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ANALYSIS

APPLICATION OF GLASSY CARBON ELECTRODE MODIFIED WITH NAFION/MWCNTS FOR SENSITIVE VOLTAMMETRIC DETERMINATION OF THYMOL

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Abstract: The glassy carbon electrode modified with Nafion and multi-walled carbon nanotubes (Nafion/MWCNTs), applied for the determination of thymol using differential pulse voltammetry in phosphoric acid and methanol is presented. The calibration graph obtained for thymol is linear from 0.5 µM (75 µg/L) to 5 µM (750 µg/L) for a preconcentration time of 15 s, with correlation coefficient of 0.998. For a GC-Nafion/MWCNTs electrode the detection limit for a preconcentration time of 30 s is as low as 7.5 µg/L. The repeatability of the method at a concentration level of the analyte as low as 75 µg/L, expressed as RSD is 3.9% (n = 5). The proposed method was successfully applied and validated by studying the recovery of thymol from urine, dental mouthwash and liquid anti-ace.

Keywords: thymol, MWCNTs, Nafion, voltammetry

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpene phenol derivative of cymene, $C_{10}H_{14}O$, isomeric with carvacrol. It has been reported many plants which contain thymol as major component like: *Thymus vulgaris*, *Origanum vulgare*, *Trachyspermum ammi* and many others (1-5). Thymol is an active ingredient in pesticide products registered for use as animal repellents, fungicides/fungistats, medical disinfectants, tuberculocides, and virucides. These products are used on a variety of indoor and outdoor sites, to control target pests including animal pathogenic bacteria and fungi, several viruses including HIV-I, and birds, squirrels, beavers, rats, mice, dogs, cats and deer. Thymol also has many non-pesticidal uses, including use in perfumes, food flavorings, mouthwashes, pharmaceutical preparations and cosmetics (6). Thus a sensitive, specific, fast and cheap method of determining thymol is necessary for studying presence of thymol in various medical samples.

A number of analytical methods have been reported for the determination of thymol such as: gas chromatography (7-9), high performance liquid chromatography (10, 11), thin-layer chromatography (12), multivariate response surface methodology (13) and flow injection spectrophotometry (14). However, these methods are usually time consuming and require complicated pretreatment. On the other hand, voltammetric techniques are rapid, relatively chip and highly sensitive. In the group of voltammetrics methods various working electrodes for the determination of thymol such as: glassy carbon electrode (15), single-walled carbon nanotubes screen-printed electrodes (16), CeO_2 nanoparticle-decorated graphene hybrid film electrode (17) are used.

The aim of this work was to study the high sensitive determination of thymol by means of linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) with the use of glassy carbon (GC) electrode modified with Nafion/multi-walled carbon nanotubes (Nafion/MWCNTs). The new procedure was examined and successfully utilized for the determination of a low thymol concentration in urine, dental mouthwash and liquid anti-ace. Potential interferences from selected metal ions, citric acid and surface-active substances were checked.

EXPERIMENTAL

Apparatus and software

A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-

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ANKO, Poland) were used for all voltammetric measurements. The classical three-electrode quartz cell, volume 20 mL, consisting of a GC electrode (diameter 3 mm, Mineral, Poland) modified with Nafion/MWCNTs as the working electrode, a double junction reference electrode Ag/AgCl/KCl (3 M) with replaceable outer junction (3 M KCl) and a platinum wire as an auxiliary electrode were used. pH measurements were performed with laboratory pH-meter (N-512 elpo, Polymetron, Poland). Stirring was performed using a magnetic bar. All experiments were carried out at room temperature. The MTM-ANKO *EAGRAPH* software enabled electrochemical measurements, data acquisition and advanced processing of the results.

Chemicals and glassware

All reagents used were of analytical grade. KH_2PO_4, K_2HPO_4 were obtained from Merck and H_3PO_4 was obtained from CHEMAN (Poland). In measurements a 0.1 M phosphate solutions were used. Standard stock solutions of thymol (0.01 M) were prepared by dissolving thymol (local source) in methanol. Solutions with lower thymol concentrations were made by appropriate dilution of the stock solution. The multi-walled carbon nanotubes (purity >95%, diameter 40-60 nm, length 5-15 μm) were obtained from Nanostructured & Amorphous Materials Inc. (USA). Nafion 5 wt. % solution in a mixture of lower aliphatic alcohols and water was purchased from Aldrich.

Prior to use, glassware were cleaned by immersion in a 1 : 1 aqueous solution of HNO_3, followed by copious rinsing in distilled water.

Preparation of the electrode

Prior to modification, the GC electrode was mechanically polished with Al_2O_3 (0.05 μm), and then rinsed and sonicated 5 min in distilled water. Next, 10 mg of MWNTs was added to 10 mL ethanol and Nafion (final Nafion concentration 0.1%), and then sonicated for 2 h to obtain homogenous suspension. The prepared GC electrode was coated with 10 μL of homogenous Nafion/MWCNTs and allowed to evaporate the solvent at room temperature in the air.

Standard procedure of measurements

The electrochemical behavior of the Nafion/MWCNTs glassy carbon modified electrode was investigated using cyclic voltammetry. The voltammograms were recorded in the potential range from -200 to 1350 mV. Before each registration scan, the potential of 1350 mV (3 s) was applied to clean the surface of the electrode. The electrode conditioned in this way was used to determine thymol in the supporting electrolyte: 0.1 M phosphoric acid (total volume 10 mL) contained in a quartz voltammetric cell. In the case of DPV measurements the potential of the electrode was changed in the following sequence: cleaning potential 1350 mV for 3 s and preconcentration potential E_{acc} = -50 mV for t_{acc} = 10 s. During the preconcentration step thymol was collected while the solution was being stirred (ca. 500 rpm) using a magnetic stirring bar. Then, after a rest period of 3 s, a differential pulse voltammogram was recorded in the anodic direction from 450 to 1350 mV. The other experimental parameters were as follows: step potential, 6 mV; pulse potential, 50 mV; time step potential, 40 ms (20 ms waiting + 20 ms sampling time). The measurements were carried out from undeaerated solutions. Quantitative measurements were performed using the standard addition procedure.

Sample preparation

Urine

For DPV determination of thymol in urine, 250 μL of the fresh sample was added directly into voltammetric cell with supporting electrolyte (total volume 10 mL).

Dental mouthwash and liquid anti-ace

For the determination of thymol in dental mouthwash and liquid anti-ace, at first the products were 10-fold diluted with methanol and next, 50 μL of the samples were added to the voltammetric cell.

RESULTS AND DISCUSSION

Cyclic voltammetry studies

Effect of the scan rate

The influence of the scan rate (v) on the thymol peak at the GC electrode modified with Nafion/MWCNTs was investigated in the range of 10 mV/s to 500 mV/s (Fig. 1). The peak current *vs.* square root of scan rate (Fig. 2) gave a straight line up to 500 mV/s. The obtained linear regression equation is:

$$I_p = 2.7v^{1/2} - 1.1 \text{ [μA]}, \qquad r = 0.997$$

This suggests that the process of electrode reaction is controlled by diffusion of thymol.

The anodic peak potential was shifted in the positive direction with the increasing scan rate. The peak potential *vs.* ln scan rate gave a straight line (Fig. 3). The obtained linear regression equation is:

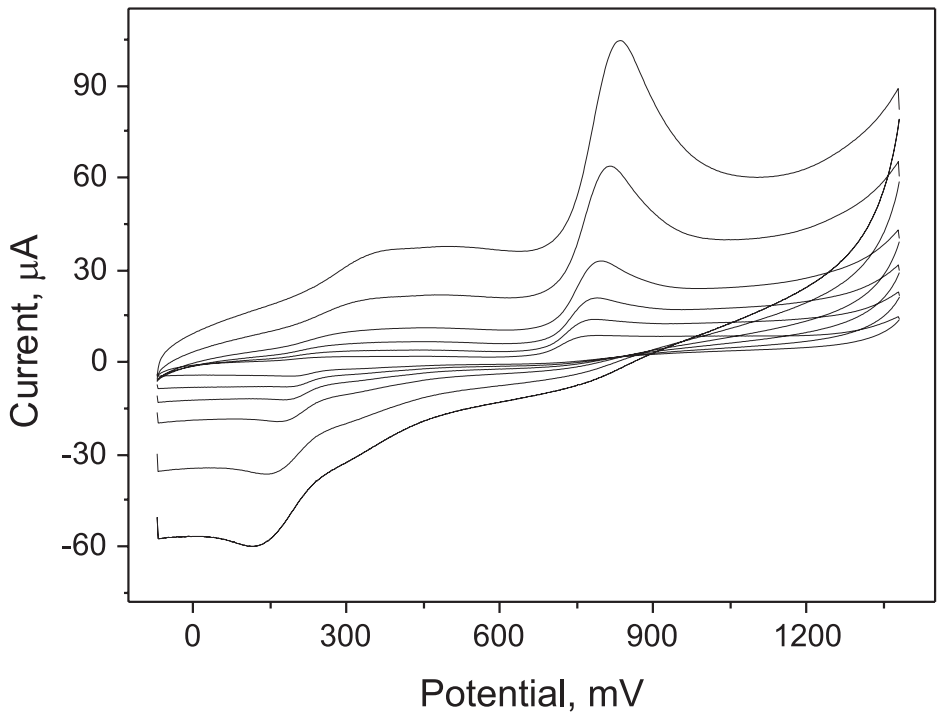

Figure 1. The cyclic voltammograms obtained for 0.25 mM thymol at the GC electrode modified with 10 μL Nafion/MWCNTs in 0.1 M H_3PO_4. Scan rate in the range from 10 to 500 mV/s

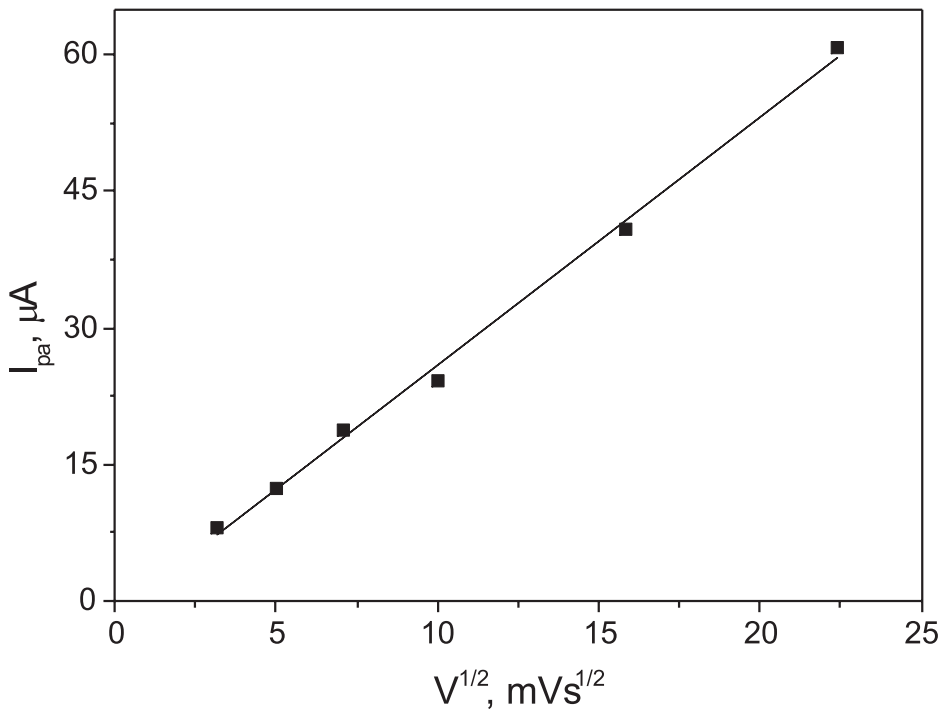

Figure 2. Dependence of the thymol peak current on square root of scan rate in the range from 10 to 500 mV/s for 0.25 mM thymol in 0.1 M H_3PO_4

$E_p = 20.3\ ln(v) + 747$ [mV], r = 0.997

Based on the theory for an irreversible electrode reaction the following equation holds (18):

$$E_p = E^0 + \frac{RT}{\alpha n F}\left[0.780 + \ln\left(\frac{D_0^{1/2}}{k_s}\right) + \ln\left(\frac{\alpha n F v}{RT}\right)^{1/2}\right] \quad (1)$$

where E^0 is the formal potential, a is the transfer coefficient, n is the number of electrons involved in the charge-transfer step, k_s is the standard rate constant, F is the Faraday constant, D is the diffusion coefficient, R and T have their usual meaning. From the slope of E_p *vs. ln(v),* $\alpha n = 0.63$ could be obtain

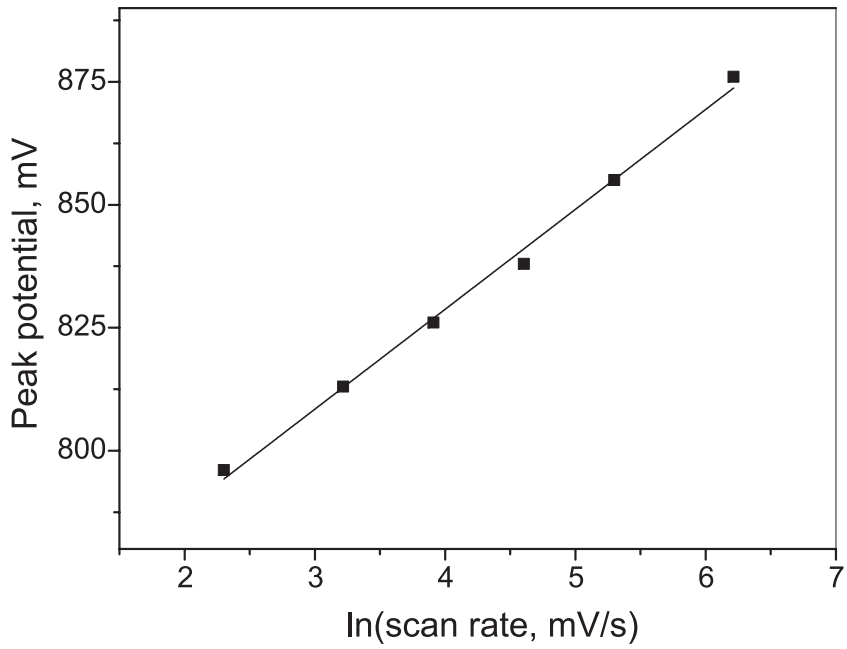

Figure 3. Dependence of the thymol peak potential on ln of scan rate in the range from 10 to 500 mV/s for 0.25 mM thymol in 0.1 M H_3PO_4

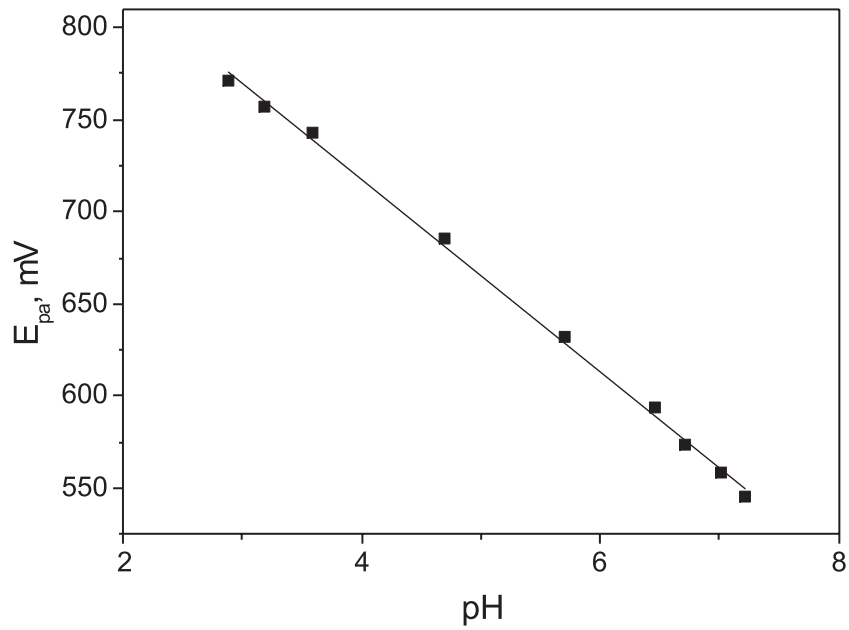

Figure 4. Dependence of the thymol peak potential on pH in the pH range from 2.9 to 7.2 for 0.25 mM thymol in 0.1 M phosphate buffers

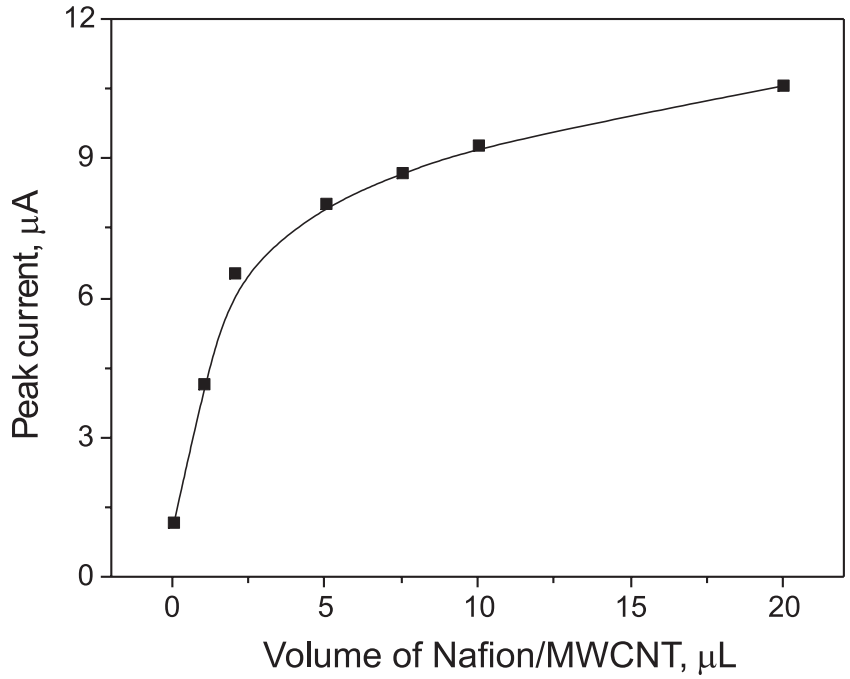

Figure 5. Dependence of the peak current on volume of Nafion/MWCNTs on GC electrode in the range of 0 to 20 μL for 25 μM thymol in 0.1 M H_3PO_4. Instrumental parameters: ΔE = 50 mV, E_s = 6 mV, t_w, t_s = 20 ms. Preconcentration potential -50 mV, preconcentration time 10 s, stirring rate 500 rpm

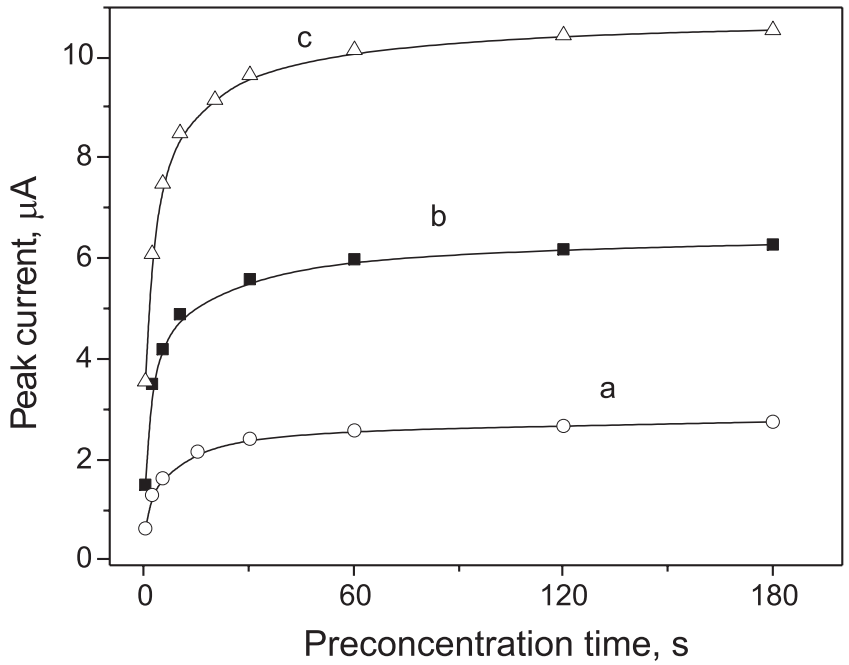

Figure 6. Dependence of the peak current on preconcentration time in the range from 0 to 180 s for (a) 2.5; (b) 10 and (c) 25 mM thymol in 0.1 M H_3PO_4. All other conditions are as in Figure 5

and the number of the electron transfer for α assuming 0.5 could be calculated to 1.

Effect of the pH values

The influence of the pH value on the peak potential at the GC electrode modified with Nafion/MWCNTs was investigated in the pH range from 2.9 to 7.2 (Fig. 4). The thymol peak potential shifted negatively as the pH increased, indicating that the electrode process involved proton participation. The dependence between the peak potential and the pH is linear and the obtained equation is:

$$Ep = -52.2\ pH + 926\ [mV], \quad r = 0.998$$

The slope of this relationship is close to the expected theoretic value of 59.1 mV/pH and suggests that the equal numbers of protons and electrons are involved in the electrode reaction.

Influence of DPV parameters on technique on thymol peak

The important parameters of the DPV technique are pulse amplitude (ΔE), potential step amplitude (E_s), waiting time (t_w) and sampling time (t_s). Consequently, these parameters were investigated. To optimize the conditions for thymol measurements, the following instrumental parameters were systematically varied: *DE* in the range 5 - 100 mV (both positive and negative mode), E_s in the range 1 - 7 mV, t_w and t_p from 10 to 60 ms.

The best results were obtained for the amplitude of 50 mV (the peak current was ~8.5 mA for 25 mM thymol). Higher pulse amplitude (>50 mV) caused major growth of the background current. For further work, the pulse amplitude of 50 mV was applied.

Changes of the step potential cause influence on peak current. For a step potential equal to 1 mV the peak current was 2.2 µA, and for a step potential of 7 mV the peak current was 9.4 µA. The step potential of 6 mV was applied in further work.

The waiting time and sampling time were changed in the range from 10 to 60 ms. The best result was obtained for waiting time and sampling time of 20 ms, and this was the value chosen for further work.

Influence of the volume of Nafion/MWCNTs on thymol peak

The mixture of Nafion/MWCNTs coated on the GC electrode is necessary to obtain high sensitive determination of thymol. The thymol peak current depends on the volume of Nafion/ MWCNTs (Fig. 5). For bare GC electrode the thymol peak current was 1.1 µA. The presence and increase of the amount of Nafion/MWCNTs on the GC electrode is accompanied by an increase of the thymol peak. The optimal volume of Nafion/ MWCNTs was for 10 µL (with the peak current reaching values about 9 mA

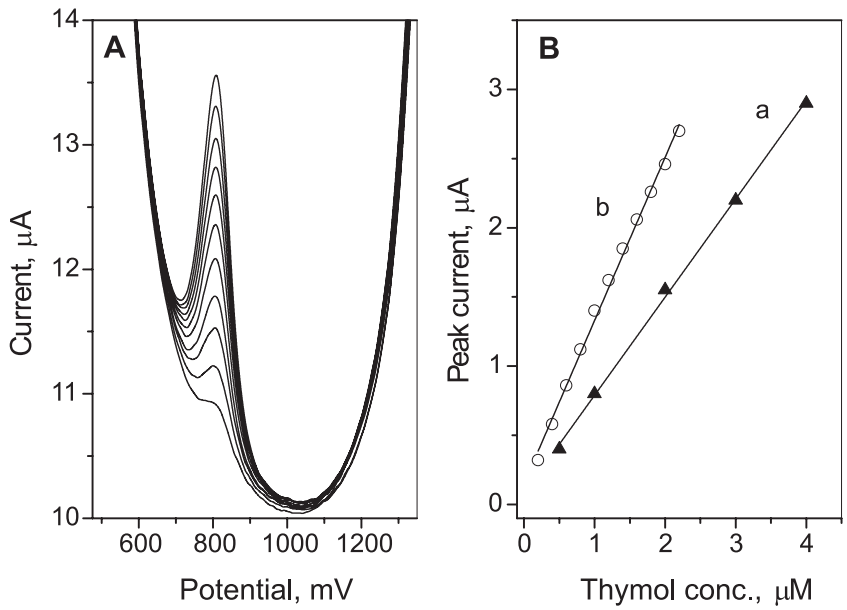

Figure 7. **A** – The DP SV thymol calibration voltammograms from 0.2 to 2.2 mM obtained for preconcentration time 30 s in 0.1 M H_3PO_4, **B** – thymol calibration curves obtained for preconcentration time: (a) – 15 and (b) – 30 s. All other conditions are as in Figure 5

Table 1. Results of thymol determination in various samples (n = 3).

Thymol added	Thymol found $\bar{x}$ ± s (recovery %)			
	Urine (μM)	I - Dental mouthwash[1] (mg/100 mL)	II - Dental mouthwash[1] (mg/100 mL)	Liquid anti-ace[2] (mg/100 mL)
0	0	62.3 ± 4.1	64.1 ± 2.9	51.1 ± 3.7
0.5 μM	0.44 ± 0.05 (88)	-	-	-
1.5 μM	1.38 ± 0.11 (92)	-	-	-
50 mg/100 mL	-	116.7 ± 6.1 (103)	116.4 ± 5.8 (102)	94.9 ± 6.7 (94)

[1] product declared 60 mg/100 mL; [2] product declared 50 mg/100 mL

for 25 μM of thymol). Higher volumes of Nafion/MWCNTs cause major increase in a background current. The presence of Nafion/MWCNTs also had an influence on the peak potential. For bare GC electrode the DPV thymol peak potential was 876 mV and for modified electrode with 10 μL Nafion/ MWCNTs the thymol peak potential was 800 mV. The negative shift of the thymol peak potential suggests catalytic effect caused by Nafion/ MWCNTs. For further work, the volume of 10 mL was used.

Influence of preconcentration potential and time on thymol peak

Influence of preconcentration potential and time are usually important factors on the sensitivity and detection limit of the stripping methods. In the case of thymol determination the preconcentration potential has no influence on the peak current. For further work, the -50 mV preconcentration potential was applied.

The changes in magnitude of the thymol current *vs*. preconcentration time are presented in Figure 6. The peak current increased with the increase of the preconcentration time for (a) – 2.5 μM thymol from 0.45 μA (t_{acc} = 0 s) to 2.6 μA (t_{acc} = 180 s), for (b) – 10 μM thymol from 1.6 μA (t_{acc} = 0 s) to 6.4 μA (t_{acc} = 180 s) and for (c) – 25 μM thymol from 3.6 μA (t_{acc} = 0 s) to 10.6 μA (t_{acc} = 180 s), respectively. For a preconcentration time higher than 60 s, practically no increase of the thymol peak current was observed. The thymol peak potential is independent on either the preconcentration potential (E_p = 800 mV).

Influence of supporting electrolyte composition

Determination of thymol on GC electrode modified with Nafion/MWCNTs requires an acidic conditions in order to obtain a well-shaped and high peak. The best results were obtained in phosphoric acid. To improve the solubility of thymol, additionally methanol to the supporting electrolyte was added. For further measurements, the 0.1 M H_3PO_4 and 500 uL methanol was applied (good relation the thymol signal to the background current).

Interferences

The examined ions, such as: Ca(II), Mg(II) in a 100-fold excess, and Zn(II), Mn(II) in a 100-fold excess as well as Pb(II), Cd(II), Cu(II) in a 2-fold excess did not interfere. Organic compounds such as: citric acid in a 20-fold excess and glucose 25 mg/L did not interfere.

The surface-active compounds are usually a source of strong interferences in voltammetric methods. A non-ionic surface-active compound (Triton X-100) was investigated in this respect. For 0.5 mg/L of Triton X-100 concentration, no suppress of the signal was observed. Higher concentration of Triton X-100 caused suppression the signal e.g., for 2.5 mg/L of Triton X-100 by 30% and for 6 mg/L of Triton X-100 by 55%.

Analytical performance

The differential pulse stripping voltammetry (DP SV*)* voltammograms of thymol for the 0.2-2.2 μM concentration range and preconcentration time of 30 s are presented in Figure 7.

The detection limit obtained for short preconcentration time (15 s) was 0.1 μM with the linearity up to 5 μM (slope for regression line was 0.71 ± 0.02 [μA/μM], intercept 0.08 ± 0.04 μA, correlation coefficient 0.998). A longer preconcentration time results in a lower detection limit (for example, when the preconcentration time of 30 s was used during measurement, the detection limit was 0.06 μM. The

slope for regression line was [μA/μM]: 1.18 ± 0.02, intercept [μA]: 0.15 ± 0.06, the correlation coefficients 0.998 and the linearity was up to 3 μM.

To validate the method, the urine, dental mouthwash and liquid anti-ace were investigated.

The samples, spiked with thymol were analyzed according to the described procedure using the GC electrode modified with Nafion/MWCNTs. Determinations of thymol were performed using the standard addition method (two additions of the standard solution). Results from thymol determination are presented in Table 1. The recovery of thymol ranged from 88 to 103%. The analytical usefulness, of the presented method for the determination of thymol in samples was confirmed.

CONCLUSIONS

The presented DPV method for the electrochemical determination of thymol using a GC electrode modified with Nafion/MWCNTs allows to determine thymol at trace levels, in concentrations as low as 0.05 μM (7.5 μg/L) for a preconcentration time of 30 s. The reproducibility of the method is good, i.e., when measured as RSD is 3.9%. Acceptable recovery (88–103%) shows that the proposed method can be used for the determination of thymol in urine, dental mouthwash and liquid anti-ace.

The preparation of GC electrode modified with Nafion/MWCNTs is very simple, short and economically acceptable. The obtained results confirm that presented method may be used into out-of-laboratory systems.

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