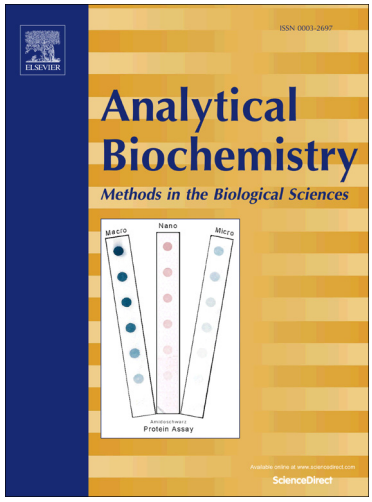

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Sensitive voltammetric determination of thymol in essential oil of *Carum copticum* seeds using boron-doped diamond electrode

Short title:

Determination of thymol in essential oil

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Abstract

Essential oil of *Carum copticum* seeds, obtained from 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 vs. silver-silver chloride-potassium electrode (3M). Under optimum experimental conditions, a plot of peak height against concentration of thymol was found to be linear over the range of 4 – 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 *Carum copticum* seeds) was evaluated. Thus, the proposed method displays a sufficient selectivity toward thymol with detection limit of 3.9 μM and it was successfully applied for the determination of thymol in essential oil of *Carum copticum* seeds. Prussian blue Method was used for validation of the proposed elactroanalytical method.

Keywords: Carum copticum seeds, thymol, boron-doped diamond electrode

1. Introduction

Carum copticum is a grassy, annual plant in the Umbellifarae family, which grows in the east of India, Iran and Egypt with white flowers and small, brownish seeds. Constituent of its seed are: an aromatic volatile essential oil, and a crystalline substance 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 posses antiallergic, antibacterial, anthelmintic, anti-fungal, hypocholesteraemic, bronchodilator and cholinergic activities [8, 9].

Thymol crystallizes easily from the oil extracted from the *Carum 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 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) was discovered by the end of 20^{th} 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 as well asminimal noise and low residual current [15].

In consequence, the conceptual suitability of electrochemistry for the determination of the organic compound as well as the inherent advantages of boron-doped diamond electrode, offer an attractive pool of sensitive, economical and disposable tools for the detection of the thymol. Indeed, 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 is to explore the analytical possibilities of commercial tools based on boron-doped diamond electrode for fast determination of thymol in essential oil from *Carum copticum* seeds. The various experimental and instrumental parameters were evaluated and optimal conditions were obtained. Besides, the effect of important interferences was investigated. The proposed method was successfully applied for the determination of thymol in essential oil extract.

2. Experimental

2.1.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 buffers were adjusted with sodium hydroxide (0.2 M).

Cyclic voltammetric and square-wave voltammetric measurements were performed using an electrochemical system AUTOLAB PGSTAT 302N, Methrom Autolab B.V., (The Netherlands) controlled by the corresponding software (NOVA 1.10). The cell (10 mL) consisted of three-electrode system, boron-doped diamond electrode (inner diameter of 3 mm; Windsor Scientific Ltd., Slough, Berkshire, United Kingdom), an Ag/AgCl (saturated KCl) reference electrode and Pt counter electrode. All figures were prepared using Origin 8.5. All potentials reported in this paper were obtained vs. 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 amplitude70 mV) for square-wave voltammetry.

For Prussian blue Method the different volumes of thymol were prepared in range as for 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. Seven minutes later absorbance was measured at 700 nm.

2.2. Sample preparation

Dried seeds of *Carum copticum* were purchased from a local herbal store. The plant material was ground into coarse powder by electrically driven grinder and was soaking 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 fridge at 4^{o}C.

3. Results and discussion

3.1.Electrochemical behavior of thymol at different pH and scan rates

Voltammetric behavior of thymol at boron-doped diamond electrode was evaluated by using cyclic voltammetry in different Britton-Robinson buffers. Thymol in concentration of 0.1 mM provided singe and oval-shaped irreversible oxidation peak at +1.13 V (Fig. 1). Inset of Fig. 1 presents a 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 pH of BR buffer on every three pH units. The highest peak currents were obtained from pH 2 to pH 4. pH 4 was selected as suitable for further experiments, as more acidic media can have influence on real sample composition, and require the use of higher amount of chemicals to adjust the media. Using this pH, the effect of scan rate (Fig. 2) and dependence between peak current and square root of scan rate (inset of Fig. 2) was evaluated. With increasing scan rate 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 diffusion-controlled nature of the electrode process.

Here Fig. 1.

Here Fig. 2.

3.2.Optimization of square wave voltammetry parameters

For determination of thymol square-wave voltammetry (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 set-up for determination of thymol. All experiments were carried out in 0.1 mM thymol in BR buffer solution at pH 4.

Firstly, the influence of modulation amplitude on the peak current was studied in the range from 10 to 100 mV at fixed frequency of 20 Hz and step potential of 5 mV. It was found that the oxidation peak of thymol slightly shifted to the less positive potentials with increasing amplitude (data not shown). The modulation amplitude of 70 mV was selected as an optimal for determination of thymol thank to convenient peak current and potential. In the case of frequency, the peak current decreased with increase of frequency from 10 to 70 Hz. The most

suitable peak current was observed at 10 Hz at fixed value of modulation amplitude at 70 mV. For all further experiments, SWV with optimized operating parameters was applied.

3.3.Calibration curve and determination of thymol

Calibration curve was constructed by plotting of peak current against thymol concentration in the range from 4 to 100 μM and the oxidation peak increased proportionally in this concentration range. The resulting voltammograms are presented at Fig. 3. From Fig. 4 it can be concluded that there were two linear ranges for determination of thymol. First range was linear from 4 to 20 μM (inset A in Fig. 4) and could be described by equation $I\ (\mu A) = (0.784 \pm 0.018)\ c\ (\mu M) - (3.026 \pm 0.190)$, $R^2 = 0.9964$, and the second one was linear in the range from 20 to 100 μM (inset B in Fig. 4) and could be described by equation $I\ (\mu A) = (1.334 \pm 0.014)\ c\ (\mu M) - (14.572 \pm 0.803)$, $R^2 = 0.9993$. The detection limit was found to be 3.9 μM and this low value confirms the high sensitivity of proposed method. At the same concentration level under proposed experimental conditions the repeatability of 7 measurements was examined. The relative standard deviation of these measurements was 2.7 %, and this value verified an excellent repeatability of proposed method. This fact confirms that proposed procedure proved to be suitable for precise detection and quantification of thymol.

Here Fig. 3.

Here Fig. 4.

3.4. Interferences studies

The influence of possible interfering biomolecules and selectivity of proposed method were investigated under found operating conditions. Based on so far reported articles from this area which deal with analysis of essential oil from *Carum copticum* seeds, the most common interferences are p-cymene (around 15 %) and γ-terpinene (around 30 %). All other biomolecules are usually present at concentration level of 1 % or less [9]. In Fig 5.the CV voltammograms of all three selected compounds in concentration of 0.1 mM are displayed. As can be seen from this figure, p-cymene and γ-terpinene do not give signals in CV record under the same experimental conditions. Inset A and B of Fig 5. shows SW voltammograms of thymol without and with presence of selected interferences at same concentration level. It can

be concluded that there was no influence of these biomolecules on the determination of thymol.

Here Fig. 5.

3.5. Analytical application

Based on the results described in previous part, the developed method is very sensitive and sufficiently selective, and therefore, it could be applied for the determination of thymol in essential oil of *Carum copticum* seeds. Essential oil was extracted with previously described procedure. According to the literature [9] extraction yield based on SFE varied in the range of 1.0 to 5.8 %, and the content of thymol in essential oil varied in the range from 45 to 55 %. The extraction yield, based on our extraction was 4.2 % (w/w). 100 µL of extracted oil was added in buffer solution to make 10 mL and then directly analyzed.The procedure was repeated 3 times. The obtained result expressed by relative standard deviation of 3 determinations is summarized in Table 1. It is obvious, that determined values well corresponded with the content obtained by other authors and that the proposed method could be serve as reliable method for the analysis of thymol in natural samples. The illustrative SW voltammograms for thymol determination in the essential oil from *Carum copticum* seeds are shown in Fig. 6.

Table 1.Content of thymol in essential oil of *Carum copticum* obtained by SWV at BDD electrode under optimized parameters.

Sample	Found	Found by PB method	Expected [9]
Essential oil from *Carum copticum*	(52 ± 1.2) %	(51 ± 0.8) %	45 - 55 %

Here Fig. 6.

4. Conclusions

For the first time, an application of BDD electrode with SWV technique for sensitive, simple, fast and cheap analytical determination of thymol in essential oil from *Carum copticum* seeds was presented. The procedure enables the determination of thymol with sensitivity below the maximum content in essential oil, and without any difficult sample preparation (just dilution). The selectivity of method appears to be sufficient as there were no influence of other biomolecules present in essential oil. It can be concluded that the proposed SW voltammetric procedure is sensitive, fast, green and effective tool in analysis of thymol and other monoterpene phenols and may represent the electrochemical alternative to other more expensive and complicated analytical procedures.

5. Acknowledgements

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Figure captions:

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

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

Fig. 3 SW voltammograms obtained for different concentrations of thymol (from 4 to 100 μM) at boron-doped diamond electrode under optimized experimental conditions.

Fig. 4 Calibration curves obtained by plotting current versus concentration of thymol under optimized experimental conditions.

Fig. 5 CV voltammograms of thymol, p-cymene and γ-terpinene in concentration of 0.1 mM at BDD electrode in BR buffer solution pH 4.5, baseline is marked with dots; Inset figures present SW voltammograms of thymol (0.1 mM) at optimized experimental conditions in absence (A) and presence (B) of p-cymene and γ-terpinene as interferences in same concentration level.

Fig. 6 SW voltammograms for determination of thymol in essential oil from *Carum copticum* using proposed electroanalytical procedure obtained by standard addition method. S-sample, 1-3 curves present addition of thymol standard solution.

Figure 1

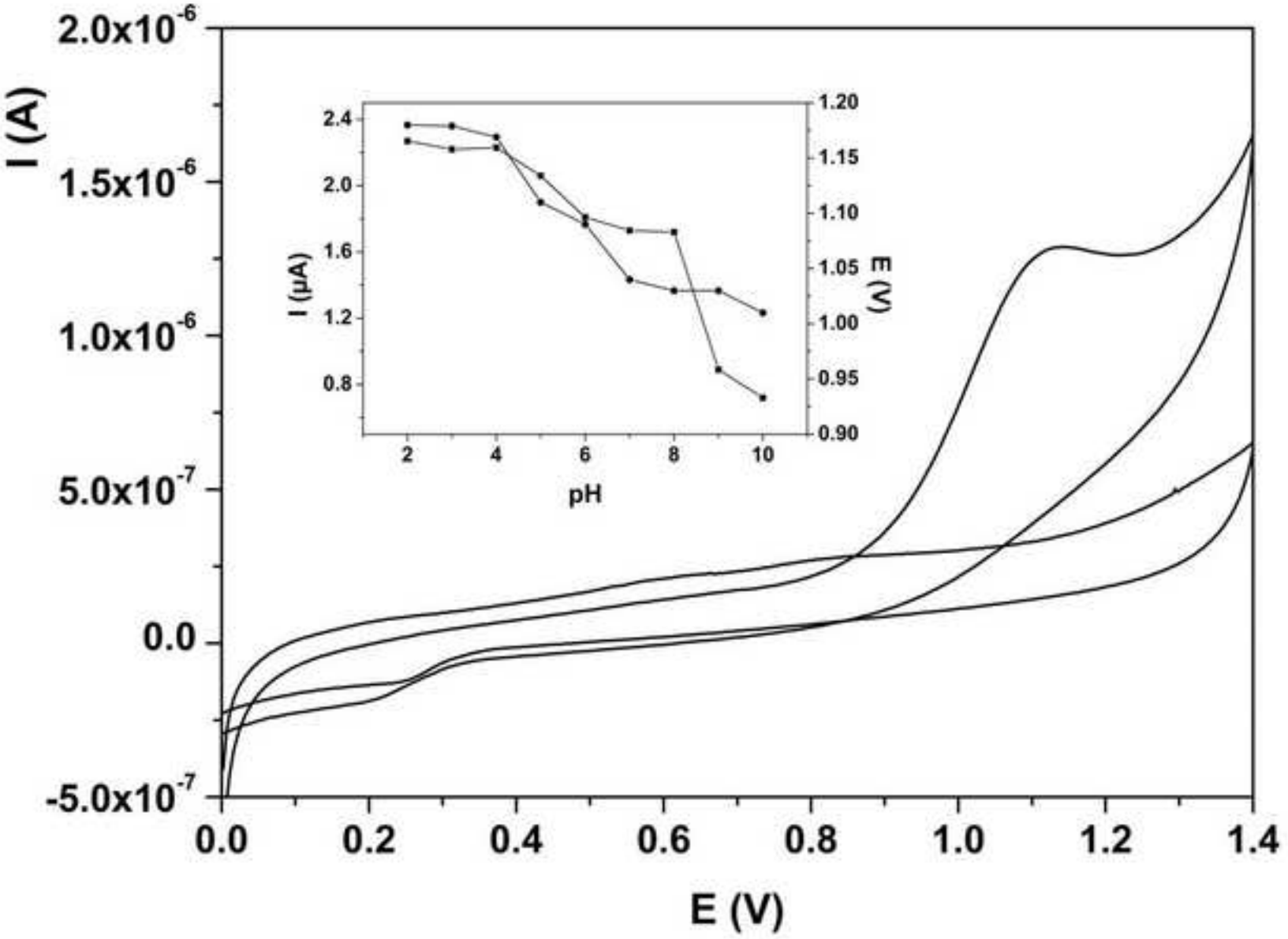

Figure 2

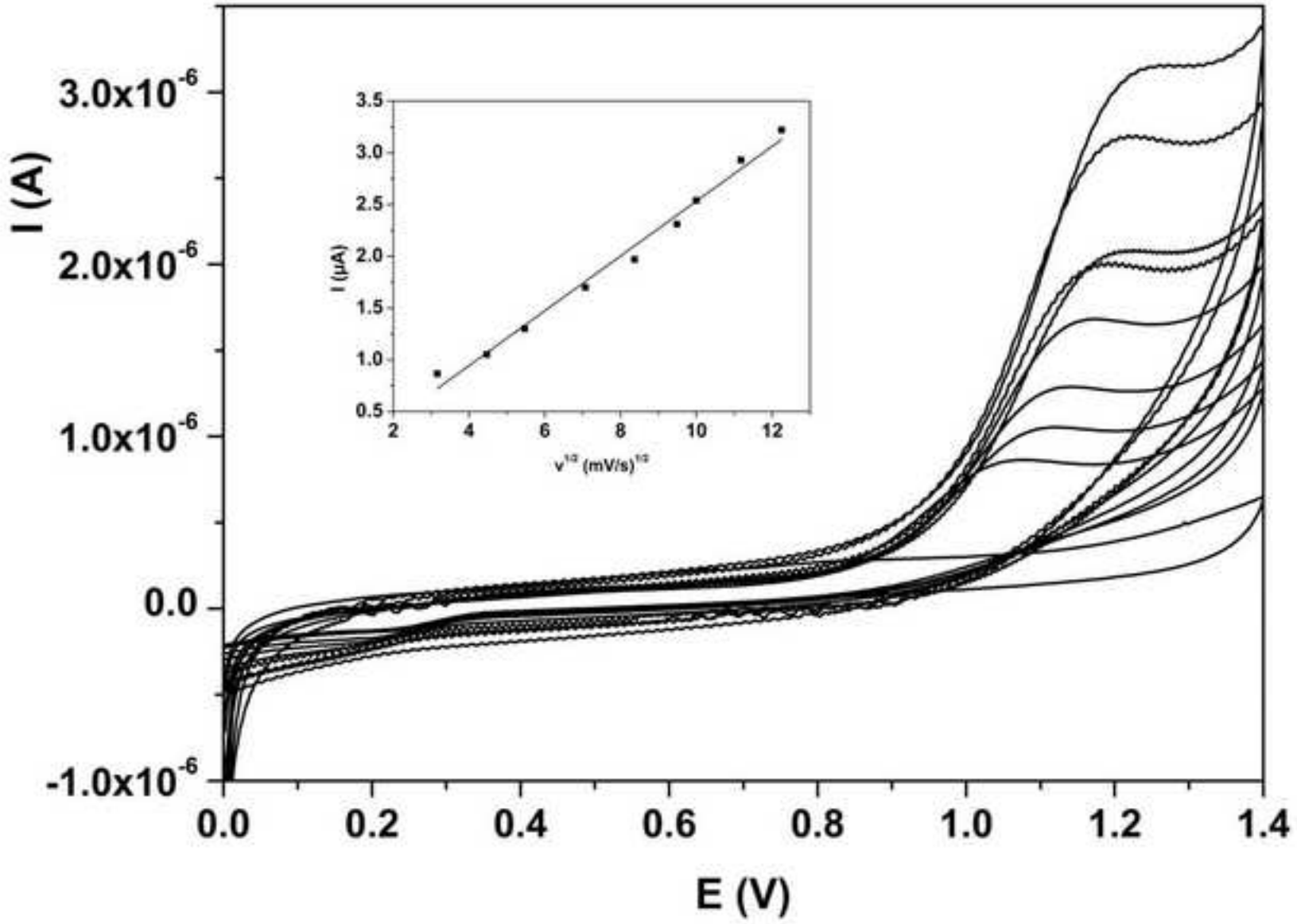

Figure 3

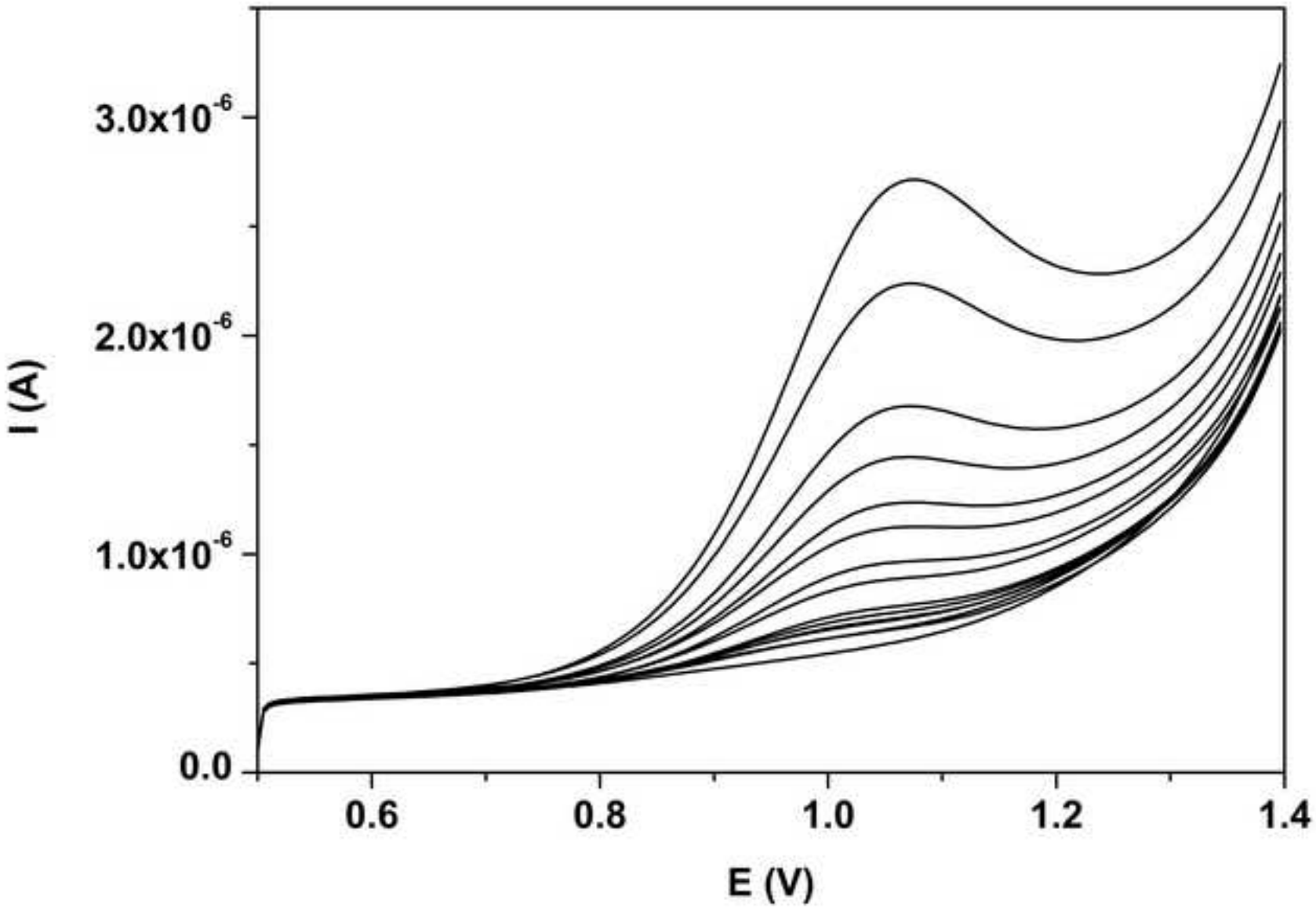

Figure 4

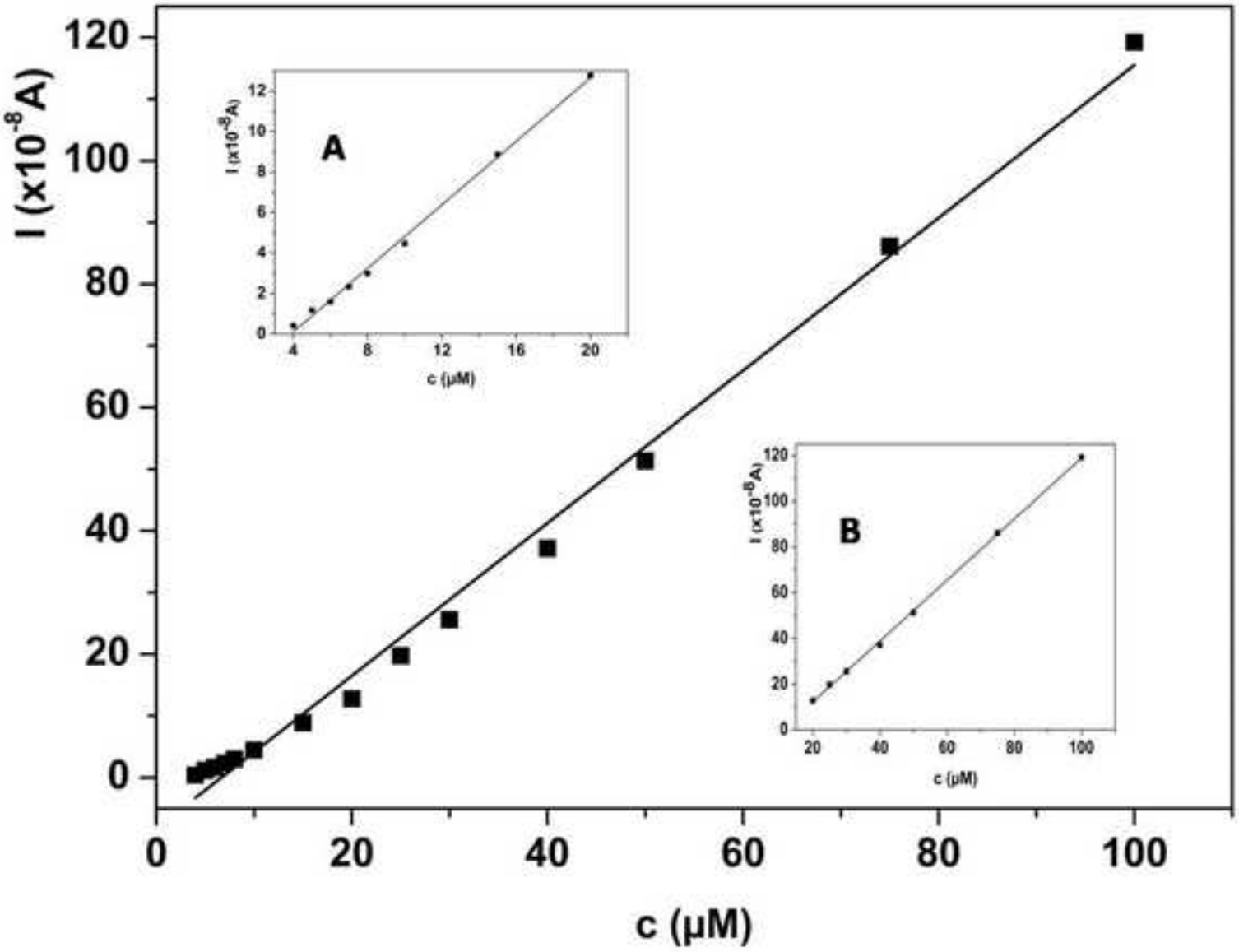

Figure 5

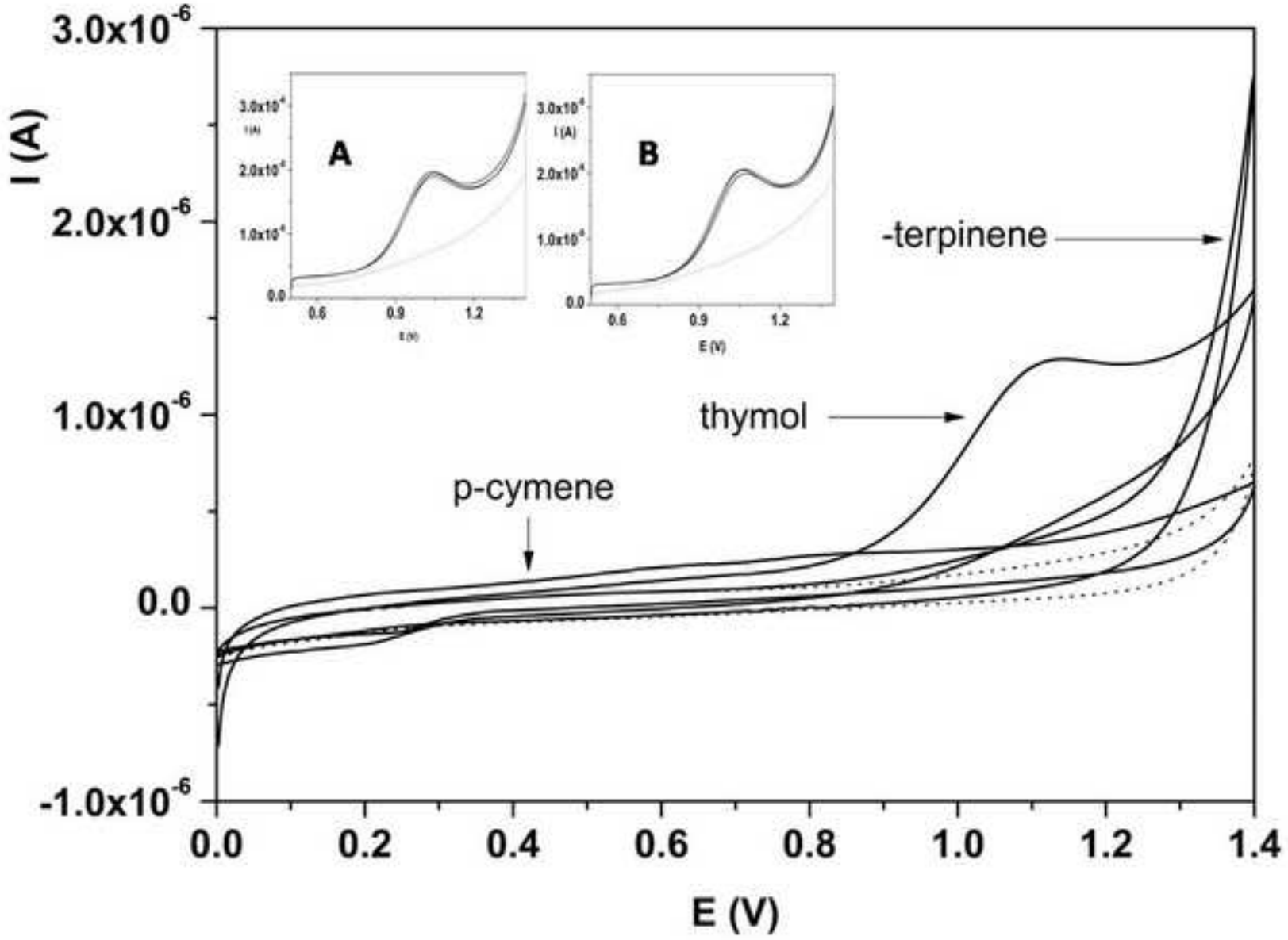

Figure 6