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Sensitive voltammetric sensor for thymol and carvacrol based on the electropolymerized thymolphtalein

Guzel Ziyatdinova * and Natalia Chernousova

Analytical Chemistry Department, Kazan Federal University, Kremleyevskaya, 18, 420008 Kazan, Russia; chernousovanata@gmail.com

* Correspondence: Ziyatdinovag@mail.ru

Abstract: Thymol and carvacrol (isopropylmethylphenols) are natural phenolic monoterpenoids with antibacterial, antifungal, insecticidal, and antioxidant properties. Their dose-dependent antioxidant effect requires control in real samples. Various modes of voltammetry have been successfully developed for thymol and carvacrol quantification. Glassy carbon electrode (GCE) modified with multi-walled carbon nanotubes (MWCNTs) and electropolymerized thymolphthalein has been developed for this purpose. Conditions of thymolphthalein electropolymerization (monomer concentration, number of cycles, parameters of electrolysis) providing the best response to thymol have been found. Scanning electron microscopy and electrochemical methods confirm the effectivity of the sensor developed. In differential pulse mode, the sensor gives a linear response in the ranges of 0.050-25 and 25-100 μM for thymol and 0.10-10 and 10-100 μM for carvacrol with detection limits of 0.037 and 0.063 μM, respectively that is significantly improved compared to reported earlier. Sensor developed is selective to isopropylmethylphenols in the presence of typical interferences (inorganic ions, saccharides, ascorbic acid) and other phenolics (caffeic, chlorogenic, gallic and rosmarinic acids, quercetin, and rutin). Sensor has been applied for the evaluation of total isopropylmethylphenols in oregano and thyme spices using single sonication assisted extraction with methanol. Voltammetric sensor data agree well with the independent spectrophotometric quantification.

Keywords: electrochemical sensors; voltammetry; polymer-modified electrodes; electropolymerization; phthalein dyes; thymol; carvacrol; spices; food analysis

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1. Introduction

Thymol and carvacrol (isopropylmethylphenols) are phenolic monoterpenoids with antibacterial, antifungal, insecticidal, and antioxidant properties [1–4]. Thyme, oregano, and other culinary and medicinal herbs are their natural sources [5]. Similar to other natural phenolics, isopropylmethylphenols show the prooxidant activity at high concentrations [6] that leads to formation of free radicals which able to provide harmful effect on living cells. Therefore, the control of thymol and carvacrol contents in real samples is required.

The presence of phenolic hydroxyl groups in the thymol and carvacrol structures makes them active at the electrode surface, allowing the application of voltammetry for their quantification. Similarity of structure leads to the almost the same oxidation potentials of both compounds. Therefore, total isopropylphenols determination is usually performed.

Traditional carbon-based electrodes [7–9] and boron-doped diamond electrodes [10,11] are applied for these purposes. Recently, chemically modified electrodes have been developed for the determination of isopropylmethylphenols improving its sensitivity. Multi- [12,13] and single-walled carbon nanotubes [14], graphene oxide

nanosheets [15], monodisperse Ag@C@Ag core-double shell spheres [16], MnY nanozeolite [17], and CeO_2 nanoparticles in combination with graphene [18] and Brij® 35 micellar medium [19] are successfully used as sensing layers for thymol determination. Carvacrol is less studied in electroanalysis. To date, only two electrochemical sensors for carvacrol based on the multi-walled carbon nanotubes (MWCNTs) [20] and La_2O_3/Co_3O_4 nanocomposite [21] have been reported.

Thus, further development of electrochemical sensors for isopropylmethylphenols is of practical interest. Polymer-modified electrodes that are widely developed for other natural phenolic compounds [22] are fully out of consideration in application to thymol and carvacrol. The current work is focused on the development of sensitive voltammetric sensor for isopropylmethylphenols determination using electrode modified with MWCNTs and electropolymerized thymolphtalein.

2. Materials and Methods

Thymol (99.5% purity), thymolphthalein (95%) from Sigma (Steinheim, Germany), and carvacrol (98%) from Aldrich (Steinheim, Germany) were used. Their standard 10 mM solutions were prepared in methanol (c.p. grade) in 5.0 mL flasks. Ascorbic (99%), gallic (99%), caffeic (98%) and rosmarinic (98%) acids, quercetin dihydrate (95%) from Sigma (Steinheim, Germany), and chlorogenic acid (95%) from Aldrich (Steinheim, Germany) have been used in the interference study. Their 10 mM stock solutions in methanol were prepared in 5.0 mL flasks. Less concentrated solutions were obtained by the exact dilution.

MWCNTs (outer diameter 40–60 nm, inner diameter 5–10 nm and 0.5–500 μm length) from Aldrich (Steinheim, Germany) were used as a platform for further electrodeposition of polythymolphthalein. Homogeneous 0.5 mg mL^{-1} suspension of MWCNTs was prepared in 1% sodium dodecylsulfate (Panreac, Barcelona, Spain) by 30 min of sonication in an ultrasonic bath (WiseClean WUC-A03H (DAIHAN Scientific Co., Ltd., Wonju-si, Republic of Korea).

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 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.

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

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

3. Results

3.1. Electrodeposition of polythymolphthalein at the MWNTs-modified electrode

GCE was modified with MWCNTs by a drop casting method. Then, the electrochemical deposition of polythymolphthalein was performed in a potentiodynamic mode. Irreversible single-step electrooxidation of thymolphthalein at 0.594 V has been observed in neutral medium (Figure 1). Thymolphthalein undergoes one electron and one proton oxidation with participation of thymol fragments forming phenoxyl radical that can further dimerize and polymerize that agrees well with the reported data for thymolphthalein [23] and bromothymol blue [24]. The dramatic decrease of the oxidation current with the increase of the number of cycles confirms the formation of insulating polymeric coverage that is typical for the electropolymerization of phenolics [25].

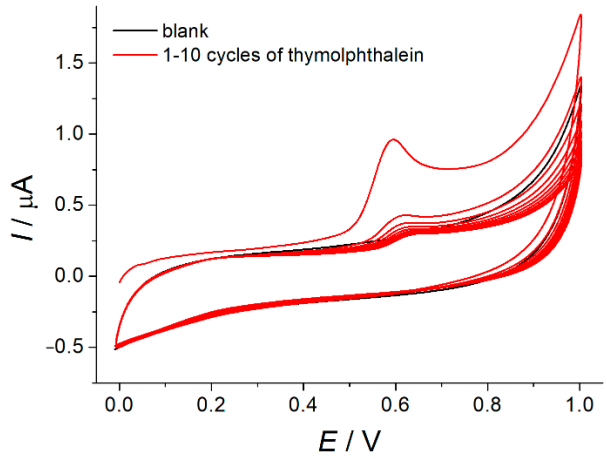

Figure 1. Electropolymerization of 10 μM thymolphthalein at the MWNT/GCE in phosphate buffer pH 7.0. $\upsilon = 100$ mV s^{-1}.

The electropolymerization conditions of thymolphthalein have been optimized on the basis of the voltammetric response of 10 μM thymol. The best characteristics of the analyte have been registered on the polymer-modified electrode obtained by 10-fold potential cycling from 0.0 to 1.0 V with the scan rate of 100 mV s^{-1} in the 10 μM monomer solution in phosphate buffer pH 7.0. Comparison of the voltammetric characteristics of thymol on bare GCE, MWCNTs/GCE and polythymolphthalein/MWCNTs/GCE (Table 1) clearly indicates the effectivity of the developed electrode. Furthermore, capacitive currents are significantly less on the polymer-modified electrode vs. MWCNTs/GCE.

Table 1. Voltammetric characteristics of 10 μM thymol at the bare and modified GCE in Britton-Robinson buffer pH 7.0.

Electrode	E_{ox} (V)	I_{ox} (μA)
GCE	0.655	0.16 ± 0.01
MWCNTs/GCE and	0.564	0.26 ± 0.01
Polythymolphthalein/MWCNTs/GCE	0.564	0.50 ± 0.02

Polymer-modified electrode shows significant increase in the effective surface area (88 ± 5 mm^2 vs. 8.9±0.3 mm^2 for GCE) and electron transfer rate (electron transfer resistance is 9.9-fold less than for the GCE).

3.2. Electrooxidation of isopropylmethylphenols at the polythymolphthalein/MWCNTs/GCE

Varying supporting electrolyte pH in the range of 2.0–12.0, the voltammetric characteristics of thymol and carvacrol have been studied under conditions of cyclic voltammetry. The decrease of the oxidation potentials of both compounds at pH 2.0–11.0 confirms the participation of protons in the electrode reaction. Then, pH independent oxidation occurs that agree well with thymol and carvacrol ionization constants [26]. Oxidation currents are also decreased with the increase of Britton-Robinson buffer pH. Therefore, pH 2.0 has been chosen for further investigations.

Diffusion-controlled two electron oxidation of thymol and carvacrol has been confirmed on the basis of cyclic voltammetry data at various potential scan rates. An irreversible process has been observed for both compounds and anodic transfer coefficients of 0.40 and 0.44 have been calculated for thymol and carvacrol, respectively.

3.3. Quantification of isopropylmethylphenols using a polythymolphthalein-based sensor

The electrode created has been used as a voltammetric sensor for thymol and carvacrol. The differential pulse voltammetric response of the sensor (Figure 2) increases linearly with the growth of the isopropylmethylphenol concentration in the ranges of

0.050–25 and 25–100 μM for thymol and 0.10–10 and 10–100 μM for carvacrol with detection limits of 0.037 and 0.063 μM, respectively. The oxidation potentials of thymol and carvacrol are 0.81 and 0.83 V, respectively, making impossible their simultaneous determination. Similar to other electrochemical approaches, the total contents of isopropylmethylphenols can be evaluated. The analytical characteristics obtained are improved *vs*. those reported for other voltammetric sensors [12–21]. The high precision of the developed sensor has been confirmed by recovery of 99-100.1% for the determination of thymol and carvacrol in model solutions. The relative standard deviation is less than 5%, which indicates good reproducibility of the sensor response as far as the electrode surface has been renewed after each measurement.

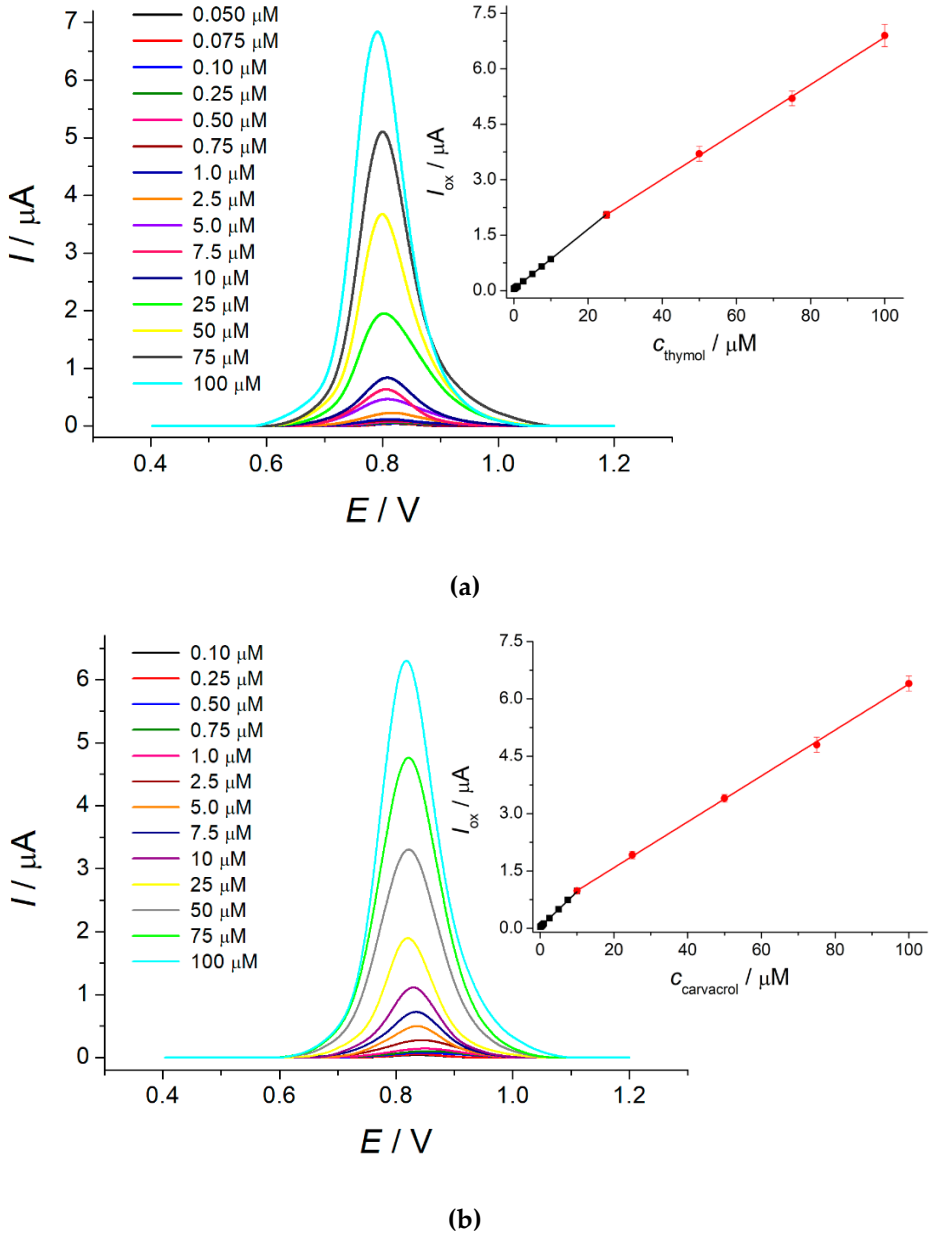

Figure 2. Differential pulse voltammograms of isopropylmethylphenols at the polythymolphthalein/MWCNTs/GCE in Britton-Robinson buffer pH 2.0: (**a**) thymol; (**b**) carvacrol. ΔE_{pulse} = 100 mV, t_{pulse} = 25 ms, υ = 20 mV s^{-1}. Inserts are the corresponding calibration graphs.

The sensor response to isopropylmethylphenols is selective in the presence of typical interferences (1000-fold excesses of K^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^- и SO_4^{2-}, 100-fold excesses

of saccharides, ascorbic acid) and other phenolics (50-fold excesses of caffeic, chlorogenic, and rosmarinic acids, 1.0 μM of gallic acid, and 25-fold excess of quercetin and rutin). The data obtained allow to use sensor in the analysis of plant materials.

3.4. Quantification of total isopropylmethylphenols in thyme and oregano spices

The sensor developed has been tested on thyme and oregano spices being the major source of isopropylmethylphenols. Single sonication assisted extraction with methanol for 5 min has been used. Spice/methanol ratio providing highest yield of isopropylmethylphenols is 1:40 for oregano and 1:30 for thyme.

The voltammetric determination of total isopropylmethylphenols has been compared with an independent spectrophotometric method [27] (Figure 3). The results obtained agree well and the F-test confirms similar accuracy of the methods (*F*-criterium values of 1.75–6.09 are less than the critical value 6.59).

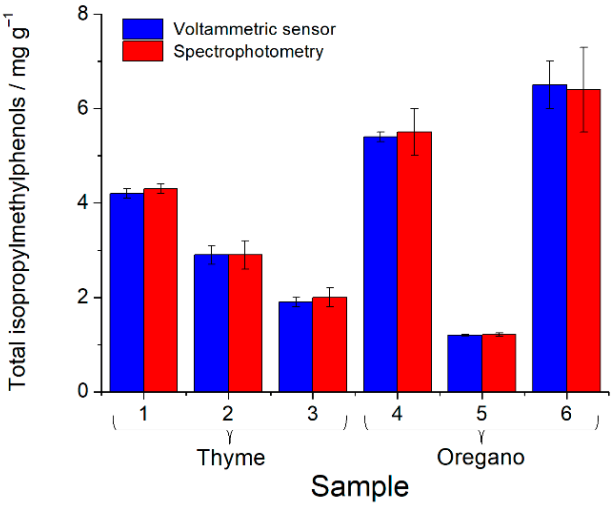

Figure 3. Quantification of total isopropylmethylphenols in thyme and oregano spices.

5. Conclusions

A sensitive and selective voltammetric sensor based on MWCNTs and electropolymerized thymolphthalein has been developed for the determination of thymol and carvacrol. The application of MWCNTs significantly increases the conductivity of the electrode and its surface area providing a higher load of polymeric coverage. The polymeric film provides structural similarity to the analytes and the porous structure leading to the increase of the determination sensitivity. The analytical characteristics achieved are the best one among reported to date. The sensor is simple, reliable, cost-effective, and can be applied for the routine analysis for the fast screening of the plant materials.

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