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Voltammetric Determination of Thymol in Oregano Using CeO_2-Modified Electrode in Brij® 35 Micellar Medium

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Abstract Glassy carbon electrode (GCE) modified with CeO_2 nanoparticles dispersed in 0.01 M Brij® 35 (CeO_2-Brij® 35/GCE) has been developed for the determination of thymol in micellar medium. Scanning electron microscopy (SEM) data confirm immobilization of the nanomaterial on the electrode surface. The electrooxidation of thymol on CeO_2-Brij® 35/GCE is an irreversible diffusion-controlled process with participation of two electrons and two protons. Differential pulse voltammetry has been used for the quantification of thymol. The linear dynamic range of the thymol determination is 0.700–10.1 and 10.1–606 μM with the limits of detection and quantification 0.20 and 0.65 μM, respectively. The approach developed has been applied for the quantification of thymol in oregano spices using preliminary micellar extraction with Brij® 35. The results of voltammetric determination are in good agreement with the data of standard spectrophotometric method.

Keywords Chemically modified electrodes · Nanoparticles · Micellar media · Differential pulse voltammetry · Thymol · Food analysis

Introduction

Thymol (2-isopropyl-5-methylphenol) is a natural phenolic compound of wide spectrum of biological activity such as antimicrobial, antioxidant, antinociceptive, local anesthetic, and anti-inflammatory in vitro (Braga et al. 2006; Haeseler et al. 2002; Falcone et al. 2005; Yanishlieva et al. 1999). As an antioxidant, it plays an important role in the inhibition of liposome phospholipid peroxidation in a concentration-dependent manner (Aeschbach et al. 1994). Therefore, thymol is successfully used as a preservative and an active principle in perfumes, foodstuff, mouthwashes, pharmaceuticals, and cosmetics (Piech and Paczosa-Bator 2015). On the other hand, thymol is a major component of many plants like *Thymus vulgaris*, *Origanum vulgare*, and *Trachyspermum ammi* (Evans 2009; Haque et al. 2012; Karami-Osboo et al. 2010), giving a significant contribution to their biological activity. These plants are widely used as spices for cooking and can be considered as one of the sources of antioxidants in human diet. Thus, the control of thymol concentration in real samples is of practical interest.

Different types of chromatography (Haque et al. 2012; Alekseeva 2009; Hajimehdipoor et al. 2010; Kiyanpoura et al. 2009; Abu-Lafi et al. 2008; Vinas et al. 2006; López et al. 2011) and spectrophotometry (Al-Abachi and Al-Ward 2012; Razzaq and Mohammed 2014; Backheet 1998) have been developed for the determination of thymol. The main disadvantages of these methods are time-consuming procedure and complicated sample pretreatment. In particular, the solid-phased microextraction with nanomaterial-containing composites is actively developed in the last years (Roosta et al. 2015; Ghiasvand et al. 2015; Fiori et al. 2013).

Being a phenolic compound, thymol undergoes oxidation reactions that allow the use of electrochemical methods for its quantification. The advantages of electrochemical methods, such as rapid response, cost-efficiency, high sensitivity, and low detection limits as well as the possibility to improve the selectivity using suitable electrode and conditions, enlarge their applicability for the samples with complex matrix, plant materials, for instance.

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The electrochemical methods developed for the quantification of thymol are based on its oxidation on the glassy carbon electrode (GCE) (Lau et al. 1998; Michelitsch et al. 2004), boron-doped diamond electrode (Stanković 2015), glassy carbon microbead-based electrode under conditions of flow-injection analysis with coulometric detection (Mika et al. 2015), as well as glassy carbon paste electrode (Zima et al. 2007) and GCE (Cantalapiedra et al. 2014) as detectors in high-performance liquid chromatography. The common approach to increase the selectivity and sensitivity of determination is the use of chemically modified electrodes. The most applied modifiers of the electrode surface are nanomaterials, in particular carbon nanomaterials and metal (or their oxides) nanoparticles. So, GCE modified with Nafion and multi-walled carbon nanotubes (Piech and Paczosa-Bator 2015), monodisperse Ag@C@Ag core-double shell sphere nanocomposite-modified electrode (Gan et al. 2015), graphene oxide nanosheet-modified GCE (Behpour et al. 2014), and GCE with CeO_2 nanoparticle-decorated graphene hybrid film (Zhao et al. 2013) have been successfully used for thymol determination in real samples like honey and thyme plant.

Thymol has poor solubility in water. Therefore, organic solvents or water-organic media (usually methanol) are used for its quantification. Surfactant-based water media could be a good alternative to organic solvents as has been shown earlier on β-carotene (Ziyatdinova et al. 2012b), α-tocopherol (Jaiswal et al. 2001; Ziyatdinova et al. 2012a), retinol (Ziyatdinova et al. 2010), and eugenol (Ziyatdinova et al. 2013). Surfactants effect on the electrochemical response of compounds facilitating target analyte solubilization and adsorption on the electrode (Ziyatdinova et al. 2012c).

The present work is devoted to the development of new voltammetric method for thymol determination based on its oxidation of GCE modified with CeO_2 nanoparticles in non-ionic surfactant Brij® 35 micellar medium being investigated for the first time. The parameters of thymol oxidation on the modified electrode have been studied. The approach developed has been applied for the quantification of thymol in micellar extracts of oregano spices.

Materials and Methods

Chemicals and Reagents

Thymol (99.5 %) was purchased from Sigma-Aldrich (Germany). Its 0.01 M stock solution was prepared by dissolving an appropriate amount in 5.0 mL of 0.1 M Brij® 35 (Sigma-Aldrich, Germany). Dispersion of CeO_2 nanoparticles in water (10 % wt.) with particle size <25 nm was obtained from Sigma-Aldrich (Germany). The working dispersion in 0.01 M Brij® 35 with concentration 1.0 mg mL^{-1} was obtained by appropriate dilution.

0.1 M phosphate buffer (PB; pH 4.0–8.0) was tested as a supporting electrolyte. All other chemicals were analytical reagent grade purity and used as received. Double-distilled water was used for the measurements. The experiments were carried out at ambient temperature (25 ± 1 °C).

Apparatus

Voltammetric measurements were performed on a potentiostat/galvanostat μAutolab type III with the software GPES, version 4.9.005 (Eco Chemie B.V., Utrecht, Netherlands). The electrochemical cell consisted of the working GCE (6.07-mm^2 geometric surface area) or CeO_2-modified GCE, silver-silver chloride saturated KCl reference electrode, and counter electrode (platinum wire).

Scanning electron microscopy (SEM) of the electrode surfaces was performed using tabletop scanning electron microscope TM-1000 (Hitachi, Japan).

An "Expert-001" pH meter (Econix-Expert Ltd., Moscow, Russia) equipped with the glass electrode was used for pH measurements.

Procedures

Preparation of the Modified Electrode The GCE was carefully polished with alumina (0.05 μm) on a polishing cloth and rinsed with acetone and double-distilled water before use. Electrode modification was performed by drop casting of 6 μL CeO_2-H_2O or CeO_2-Brij® 35 dispersion on the GCE surface and evaporating to dryness.

SEM SEM images of the electrode surfaces were obtained at room temperature in ambient conditions. The 6 μL of CeO_2-H_2O or CeO_2-Brij® 35 dispersion was dropped on the GCE surface and allowed to evaporate to dryness. Then SEM images were scanned at accelerating voltage of 15 kV and emission current 50.3 mA.

Voltammetry Voltammograms were recorded from 0.18 to 1.0 V with linear sweep (CV) or 0.4–0.8 V in differential pulse (DPV) mode. Prior to testing thymol, five scans were performed only with supporting electrolyte solution containing Brij® 35 for a stable background voltammogram achievement. The portion of Brij® 35 was reduced to 10 % (*v*/*v*) in all voltammetric measurements giving a final concentration of 0.01 M. Baseline correction using a moving average algorithm included in the GPES software was applied for better identification of oxidation peak in DPV.

Sample Preparation

The real samples are commercially spices of oregano available on local market. A representative portion of the milled oregano samples (0.1000 ± 0.0003 g) was weighted and preliminary ultrasonic liquid extraction with 0.1 M Brij® 35 was used (Ziyatdinova et al. 2016). Extraction conditions were varied in order to find the best thymol recovery. Then, extract was filtered and used for further measurements. The aliquot portion of extract (500 μL) was inserted in electrochemical cell containing 0.01 M Brij® 35 in supporting electrolyte, and DPVs were recorded from 0.4 to 0.8 V. Thymol concentration was recalculated per 1 g of the spice.

Data Treatment

All measurements were performed five times. Statistical evaluation was performed at significance level of 5 % by SPSS for Windows software (SPSS Inc., USA). All data were expressed as the $X \pm \Delta X$ with X as average value and ΔX as confidence interval.

Regression analysis was performed using software OriginPro 8.0 (OriginLab, USA).

Results and Discussion

SEM Characterization of the Electrodes

The surface of the bare GCE and CeO_2-modified GCE has been studied by SEM (Fig. 1). The GCE shows an unstructured smooth surface. On the modified electrodes, the top view changes significantly. The CeO_2-H_2O/GCE surface is homogeneously covered with CeO_2 nanoparticles (Fig. 1b). Their strong incorporation into the Brij® 35 film with relatively homogeneous distribution is observed for the CeO_2-Brij® 35/GCE (Fig. 1c). These data confirm a successful immobilization of the nanomaterial on the electrode surface.

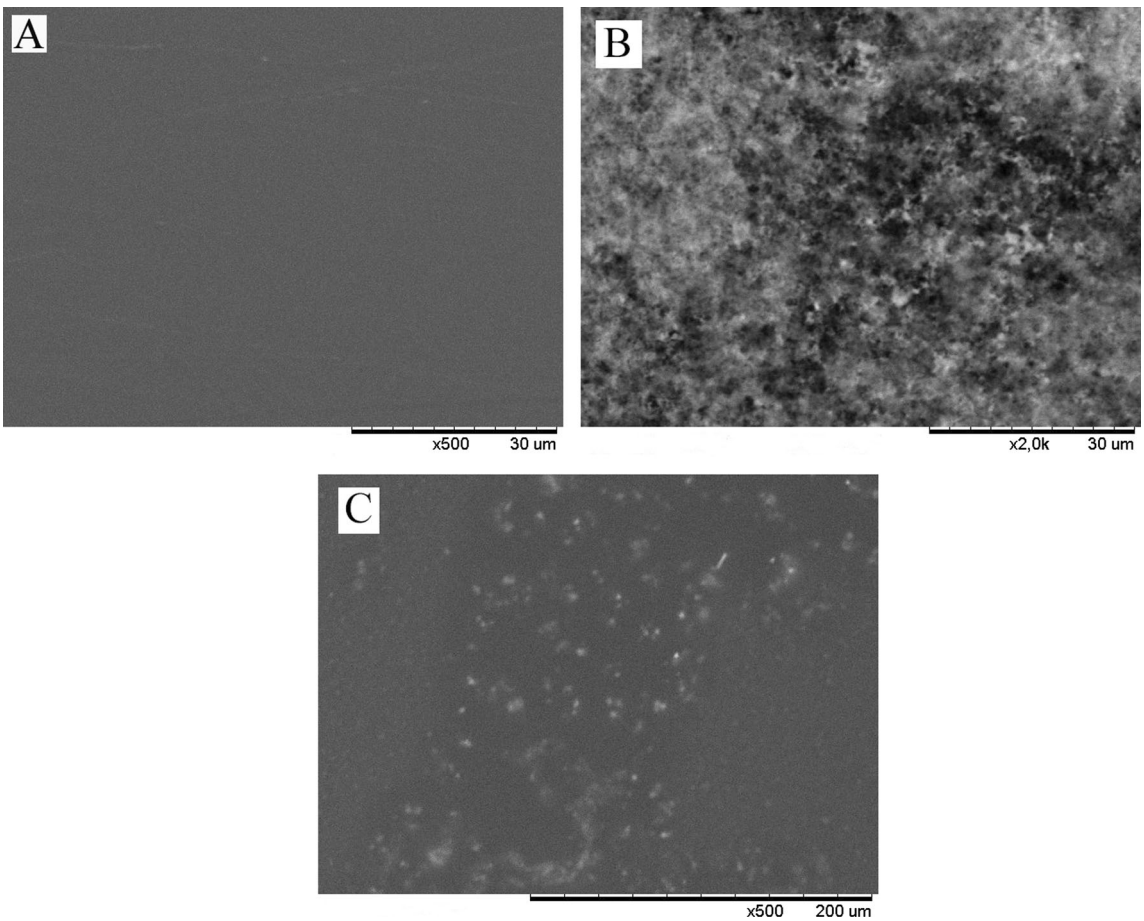

Fig. 1 SEM images of bare GCE (**a**), CeO_2-H_2O/GCE (**b**), and CeO_2-Brij® 35/GCE (**c**)

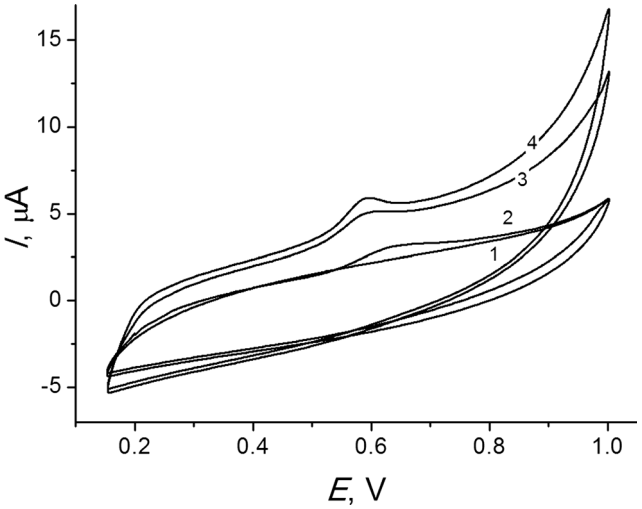

Fig. 2 CVs of 162 μM thymol on GCE (*curve 2*) and CeO_2-H_2O/GCE (*curve 3*) and CeO_2-Brij® 35/GCE (*curve 4*) in 0.01 M Brij® 35 in PB pH 7.0 (*curve 1* on GCE). Potential scan rate is 100 mV s^{-1}

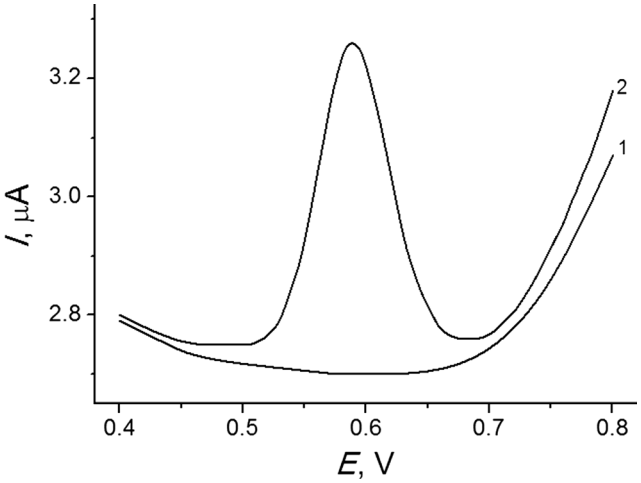

Fig. 4 DPV of 50.5 μM (*curve 2*) thymol on CeO_2-Brij® 35/GCE in 0.01 M Brij® 35 in PB pH 6.0 (*curve 1*). Pulse amplitude is 50 mV, pulse width is 50 ms, and potential scan rate is 10 mV s^{-1}

Electrooxidation of Thymol on CeO_2-Brij® 35/GCE

Voltammetric behavior of thymol has been studied in 0.01 M Brij® 35 in PB pH 7.0. Standard thymol solution was prepared in Brij® 35 micellar media providing its solubilization in water medium instead of organic solvents. Brij® 35 is electrochemically inactive in the potential window under investigations and does not affect on the CVs of supporting electrolyte.

Thymol is irreversibly oxidized on GCE, CeO_2-H_2O/GCE, and CeO_2-Brij® 35/GCE in a PB pH 7.0 that is confirmed by the absence of cathodic steps on the CVs (Fig. 2) and typical for monophenols. The oxidation signal on GCE is low (Fig. 2, curve 2) in comparison to the modified electrodes. The oxidation currents on CeO_2-H_2O/GCE are 1.7-fold enhanced (Fig. 2, curve 3) in comparison with the bare GCE due to an increase in the effective surface area of the modified electrode. The cathodic shift of thymol oxidation potential (0.59 vs. 0.63 V) is observed on CeO_2-H_2O/GCE. So far as the Brij® 35 micellar medium is used for the measurements, the CeO_2-Brij® 35/GCE has been tested. Further increase in thymol oxidation currents (1.37 ± 0.08 and 1.03 ± 0.06 μA for the CeO_2-Brij® 35/GCE and CeO_2-H_2O/GCE, respectively) at the same oxidation potential has been observed on surfactant-modified electrode (Fig. 2, curve 4). These results confirm effect of the immobilized surfactant on thymol electrooxidation parameters. The increase of the oxidation currents is caused by the higher working surface area and possible preconcentration of the analyte due to the hydrophobic interactions with the surfactant.

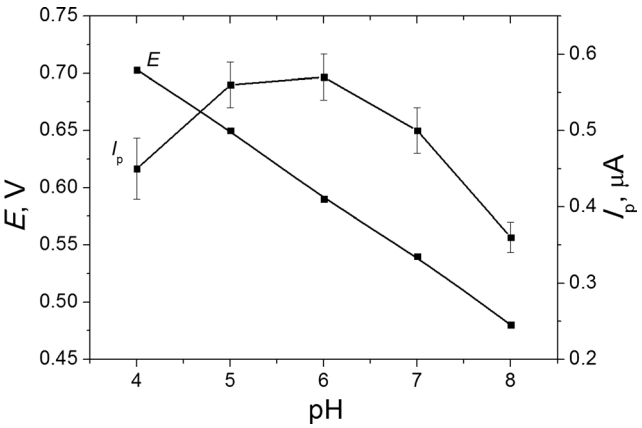

Fig. 3 Effect of supporting electrolyte pH on voltammetric characteristics of the thymol oxidation on CeO_2-Brij® 35/GCE

The effect of potential scan rate on the voltammetric behavior of thymol has been studied in the range of 10–500 mV s^{-1}. The oxidation currents of thymol are proportional to the square root of the potential scan rate (Eq. 1):

$$I_p[\mu A] = (0.014 \pm 0.024) + (0.0753 \pm 0.0019)\upsilon^{1/2}\left[\text{mV s}^{-1}\right] \quad R^2 = 0.9968 \tag{1}$$

These data mean that thymol oxidation on CeO_2-Brij® 35/GCE is a diffusion-controlled process (Nicholson and Shain 1964). Moreover, the slope value of the linear plot of $\ln I_p$ on $\ln\upsilon$ (Eq. 2) equaled to 0.47 is close to theoretical value of 0.5 indicating that the process is controlled by diffusion only (Bard and Faulkner 2001).

$$\ln I_p[\mu A] = (0.824 \pm 0.055) + (0.470 \pm 0.019)\ln\upsilon\left[\text{V s}^{-1}\right] \quad R^2 = 0.9920 \tag{2}$$

The shift of thymol oxidation potential with the increase of scan rate confirms that the heterogeneous electronic transfer is irreversible or that there is a homogeneous chemical reaction following the electrochemical reaction at the electrode surface.

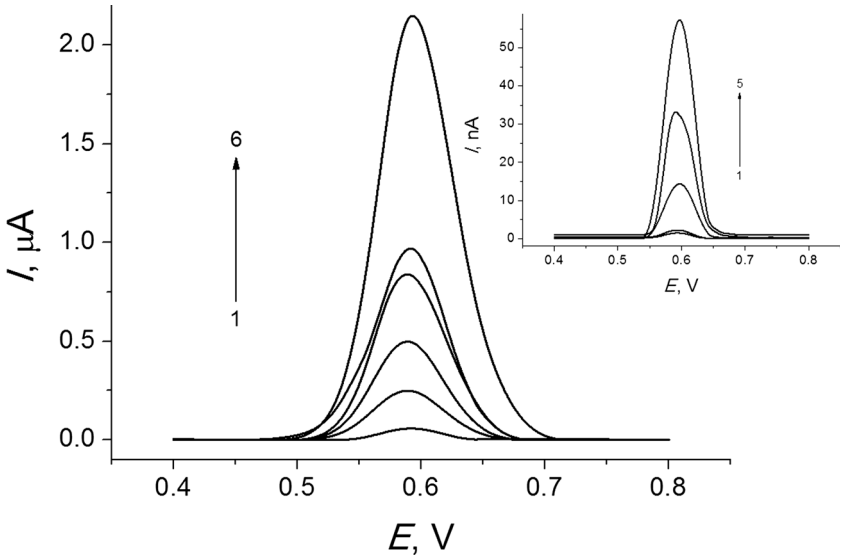

Fig. 5 Baseline-corrected DPVs of 10.1 (*curve 1*), 25.3 (*curve 2*), 50.5 (*curve 3*), 75.8 (*curve 4*), 101 (*curve 5*), and 202 (*curve 6*) μM thymol on CeO_2-Brij® 35/GCE in 0.01 M Brij® 35 in PB pH 6.0. *Inserted plot*: DPVs for 0.700 (*curve 1*), 1.01 (*curve 2*), 2.53 (*curve 3*), 5.05 (*curve 4*), and 10.1 (*curve 5*) μM of thymol. Potential scan rate is 10 mV s^{-1}. Pulse amplitude is 50 mV, pulse width is 50 ms, and potential scan rate is 10 mV s^{-1}

In this case, the number of electrons involved in reaction can be calculated according to $\Delta E_p = 30/\alpha n$ at 298 K for each 10-fold increase in υ (Scholz 2002). In general, α for a totally irreversible electrode process is assumed to be 0.5 (Bard and Faulkner 2001). The shift of thymol peak potential with increase of the scan rate is 31 ± 1 mV. Hence, the number of electrons involved in the oxidation process is 1.9 ± 0.1 on contrary to reported earlier one-electron oxidation with further participation of the radical formed in dimerization and polymerization reactions (Zhao et al. 2013; Piech and Paczosa-Bator 2015).

The effect of PB pH (4–8) has been investigated (Fig. 3). The oxidation potential of thymol is linearly decreased as the pH increased (Eq. 3) which confirms the participation of protons in the electrode process.

$$E[\mathrm{V}] = (0.9260 \pm 0.0052) - (0.0556 \pm 0.00085)\mathrm{pH} \quad R^2 = 0.9991 \tag{3}$$

The slope of 56 mV per pH unit indicates the equal number of protons and electrons participating in the electrode reaction. Thus, thymol oxidation on CeO_2-Brij® 35/GCE is a two-electron and two-proton process with formation of phenoxyl radical and phenoxonium ion that are stabilized by Brij® 35 micellar medium. The phenoxonium ion might undergo further chemical reactions (Simić et al. 2007).

The oxidation currents of thymol are decreased in basic medium that is caused by partial oxidation of the analyte by air oxygen. The highest and most stable signal is observed at pH 6.0 and used for further measurements.

Table 1 Voltammetric determination of thymol in model solutions CeO_2-Brij® 35/GCE in 0.01 M Brij® 35 in PB pH 6.0 (n = 5; P = 0.95)

Added, μg	Found, μg	RSD, %	R, %
7.6	7.5 ± 0.3	3.66	98.8
38	38 ± 2	3.69	100
114	114 ± 3	1.85	100
304	303 ± 1	0.363	99.7
911	911 ± 2	0.154	100

DPV Determination of Thymol

Thymol quantification was performed under conditions of DPV on CeO_2-Brij® 35/GCE. The effect of pulse parameters on thymol voltammetric response was evaluated. The best results (the highest and most reproducible peak currents) were observed at pulse amplitude of 50 mV and modulation time of 50 ms.

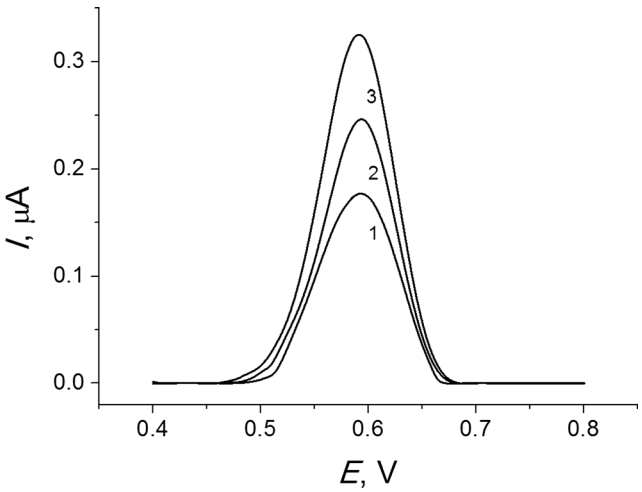

Fig. 6 Baseline-corrected DPVs of oregano micellar extract on CeO_2-Brij® 35/GCE in 0.01 M Brij® 35 in PB pH 6.0: *1*—extract; *2*—extract + 6.80 μM of thymol; *3*—extract + 13.4 μM of thymol. Pulse amplitude is 50 mV, and pulse width is 50 ms. Potential scan rate is 10 mV s^{-1}

Table 2 Determination of thymol in oregano ($n = 5$; $P = 0.95$)

Sample	Found by voltammetry, mg g^{-1}	RSD, %	Found by spectrophotometry, mg g^{-1}	RSD, %	t test[a]	F-test[b]
1	2.35 ± 0.04	1.60	2.39 ± 0.05	1.76	1.67	4.20
2	0.47 ± 0.03	5.39	0.48 ± 0.07	6.25	0.714	0.694
3	0.48 ± 0.02	3.19	0.51 ± 0.04	2.98	2.15	0.563
4	5.71 ± 0.05	0.50	5.7 ± 0.1	1.02	0.230	0.242

[a] $t_{tab} = 2.45$ at $P = 0.05$ and $df = 6$

[b] $F_{tab} = 6.94$ at $P = 0.05$ and $df_1 = 4$, $df_2 = 2$

There is a well-defined oxidation peak at 0.59 V on the DPVs of thymol on CeO_2-Brij® 35/GCE in BRB pH 2.0 (Fig. 4). The oxidation currents are linearly dependent on thymol concentration (Fig. 5) in the ranges of 0.700–10.1 and 10.1–606 μM (Eq. 4 and 5, respectively).

$$I_p[\mu A] = (-3.9 \pm 3.9) \times 10^{-4} + (66.19 \pm 0.68) \times 10^2 c_{thymol}[M] \quad R^2 = 0.9997 \quad (4)$$

$$I_p[\mu A] = (-0.020 \pm 0.024) + (108.92 \pm 0.89) \times 10^2 c_{thymol}[M] \quad R^2 = 0.9995 \quad (5)$$

The limits of detection (LOD) and quantification (LOQ) are calculated using statistic treatment ($3SD_a/b$) and ($10SD_a/b$), respectively, where SD_a is the standard deviation of the average arithmetic of 10 voltammograms of the blank solution obtained at the potential of thymol oxidation and b is the slope of the calibration graph. The LOD and LOQ are 0.20 and 0.65 μM of thymol, respectively, indicating good sensitivity of the approach developed. The analytical characteristics obtained in micellar medium using CeO_2-Brij® 35/GCE are much better or comparable with that one reported for bare and other chemically modified electrodes. The CeO_2-Brij® 35/GCE developed shows improved analytical characteristics in the oxidation of thymol compared with other electrodes.

So far as a new electrode has been prepared before each measurement, the reproducibility of thymol determination has been evaluated by five measurements at five various concentration levels using the added-found method (Table 1). The relative standard deviation does not exceed 4 %. The recovery of 99.9 ± 0.8 % obtained shows the high accuracy of the determination.

The Interference Study

The selectivity of the electrode was evaluated by testing the influences of several interfering substances on the detection of 50 μM thymol in PB pH 6.0. The electrochemical results indicate that 1000-fold higher concentrations of inorganic ions (K^+, Na^+, Mg^{2+}, Ca^{2+}, NO_3^-, Cl^-, and SO_4^{2-}) and 100-fold higher concentrations of glucose, rhamnose, sucrose, and ascorbic acid did not show the interference effect on thymol response. The interferences from other phenolic compounds could be observed in the case of real samples. Therefore, a range of natural phenolic compounds was studied. The electrode response to thymol is selective in the presence of gallic, caffeic, chlorogenic and rosmarinic acids, quercetin, rutin, and tannin. Eugenol, curcumin, and capsaicin affect thymol analytical signal at concentrations higher than 40, 10, and 30 μM, respectively, but the changes in the peak currents do not exceed 10 %.

Real Samples Analysis

The approach developed was applied for the quantification of thymol in micellar extracts of oregano spices. 0.1 M Brij® 35 was used as extractant. An intensification of the extraction was achieved using ultrasonic treatment for 10 min (Ziyatdinova et al. 2016). The component (spice/extractant) ratios of 1:20–1:60 were studied. The maximum extraction of thymol was reached at 1:40 ratio. Further increase of the extractant volume did not lead to the changes in recovery. The extract was filtered and then analyzed directly without any pretreatment.

There is a well-defined thymol oxidation peak at 0.59 V on the DPVs of oregano extracts that is confirmed by the standard addition method (Fig. 6) which showed proportional increase of the oxidation current at the same potential (Fig. 6, curves 2 and 3). The recovery values (99.7–100.8 %) confirm the accuracy of the determination and the absence of the matrix effects.

The thymol contents in different oregano spices recalculated per 1 g of dry sample are presented in Table 2. The voltammetric data correspond well to the results of spectrophotometric method (Razzaq and Mohammed 2014). The calculated t and F-test results are less than critical values of t and F at 95 % confidence level showing that variances of two populations are homogeneous and there are no significant differences in precision of voltammetry and spectrophotometry.

Conclusion

A new voltammetric method for the thymol quantification was developed. CeO_2 nanoparticles in combination with nonionic surfactant Brij® 35 as electrode surface modifier were used

for the first time. On the other hand, the Brij® 35 micellar medium was used for the measurements instead of organic solvents. The approach developed is rapid, simple, reproducible, and reliable. The analytical characteristics obtained are better than reported for other chemically modified electrodes. The renewal of the electrode surface before each measurement excludes passivation due to the adsorption of electrode reaction products. Good reproducibility of the modified electrode surface allows screen-printed electrodes to be produced for further application of the method in routine analysis of thymol-containing samples.

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Compliance with Ethical Standards

Conflict of Interest Guzel Ziyatdinova declares that she has no conflict of interest.

Endzhe Ziganshina declares that she has no conflict of interest.

Phuc Nguyen Cong declares that he has no conflict of interest.

Herman Budnikov declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Not applicable.

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