Analytical Biochemistry 486 (2015) 1–4

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Sensitive voltammetric determination of thymol in essential oil of *Carum copticum* seeds using boron-doped diamond electrode

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 17 June 2015
Accepted 18 June 2015
Available online 25 June 2015

Keywords:
Carum copticum seeds
Thymol
Boron-doped diamond electrode

ABSTRACT

Essential oil of *Carum copticum* seeds, obtained from a local shop, was extracted and content of thymol was analyzed using square-wave voltammetry at boron-doped diamond electrode. The effect of various parameters, such as pH of supporting electrolyte and square-wave voltammetric parameters (modulation amplitude and frequency), was examined. In Britton–Robinson buffer solution (pH 4), thymol provided a single and oval-shaped irreversible oxidation peak at +1.13 V versus silver/silver chloride potassium electrode (3 M). Under optimal experimental conditions, a plot of peak height against concentration of thymol was found to be linear over the range of 4 to 100 μM consisting of two linear ranges: from 4 to 20 μM (R^2 = 0.9964) and from 20 to 100 μM (R^2 = 0.9993). The effect of potential interferences such as p-cymene and γ-terpinene (major components in essential oil of *C. copticum* seeds) was evaluated. Thus, the proposed method displays a sufficient selectivity toward thymol with a detection limit of 3.9 μM, and it was successfully applied for the determination of thymol in essential oil of *C. copticum* seeds. The Prussian blue method was used for validation of the proposed electroanalytical method.

Carum copticum is a grassy annual plant in the Umbellifarae family that grows in the east of India, Iran, and Egypt with white flowers and small brownish seeds. Constituent of its seeds are an aromatic volatile essential oil and a crystalline substance called stearoptene. The stearoptene is known as *ajowan-ka-phul* (crude thymol) [1–4]. This plant has been mentioned in Iranian traditional literature to have therapeutic effect on colic, dyspepsia, diarrhea, flatulence, and indigestion [3,5–7]. The plant is also known to possess antiallergic, antibacterial, anthelmintic, antifungal, hypocholesteremic, bronchodilator, and cholinergic activities [8,9].

Thymol crystallizes easily from the oil extracted from the *C. copticum* seeds, and the remainder consists of p-cymene and γ-terpinene as major components and b-pinene, diterpene β-terpinene, and carvacrol. Thymol as phenolic antioxidant is a very interesting component. Natural antioxidants are being extensively studied with respect to their capacity to protect organisms and cells from damage induced by oxidative stress, which is considered to be a cause of degenerative processes and cancer. Thymol has an important role in inhibiting the peroxidation of liposome phospholipids in a concentration-dependent manner [10–12].

Boron-doped diamond (BDD)[1] was discovered by the end of 20th century as new carbon-based electrode material [13,14]. This electrode has the widest usable potential range from all electrode materials (up to 3 V), minimum problem with passivation pertinent to sp^3 character of diamond carbon, stability in both alkaline and acidic media, and minimal noise and low residual current [15].

In consequence, the conceptual suitability of electrochemistry for the determination of the organic compound and the inherent advantages of BDD electrode offer an attractive pool of sensitive, economical, and disposable tools for the detection of the thymol. Indeed, the determination method tends to be fast, involving little or no sample treatment, and the response is used for immediate decision making, with confirmation requiring a conventional alternative.

Therefore, the aim of this work was to explore the analytical possibilities of commercial tools based on BDD electrode for fast determination of thymol in essential oil from *C. copticum* seeds. The various experimental and instrumental parameters were evaluated, and optimal conditions were obtained. In addition, the effect of important interferences was investigated. The proposed method

[1] *Abbreviations used:* BDD, boron-doped diamond; BR buffer, Britton–Robinson buffer; CV, cyclic voltammetry; SWV, square-wave voltammetry; DPV, differential pulse voltammetry.

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http://dx.doi.org/10.1016/j.ab.2015.06.026
0003-2697/

was successfully applied for the determination of thymol in essential oil extract.

Materials and methods

Apparatus and reagents

Thymol, p-cymene, γ-terpinene, boric acid, sodium hydroxide, ethanol, acetic acid, and phosphoric acid were purchased from Sigma–Aldrich and used as received without any further purification. Stock solution (10^{-3} M) of the tested compound was prepared in ethanol/Millipore water. Calibration standard solutions were prepared from the stock solution by appropriate dilution with supporting electrolyte. The pHs of different Britton–Robinson (BR) buffers were adjusted with sodium hydroxide (0.2 M).

Cyclic voltammetry (CV) and square-wave voltammetry (SWV) measurements were performed using an electrochemical system, Autolab PGSTAT 302 N (Metrohm Autolab, The Netherlands), controlled by the corresponding software (Nova 1.10). The cell (10 ml) consisted of a three-electrode system: a BDD electrode (3 mm i.d.; Windsor Scientific, Slough, Berkshire, UK), an Ag/AgCl (saturated KCl) reference electrode, and a Pt counter electrode. All figures were prepared using Origin 8.5. All potentials reported in this article were obtained versus Ag/AgCl reference electrode at an ambient temperature. All pH values were measured with pH meter model Orion 1230.

The potential was swept over the range from 0 to +1.4 V (vs. Ag/AgCl) at different scan rates for CV and from 0.5 to +1.4 V (vs. Ag/AgCl) at the optimized instrumental parameters (step potential 5 mV, frequency 10 Hz, and modulation amplitude 70 mV) for SWV.

For the Prussian blue method, the different volumes of thymol were prepared in a range as for differential pulse voltammetry (DPV). The following mixture was added to each dilution of control solutions: 400 µl of 0.0008 M $K_4Fe(CN)_6$ and 400 ml of $FeCl_3$ in 0.1 M HCl solution. The final volume was 10 ml. Then, 7 min later, absorbance was measured at 700 nm.

Sample preparation

Dried seeds of *C. copticum* were purchased from a local herbal store. The plant material was ground into coarse powder by an electrically driven grinder and was soaked in 70% aqueous ethanol for 3 days with occasional shaking. The soaked material was filtered, and the procedure was repeated twice. The combined filtrate was evaporated, and obtained oil was stored in a refrigerator at 4 °C.

Results and discussion

Electrochemical behavior of thymol at different pHs and scan rates

Voltammetric behavior of thymol at BDD electrode was evaluated by using CV in different BR buffers. Thymol in a concentration of 0.1 mM provided a single and oval-shaped irreversible oxidation peak at +1.13 V (Fig. 1). The inset of Fig. 1 presents the dependence of peak current and peak potential of thymol from different pHs of supporting electrolyte. It can be concluded that there was no regular dependence; the values of peak potential and peak current were changed with the pH of buffer on every three pH units. The highest peak currents were obtained from pH 2 to 4. pH 4 was selected as suitable for further experiments because more acidic media can have an influence on real sample composition and require the use of a higher amount of chemicals to adjust the media. Using this pH, the effect of scan rate (Fig. 2) and

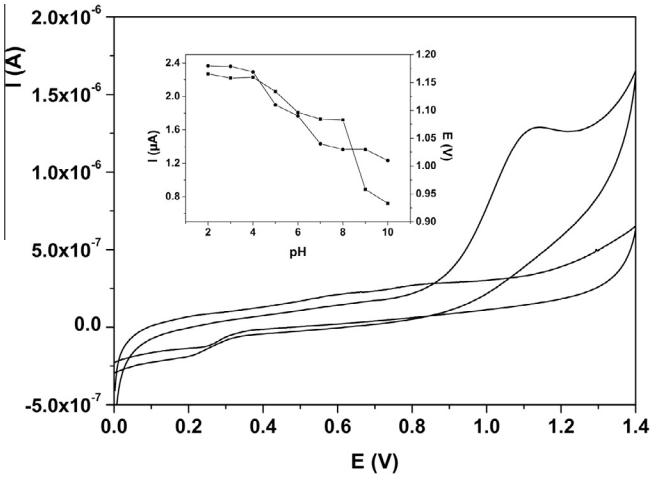

Fig.1. Cyclic voltammograms of BR buffer solution (pH 4) in the presence and absence of 0.1 mM thymol at BDD electrode with a scan rate of 30 mV/s. The inset presents the dependence between current response and pH of supporting electrolyte at the same experimental conditions. ■, peak current; ●, peak potential.

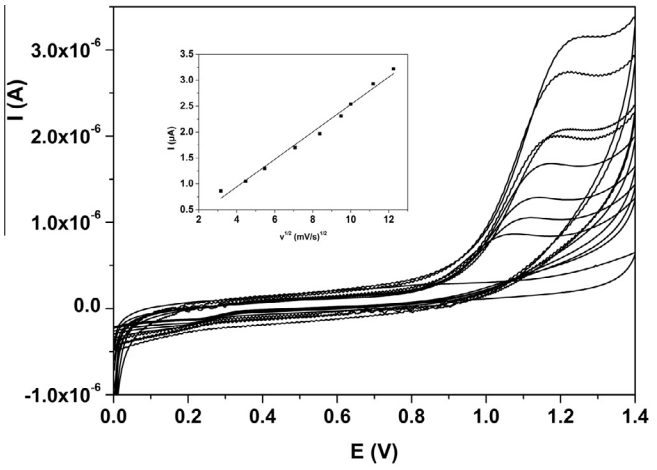

Fig.2. Cyclic voltammograms of thymol at different scan rates (10–150 mV/s) using 0.1 mM thymol in BR buffer solution (pH 4) at BDD electrode. The inset figure presents the dependence between peak current and square root of scan rate under the same experimental conditions.

dependence between peak current and square root of scan rate (inset of Fig. 2) was evaluated. With increasing scan rates, peak current becomes higher without any significant shift in peak potential. The linear Randles–Sevcik plot was obtained with linear regression equation I_p (µA) = (0.265 ± 0.011) $v^{1/2}$(mV/s)$^{1/2}$ – (0.115 ± 0.093), R^2 = 0.9963, indicating the diffusion-controlled nature of the electrode process.

Optimization of SWV parameters

For determination of thymol, SWV was selected as a suitable electroanalytical technique because of its rapidity, low background currents, and low detection limits. The optimization of SWV parameters influencing the current response of analyte is an important step in the development of electroanalytical methodology. Accordingly, the instrumental parameters such as modulation amplitude and frequency were investigated in order to optimize the experimental setup for determination of thymol. All experiments were carried out in 0.1 mM thymol in BR buffer solution at pH 4.