The data obtained proved the effectivity of the electrode surface modification with polyPGR.

3.3. Differential Pulse Voltammetric Determination of Eugenol

Optimization of pulse parameters in differential pulse mode showed that the oxidation potential of eugenol insignificantly decreased with the amplitude growth. Oxidation peak currents were changed dramatically achieving maximum at the pulse amplitude of 125 mV and pulse time of 25 ms. The one well-pronounced oxidation peak at 0.57 V was observed on the differential pulse voltammograms of eugenol in Britton-Robinson buffer pH 2.0 (Figure 2a) which currents are linearly grew in the 0.75–100 μM concentration range (the correlation coefficient was 0.9997) (Figure 2b).

The detection limit of eugenol was 0.73 μM. The analytical characteristics obtained are significantly improved compared to those reported for other chemically modified electrodes [9–13]. Moreover, the absence of preconcentration step and simple modification of the electrode surface make the method rapid and easy for realization in practice.

The accuracy of the method developed was confirmed by model solution testing for which recovery of 99.9–100.4% was obtained. High reproducibility of the method was shown on the basis of relative standard deviation values of 0.8–2.0%.

3.3.1. Selectivity Study

The key point limiting practical application of the voltammetric methods is the insufficient selectivity of the target analyte response. PolyPGR/MWCNTs-COOH/GCE selectivity toward eugenol was studied at 5.0 μM concentration. Inorganic ions, typical components of essential oils (other phenolics and terpenes) were tested as potential interferences. Corresponding oxidation potentials and tolerance limits are presented in Table 1.
Thus, the method developed showed high selectivity of eugenol determination and could be applied in the essential oils analysis, as confirmed in the further investigations.

Table 1. Oxidation potential and tolerance limits of potential interferences for the determination of 5.0 μM eugenol at the polyPGR/MWCNTs-COOH/GCE

<table>
<thead>
<tr>
<th>Interference</th>
<th>$E_{ox}$ (V)</th>
<th>Tolerance Limit (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^- и SO_4^{2-}$</td>
<td>—</td>
<td>5000</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.82</td>
<td>500</td>
</tr>
<tr>
<td>Thymol</td>
<td>0.75</td>
<td>25</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>0.76</td>
<td>50</td>
</tr>
<tr>
<td>Anethole</td>
<td>0.90</td>
<td>500</td>
</tr>
<tr>
<td>$\alpha$-Pinene</td>
<td>1.1</td>
<td>500</td>
</tr>
<tr>
<td>Limonene</td>
<td>1.3</td>
<td>500</td>
</tr>
<tr>
<td>Phytol, $\beta$-pinene, carvone, camphene, fenchene, myrcene, L-menthol, benzyl alcohol, L-borneol</td>
<td>—</td>
<td>5000</td>
</tr>
</tbody>
</table>

3.4. Real Samples Analysis

The developed voltammetric method was applied in real samples in particular essential oils from clove, cinnamon, and nutmeg. The preliminary dissolution of exact weight of essential oil in ethanol (rectificate) was used. An additional 10-fold dilution with ethanol was applied to the clove essential oil solution. 10 μL of clove and cinnamon essential oil solution and 100 μL of nutmeg essential oil solution were used for the voltammetric analysis. The eugenol content in the essential oil was presented as a mass fraction (%).

All samples demonstrated a clear oxidation peak at 0.57 V. The absence of matrix effects in the eugenol quantification was confirmed by standard addition method which gave recovery of 99–100%.

The results of the essential oil analysis are presented in Figure 3. Voltammetry data agree well with the independent spectrophotometric determination according to [14]. The absence of systematic errors and similar precision of the methods were confirmed by $t$- and $F$-tests, which are less than critical values.

Figure 3. Mass fraction of eugenol in the essential oils ($n = 5; P = 0.95$).

**Author Contributions:** Conceptualization, G.Z.; methodology, A.K. and G.Z.; validation, A.K.; investigation, A.K.; writing—original draft preparation, A.K. and G.Z.; writing—review and editing, G.Z.; visualization, A.K. and G.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


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