Electrochemical behavior of plant growth stimulator 1-naphthaleneacetic acid and its voltammetric determination using boron doped diamond electrode

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Abstract

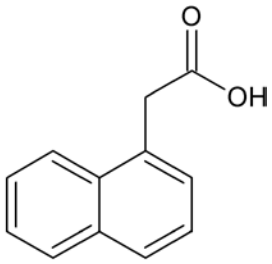
Electrochemical behavior of a plant growth stimulator 1-naphthaleneacetic acid (NAA) was firstly studied using a boron doped diamond electrode. It was found that NAA provided two irreversible anodic signals about the potential values of +1450 and +1630 mV, respectively, in an acidic medium. Products of the observed electrode reaction were identified using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (^{1}H NMR) techniques. Based on the obtained results, the oxidation mechanism of studied compound was firstly proposed. Subsequently, voltammetric method for NAA determination was developed with the following statistical parameters: *LOD* = 0.09 μg mL^{-1} and RSD_{11} = 2.1 % (10.8 μg mL^{-1}). Moreover, this work brings approach for analysis of stimulators mixtures for the first time as well. The proposed method allows the determination of NAA, IBA, and IAA in mixed formulations without application of any sophisticated separation techniques. This new method was finally applied for NAA determination in commercially available herbicide preparation.

Keywords

Plant growth stimulator, 1-Naphtaleneacetic acid, Voltammetry, Boron doped diamond electrode.

1 Introduction

1-Naphthaleneacetic acid (1-naphtylacetic acid, NAA, Scheme 1) is naphthalene derivative used as a synthetic plant growth stimulator (auxin type) utilized for various crops commonly in mixture with naturally occurring auxins like indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) or more often with synthetically prepared agents like naphthylacetamide (NAD) [1]. It is applied as a spray with concentration between 20-100 mg L^{-1} depending on the targeted plant. Therefore, its residua have been found in fruits, like apples or pears, and in the soil and water samples as well *e.g.* [1-3]. Considering the toxicity, NAA is qualified as unlikely hazardous by the WHO and highly toxic by U.S. EPA [4]. The European Union has set a maximum residua limit (MRL) for sum of NAD and NAA (expressed as amount of NAA) between 0.6 and 0.15 mg kg^{-1} for various fruits, vegetable, legumes, seeds, and others [5] and MRL for NAA of 0.05 mg kg^{-1} for citrus fruits and nuts [6].



Scheme 1 The structure formula of NAA.

Different analytical methods for precise determination of this phytohormone have been developed. The first approaches were based on a direct fluorescence or a phosphorescence determination of NAA, often simultaneously with NAD or other growth regulator, without any previous separation step [2, 7, 8] or with simple fixation of one analyte on a gel matrix [9] or in combination with a flow-injection system [3]. Nowadays, various chromatographic methods based on ultra (UPLC) and high performance liquid

chromatography (HPLC), respectively, with tandem mass [1, 10], UV [11], fluorescent [12] or diode array detection [13] were developed for precise determination of NAA.

Considering the low costs of instruments and analysis, rate of analysis or wide application range, modern electrochemical methods, especially voltammetry, represent an interesting alternative to the above-mentioned analytical techniques. To the best of our knowledge, electrochemical behavior of NAA was described only on modified carbon based working electrodes, namely on a glassy carbon electrode (GCE) modified by multi-walled carbon nanotubes film [14] or by acetylene black film [15] and on a carbon paste electrode (CPE) modified by ionic liquid [16]. NAA provided one well-developed oxidation signal on all three tested working electrodes at about +1 V *vs.* SCE. The maximum peak height was reached in a slightly acid medium (about pH value of 4) [14-16]. The used modified electrodes provided higher sensitivity than the bare ones and it was reached low limits of detection (*LOD*) in the range from 2.5×10^{-9} [14] to 1.7×10^{-7} mol L^{-1} [16]. The proposed methods were applied for analysis of soil samples or commercial formulations with very good results [14-16]. Parameters of these methods are summarized in Table 1. Moreover, two biosensors based on anti-NAA polyclonal antibody [17] or auxin binding protein immobilized on stabilized lipid films [18] were also developed for NAA determination. Sioda *et al.* focused on electrochemistry of naphthalene derivatives [19-21] including NAA [19] on GCE in the supporting electrolyte based on a mixture of acetone with water (1:1) and $(CH_3)_4N(BF_4)$. Particularly, NAA provided two oxidation signals (at about +1.6 and +2 V *vs.* Ag/AgCl (1 mol L^{-1}) reference electrode). The number of the transferred electrons for naphthalene derivatives was calculated about 2. This result led the authors to the suggestion that hydroxylic group is introduced into the aromatic nucleus while formation of particular naphthol, which was further oxidized to form a final product – naphthoquinone [19]. These results were not confirmed with any evidence (*e.g.*,

using instrumental analytical techniques suitable for identification of the oxidation products).

Table 1 Parameters of published methods for NAA determination

Electrode	Method	Electrolyte	*LOD* [mol L^{-1}]	*LDR* [mol L^{-1}]	Real sample	Ref.
MWNT/GCE	DPV	0.1 mol L^{-1} NaH_2PO_4/CA (pH 4)	2.5×10^{-9}	1.0×10^{-8}-2.0×10^{-6}	soil	4
AB/DHP/GCE	DPV	0.1 mol L^{-1} AcB (pH 3.6)	1.0×10^{-8}	4.0×10^{-8}-5.0×10^{-6}	soil	5
BPPF6/CPE	SWV	0.1 mol L^{-1} $NaClO_4$	1.7×10^{-7}	2.0×10^{-5}-4.0×10^{-4}	soil	6
NAA immunosensor	PM	PB (pH 7.4)	1.9×10^{-7}	2.7×10^{-7}-1.1×10^{-5}	tomato	7

AcB – acetate buffer solution, AB – acetylene black, BPPF6 – N-butyl-pyridinium hexafluorophosphate, CA – citric acid, CPE – carbon paste electrode, DHP –dihexadecyl hydrogen phosphate, DPV – differential pulse voltammetry, GCE – glassy carbon electrode, MWNT – multi-walled carbon nanotubes, PB – phosphate buffer solution, PM – potentiometry, SWV – square wave voltammetry.

In the present paper, we would like to elucidate an electrochemical behavior of NAA more in detail employing nuclear magnetic resonance (^{1}H NMR) and gas chromatography-mass spectrometry (GC-MS) method. The second aim of this work is to find conditions for simple and sensitive determination of NAA on bare boron doped diamond electrode (BDDE) [22-24], which represents very perspective electrode material and, unlike the above-mentioned modified electrodes and biosensors, it can be applied directly without any complicated preparation. The wide usable potential window allowing measurement even at very positive potential values together with minimal risk of electrode surface

passivation are the main advantages of BDDE [25-28]. In the past, this electrode has already been successfully used in the analysis of other plant stimulators [29, 30].

2 Experimental

2. 1 Chemicals

All of the used chemicals were of the analytical grade purity and originated from Penta, Prague, Czech Republic if not stated otherwise. All solutions were prepared in the distilled water (Milli-Q Plus system, Millipore, USA) and were stored in the dark at 4 °C in a refrigerator.

Standard solutions of 1.86 g L^{-1} NAA (0.01 mol L^{-1}), 1.752 g L^{-1} IAA (0.01 mol L^{-1}), and 2.032 g L^{-1} IBA (0.01 mol L^{-1}) were prepared by dissolution of the calculated amount of the NAA, IAA or IBA powder (>99 %, Carl Roth, Germany) in ethanol (96 %). These solutions were prepared fresh weekly. The analyzed solutions with lower stimulators concentrations were prepared daily by dilution with the supporting electrolyte. The mixture of 0.6 mol L^{-1} H_2SO_4 and 30 % ethanol (EtOH) served as a supporting electrolyte. Acetate buffer solution (AcB, 0.1 mol L^{-1}) was prepared in 100 mL volumetric flask by dilution of 0.82 g of CH_3COONa in distilled water. The value of pH 4.7 was achieved by acidification with CH_3COOH (99 %). Phosphate buffer solution (PB) was prepared by dissolution of 3.6 g of $Na_2HPO_4 \times 12\ H_2O$ (Lachema Brno, Czech Republic) in 100 mL of distilled water and the pH was adjusted to 7 with H_3PO_4 (Lachema Brno, Czech Republic). The proposed voltammetric method was applied for analysis of one growth stimulator formula – STIMULAX I (HÜ-BEN, Czech Republic) containing the mixture of active substances IAA, NAA, and IBA.

Leading electrolyte for isotachophoretic analysis consisted of the mixture with following composition: 0.01 mol L^{-1} HCl, 0.05 % HEC (hydroxyethylcellulose), histidine

of pH 5.5. The solution of 0.01 MES (morpholinoethanesulphonic acid) served as terminating electrolyte.

2. 2 Instrumentation

Electrochemical analyzer EP 100VA (HSC Servis, Slovak Republic) was employed for all voltammetric analysis. The measuring cell was in three electrodes set-up, where the BDDE (Windsor Scientific Ltd., UK, active surface area of 7.07 mm^2, inner diameter of 3 mm, resistivity of 0.075 Ω cm with a B/C ratio during deposition 1000 ppm) served as a working electrode, saturated argentochloride electrode (Ag/AgCl/KCl (sat.)) as a reference, and platinum wire as an auxiliary electrode (both from Monokrystaly, Czech Republic).

GC-MS was measured on Agilent Technologies – 6890N Gas Chromatograph, column HP-5MS, length 30 m, inner diameter 0.25 mm (GC) coupled with Agilent Network 5973 MS detector (Ionisation energy 70 eV, 33-550 Da). NMR spectra were recorded using Bruker Ascend™ 500 MHz in medium of deuterochloroform (Chloroform D >99.8% + 0.03% TMS, Fluorochem, UK).

CITP (capillary isotachophoresis) analyses were performed using electrophoretic analyzer EA 102 (Villa Labeco, Slovak Republic) with column coupling (pre-separation FEP capillary 90×0.8 mm and analytical FEP (fluoroethylene polymer) capillary 90×0.3 mm) equipped with a conductivity detector.

Values of pH were measured by pH-meter MV 870 Präcitronic. Homogenization of the solutions was performed employing an ultrasonic bath Bandelin Sonorex (Schalltec GmbH, Germany). Weighing was carried out by means of a balance Denver TB 124 A (Denver Instruments).

2. 3 Procedures

2. 3. 1 Voltammetric measurement

Cyclic voltammetry (CV) was applied for the basic study of the voltammetric behavior of NAA. The measurements were carried out from initial potential (E_{in}) −200 mV to switching potential (E_{switch}) +1800 mV with the scan rate (v) 125 mV s^{-1} or in the range from 10 to 125 mV s^{-1}. Differential pulse voltammetry (DPV) was utilized for quantitative determination of NAA. The working electrode was polarized in the range of potentials between −200 and +1800 mV with v 40 mV s^{-1}, pulse amplitude of +50 mV, and pulse wide 40 ms. The electrode pretreatment step consisting of the insertion of +2000 mV (E_{reg1}) for 10 s (t_{reg1}) followed by −200 mV (E_{reg2}) for 10 s (t_{reg2}) and again +2000 mV (E_{reg1}) for 10 s (t_{reg1}) was applied before each scan. All of the measurements were performed at laboratory temperature (23±2 °C) without removing oxygen.

Parameters of calibration curves and confidence intervals were calculated on the level of significance 0.05. Statistical parameters like limit of detection (*LOD*) and limit of quantification (*LOQ*) were calculated from the calibration dependences as 3× and 10× of standard deviation of an intercept divided by a slope.

2. 3. 2 Oxidation of 1-naphtylacetic acid

The model solution for oxidation was prepared by mixing distilled water, 96 % sulphuric acid, and NAA. The initial concentration of NAA in the electrolyzed solutions of 0.6 mol L^{-1} H_2SO_4 was 186.2 mg L^{-1} (1.0×10^{-3} mol L^{-1}). Electrochemical oxidation was carried out in the undivided chamber laboratory electrolytic cell with the volume of 0.25 L with duplicator at a temperature 25 ºC. The electrolyzed solutions were stirred by magnetic stirrer Heidolph MR Hei-Tec (250 rpm). Dimension of the used BDD anode was of 10×10×0.4 mm [31]. It was made from polycrystalline (1.2 μm) thick heavily BDD film with resistance 80±4.7 Ω (4-point measurement), which was grown by double bias enhanced hot filament chemical vapor deposition (HF CVD) technique previously

described in [32]. The active area of the BDD anode was 1.0 cm^2. The cathode of the electrolytic cell was made of a titanium rod coated a platinum film thickness of 120 μm (dimensions: 10×10×0.4 mm) with the active area of 4 cm^2. The distance of the electrodes was 35 mm.

The experiments were conducted under galvanostatic conditions using a DC Power Supply SDP–2210 (Manson, Kwai Chung, N.T., Hong Kong). Several experiments were carried out to evaluate the role of the applied reaction time on the emergence and amount of the reaction products (reaction time t = 0; 1; 2; 4; 8; 12; 24; 48; and 60 h, applied current density i = 50 mA cm^{-2}). After the reaction time elapsed, 250 mL of the reaction mixture was extracted with 2×75 mL of dichloromethane and dried by Na_2SO_4. The combined extracts were evaporated on a vacuum evaporator and analyzed using GC-MS and ^1H NMR techniques. If the oxidation of NAA took place for more than about 10-12 h, a solid sedimenting polymeric moiety was observed. In this case, the reaction mixture was filtered before extraction and the dark brown to black solid phase was removed.

2. 3. 2 Preparation of real sample for analysis

Commercially available growth stimulator preparation STIMULAX I containing 0.06 % of NAA, 0.06 % of IAA, and 0.05 % of IBA (declared by producer) was obtained in a powder form. The amount of 0.6833 g was dissolved in 100 mL of 96 % ethanol. 5 mL of the prepared sample solution was added into the electrochemical cell with 10 mL of the 0.6 mol L^{-1} H_2SO_4 and this mixture was analyzed. Standard addition method was used and 50 μL of 1.86 g L^{-1} NAA standard solution was added as standard addition 2 times at least. NAA determination was 5 times repeated and the values of average value with confidence interval as well as the relative standard deviation of repeated determination (*RSD*) were calculated.

3 Results and discussion

3. 1 Voltammetric behavior of 1-naphtylacetic acid

3. 1. 1 Dependence on supporting electrolyte composition

Since NAA is an electrochemically active compound, voltammetry was chosen for its determination. BDDE was used as a working electrode due to the wide usable potential window in a positive potential area. Because of the low solubility of the analyte, measurements were performed in 30 % EtOH in an electrochemical vessel (the organic solvent content was chosen based on our previous experience [29, 30]). Firstly, voltammetric behavior of this plant stimulator was investigated using CV at the analyte concentration of 10.8 μg mL^{-1} (5.8×10^{-5} mol L^{-1}) in acidic medium in accordance with our previously published papers [29, 30]. As it is evident from the cyclic voltammogram depicted in Fig. 1A, NAA provides two anodic peaks at the potential values about +1450 and +1630 mV on BDDE in acidic medium. No corresponding reduction signal was observed on the cathodic curve, indicating an irreversible course of the observed reaction. Furthermore, it was shown that the composition of the supporting electrolyte has a significant influence on the oxidation process. DPV with parameters specified in the caption of Fig. 1 was applied for testing of different electrolyte influence on NAA (5.9 μg mL^{-1}) signals. High and well developed peaks of NAA were recorded only in strongly acidic medium. Fig. 1B documents that the peaks height (I_p) decreased and their evaluability deteriorated with growing pH value. Simultaneously, the usable potential window in anodic area was narrowed with increasing pH which also made the signal worse. No current response corresponding to NAA oxidation was observed in the neutral and alkaline medium. Therefore, 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH was used as a supporting electrolyte for all of the following voltammetric experiments.

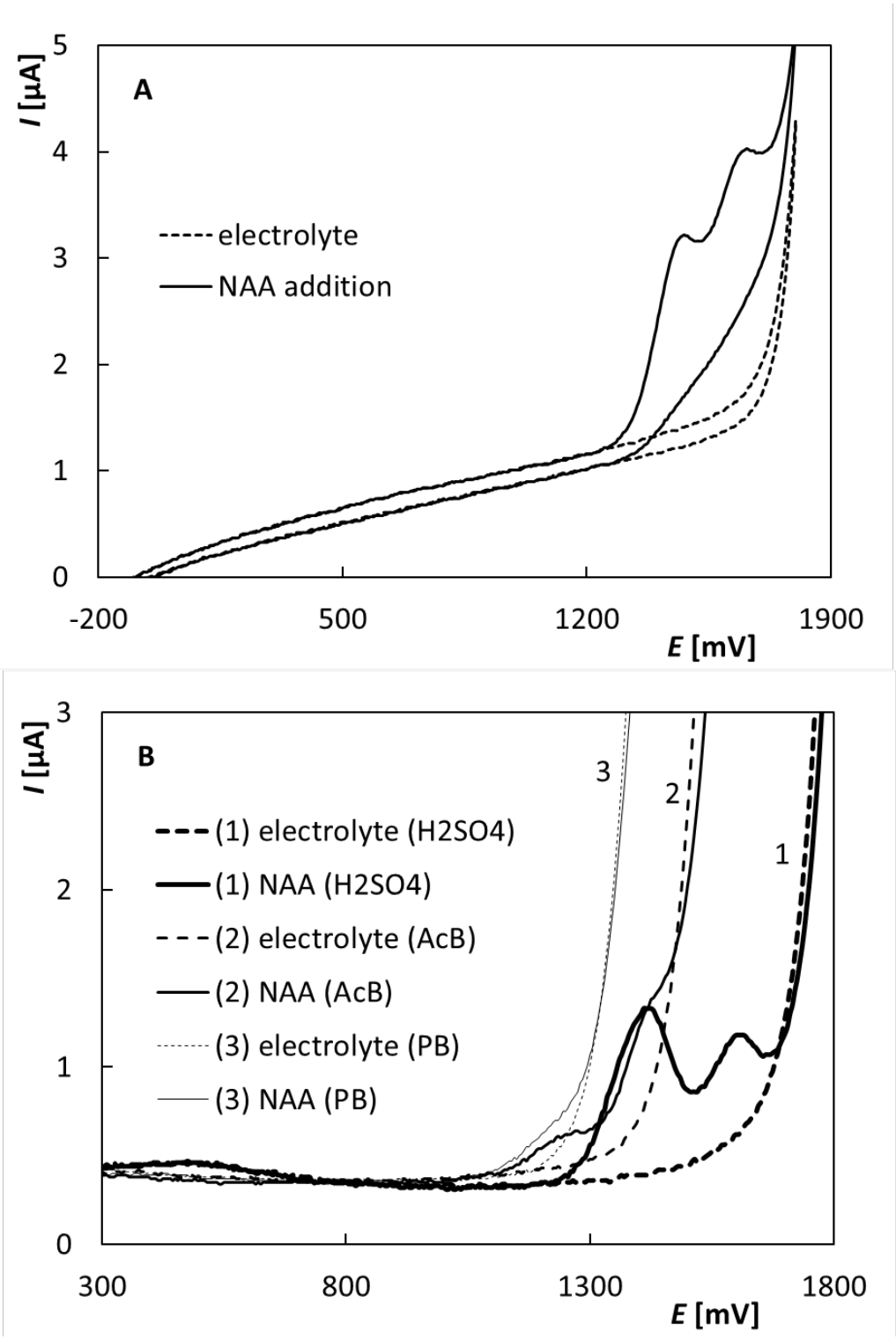


Figure 1 A – Cyclic voltammogram of NAA in 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH obtained on BDDE; E_{in} = E_{fin} = −200 mV, E_{switch} = +1800 mV, v = 125 mV s^{-1}, c_{NAA} = 10.8 µg mL^{-1}; B – DP voltammograms of NAA in dependence on pH of supporting electrolyte obtained on BDDE; E_{in} = −200 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse

amplitude = +50 mV, pulse wide = 40 ms, c_{NAA} = 5.9 μg mL^{-1}, supporting electrolyte – 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH, AcB (pH 4.7) + 30 % EtOH, PB (pH 7.0) + 30 % EtOH.

3. 1. 2 Dependence on scan rate

The investigation of voltammetric behavior of the analyzed compound in dependence on scan rate may be very useful for determining the control process of the electrode reaction and at all for elucidating the mechanism of the ongoing oxidation process. In case of NAA, cyclic voltammograms were measured in the range from 10 to 125 mV s^{-1}. It was found that both oxidation signals shifted to more positive potential values with increasing *v*. The first NAA signal significantly increased with growing scan rate, but the appropriate dependence of I_p on *v* was not linear. On the other hand, the dependence of I_p on square root of *v* shows a linear course (Fig. 2A, eq. (1)) which corresponds to diffusion as the controlling process of the reaction. This conclusion was also confirmed by the results of the logarithmic dependence (Fig. 2B) described by the equation (2) with the relevant correlation coefficient. The value of the slope (0.455±0.012) is approaching to the theoretical value (0.5) for the diffusion controlled process [33]. The second NAA anodic peak was too close to the end of the usable potential window, which made it impossible to evaluate properly the values of I_p for objective scan rate influence assessment. However, it seems that *v* does not have a significant effect on peak height, which would correspond to kinetics as a controlling process.

$$I_p\ [\mu A] = (0.2043 \pm 0.0045)\ (v\ [\mathrm{mV\ s^{-1}}])^{1/2} + (0.096 \pm 0.034),\ r = 0.999 \quad (1)$$

$$\log\left(I_p\ [\mu A]\right) = (0.455 \pm 0.012) \log\ (v\ [\mathrm{mV\ s^{-1}}]) + (-0.584 \pm 0.020),\ r = 0.999 \quad (2)$$

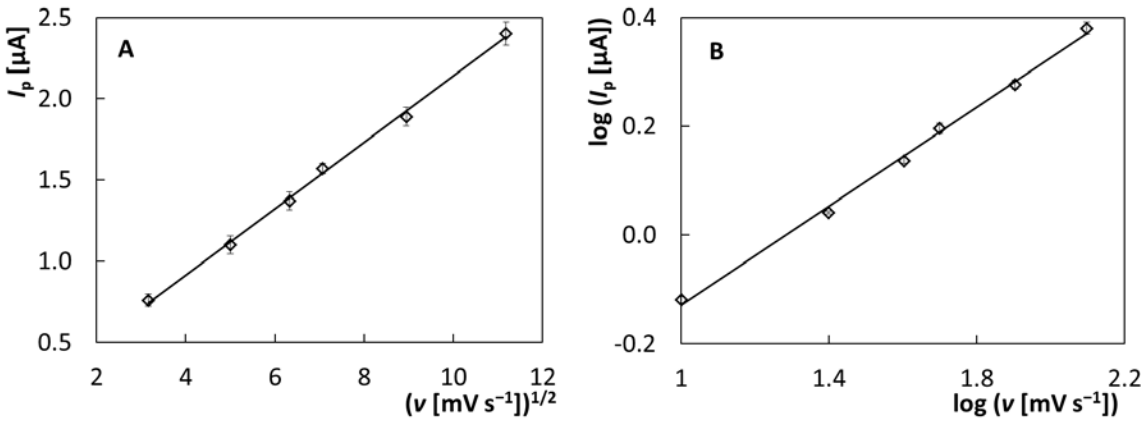


Figure 2 Dependences of I_p on v (A) and log (I_p) on log (v) for NAA obtained on BDDE; method – CV, supporting electrolyte – 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH, E_{in} = E_{fin} = −500 mV, E_{switch} = +1800 mV, v = 10, 25, 40, 50, 80, and 125 mV s^{-1}, c_{NAA} = 10.8 µg mL^{-1}.

3. 2 Mechanism of the electrochemical oxidation of 1-naphtylacetic acid

Electrochemical oxidation mechanism of NAA has not been described in the literature yet. Therefore, our research was focused also in that direction, when the electrolyzed solutions of NAA were analyzed by different instrumental analytical techniques and the oxidation mechanism was proposed. The applied procedure of electrolysis was described in detail in the experimental part. The reaction mixture was electrolyzed for various time periods. After electrolysis, each sample was extracted 2 times by 100 mL of dichloromethane, dried over Na_2SO_4 and evaporated. The obtained products were analyzed by GC-MS and ^{1}H NMR in chloroform. Analysis by GC-MS showed presence of several compounds whose abundance changed in dependence on the electrolysis time. In addition to starting NAA **1** (*m*/*z* 186) these compounds were naphtalene-1-ylmethanol **2** (*m*/*z* 158), naphthalene-1-carbaldehyde **3** (*m*/*z* 156) accompanied by traces of 1,4-naphtochinone (*m*/*z* 158) and 1-methylnaphthalene (m/z 142). Prolongation of the electrolysis led to formation of increasing amount of insoluble polymeric products. Fig. 3 illustrates changes in ^{1}H NMR

spectra in dependence on time of electrolysis and compares them with the spectra of standards. Kinetic curves obtained from these data are illustrated in Fig. 4.

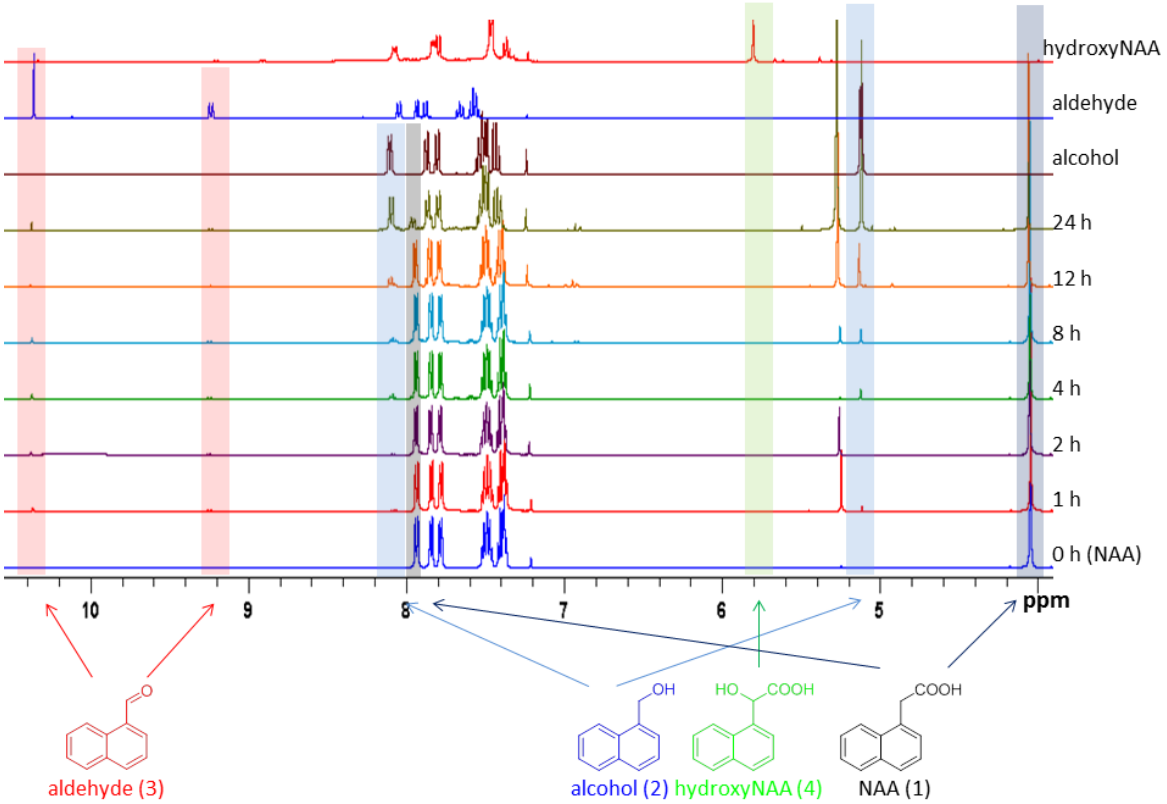


Figure 3 Time changes of ^{1}H NMR spectra of the reaction mixtures after electrolysis. Upper part shows spectra of independently synthetized standards.

Fig. 3 and 4 show increasing abundance of alcohol **2** in the reaction mixture while abundance of aldehyde **3** remains practically constant. These suggests follow oxidation sequence NAA **1** → alcohol **2** → aldehyde **3** → insoluble polymeric products, when rate of alcohol **2** oxidation to aldehyde **3** is lower than rate of oxidation of aldehyde **3** to polymeric products. Proposed oxidation mechanism is depicted in Scheme 2.

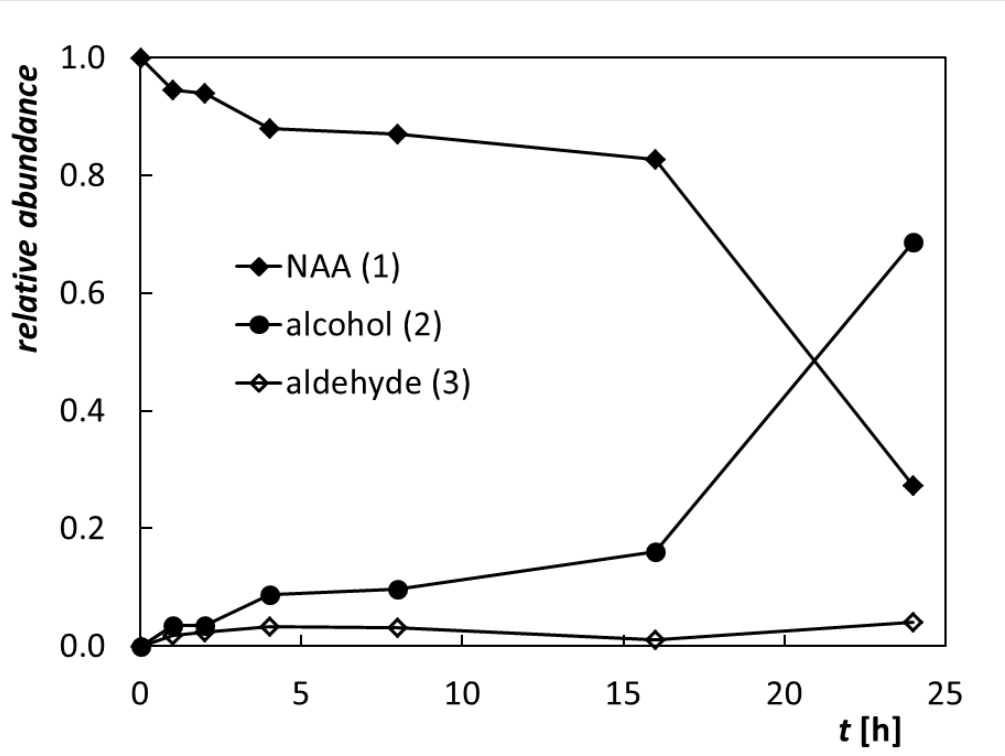
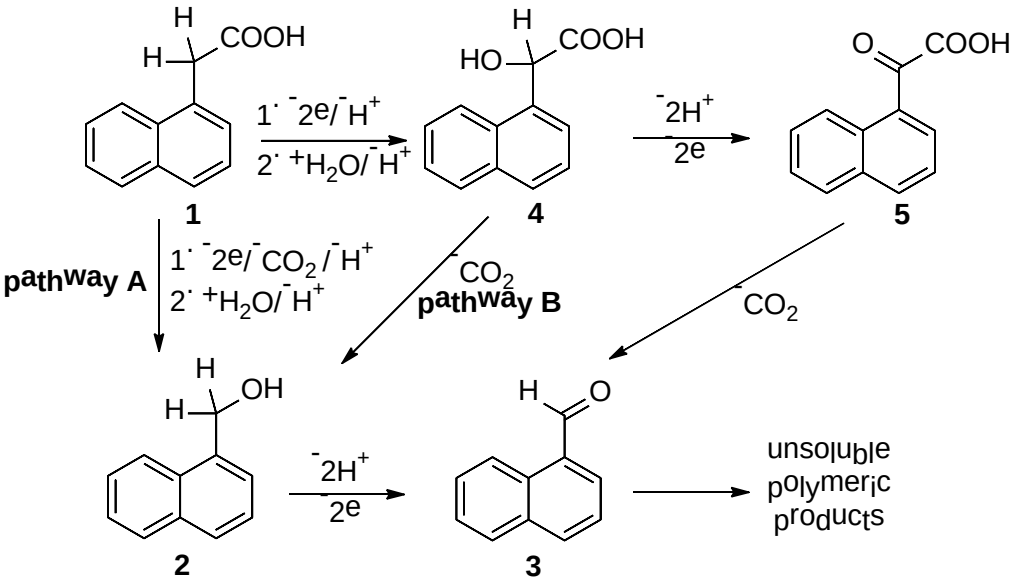


Figure 4 Time dependence of relative abundances of individual components obtained from ^{1}H NMR experiments.



Scheme 2 Possible oxidation pathways of NAA.

Voltammetric curves for oxidation of NAA show two signals at potentials +1450 mV and +1630 mV. Comparison of those voltammograms with voltammograms of individual standards **2** and **3** shows that first peak at +1450 mV corresponds to the oxidation of alcohol **2** to aldehyde **3**, while the potential for oxidation of aldehyde **3** is identical to the potential of the second peak at +1630 mV. Thus, two processes occur at first potential – oxidation of NAA **1** to alcohol **2** and oxidation of alcohol **2** to aldehyde **3**. Second peak corresponds to the oxidation of aldehyde **3** to polymeric products.

There are two possible pathways leading to alcohol **2** (Scheme 2). The first one involves decarboxylation of NAA **1** on a surface of the electrode giving alkyl radical, which subsequently forms alcohol **2** [34] (pathway A). The second one involves oxidation of NAA **1** to 2-hydroxy-2-(naphtalene-1-yl)acetic acid **4** followed by its decarbonylation (pathway B). The compound **4** was synthetized by independent way [35] and electrolyzed for verification of its possible role as a reaction intermediate. Its oxidation potential has identical value to the value of the first peak in oxidation of NAA. Furthermore, reaction mixture shows analogical composition to the mixtures obtained after electrolysis of NAA. This indicates compound **4** to be possible reaction intermediate. Since the signals of **4** were not observed in MS or ^{1}H NMR spectra of reaction mixtures obtained after electrolysis of NAA, this intermediate would have to be very reactive one. To conclude, reaction pathway A (Scheme 2) seems to be more probable, however pathway B cannot be excluded. Our findings show that oxidation proceeds on the side chain of NAA and not on the aromatic skeleton as suggested by Sioda *et al* [19].

3. 3 Voltammetric determination of 1-naphtylacetic acid

3. 3. 1 Analytical performance

DPV was chosen for the development of a novel voltammetric method for NAA determination with respect to very good sensitivity. At the beginning, basic parameters of this method, such as scan rate, pulse height, and pulse width, were optimized. All these measurements were realized in the supporting electrolyte of 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH and with the concentration of NAA in voltammetric cell 10.8 μg mL^{-1}. Scan rate was tested in the range from 10 to 50 mV s^{-1}, pulse height from +10 to +80 mV, and pulse width from 10 to 60 ms. Based on these experiments, the following parameters were used for all further measurements: v 40 mV s^{-1}, pulse height +50 mV, and pulse width 40 ms. The optimal values were chosen not only because of the highest I_p, but also with regard to the shape and evaluability of NAA current peak.

After the optimizing of DPV parameters, the procedure of BDDE pretreatment was inserted before each particular measurement assuring a surface activation as well as regeneration. According to the literature [25, 36], the electrode can be used with O-terminated or H-terminated surface which can be obtained by insertion of positive or negative pretreatment potential for a particular time. Based on our previous experiments [29, 30], the following pretreatment step consisting from insertion of +2000 mV for 10 s followed by −200 mV for 10 s and again +2000 mV for 10 s was applied before each scan. This ensured that the measurement was carried out on the O-terminated surface of the electrode and at the same time, the possible passivation was suppressed. The suitability of this procedure was verified by 11 repeated measurement of 10.8 μg mL^{-1} NAA when the calculated value of the relative standard deviation (RSD_{11}) of I_p of the first peak was 2.1 %.

3. 3. 2 Analysis of model solutions of 1-naphtylacetic acid

Using BDDE as a working electrode and the above mentioned optimized DPV conditions, various concentration dependences for NAA were measured employing model solutions. In

Fig. 5 an example of the obtained DP voltammograms recorded in concentration range from 1.2 to 10.8 μg mL^{-1} is shown. It is evident, from the inserted dependences of I_p on c_{NAA} and also from the appropriated equations (3) for the first peak and (4) for the second one, that both analyte signals increased linearly with growing concentration in the whole studied range. This results meant that both observed oxidation signals could serve for NAA determination but the first one enabled better sensitivity as evidenced by the 2 times higher value of the slope (0.09903±0.00042 μA mL μg^{-1}). Also the calculated values of *LOD* and *LOQ*, summarized for both peaks in Table 2, indicated that the first peak was more suitable for NAA determination, because it allowed to achieve lower values (*LOD* = 0.09 μg mL^{-1}).

$$I_p\ [\mu A] = (0.09903 \pm 0.00042)\ (c\ [\mu g\ mL^{-1}]),\ r = 1.000 \quad (3)$$

$$I_p\ [\mu A] = (0.05791 \pm 0.00089)\ (c\ [\mu g\ mL^{-1}]) + (-0.0186 \pm 0.0060),\ r = 0.999 \quad (4)$$

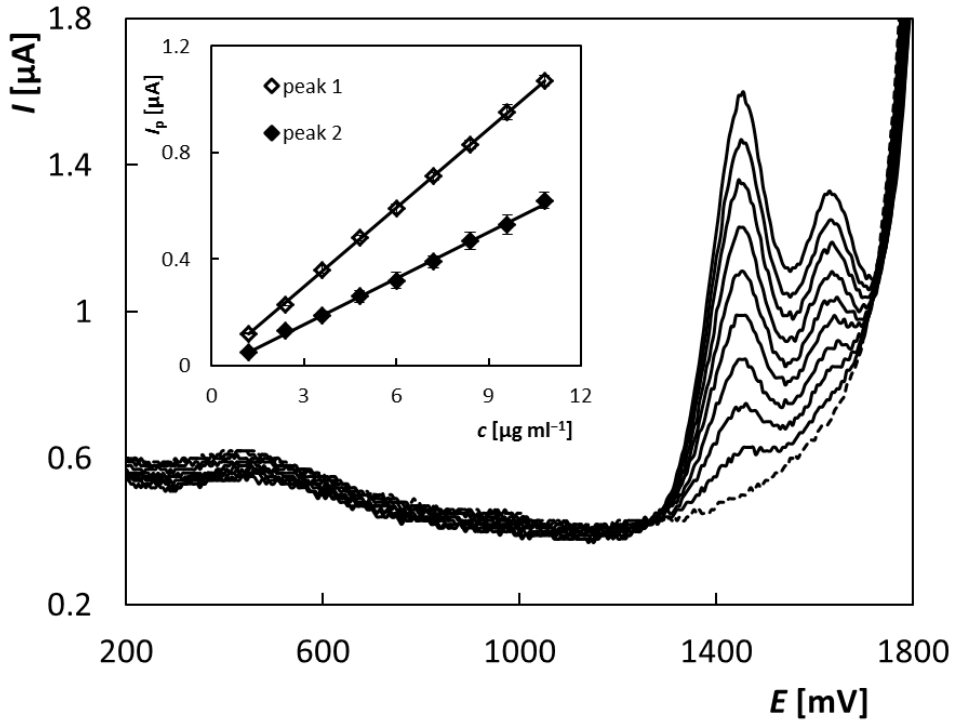


Figure 5 DP voltammograms of NAA in dependence on its concentration recorded on BDDE; supporting electrolyte – 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH, E_{in} = −200 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse amplitude = 50 mV, pulse wide = 40 ms, c_{NAA} = 1.2-

10.8 μg mL^{-1}; ***Inset*** – Dependences of I_p on c_{NAA} for both current signals, I_p evaluated after baseline correction.

Table 2 Statistical parameters for the developed voltammetric method of NAA determination using BDDE

	LOD [μg mL^{-1}]	*LOQ* [μg mL^{-1}]	RSD_{11}* [%]
peak 1	0.09	0.3	2.1
peak 2	0.18	0.6	5.2

*calculated for NAA concentration 10.8 μg mL^{-1}

The applicability of this method for NAA determination was verified by repeated determination of the tested plant stimulator in model solutions with concentration 1.2 μg mL^{-1}. The analysis was performed using the standard addition method and it was 5 times repeated. Calculated statistical parameters as average values with confidence intervals, recoveries and RSD_5 are summarized in Table 3. It is evident, that new method provides reliable results.

Table 3 Statistical parameters for the developed voltammetric method of NAA determination using BDDE

	Added [μg mL^{-1}]	Found [μg mL^{-1}]	Recovery [%]	RSD_5 [%]
peak 1	1.2	1.196±0.014	97.5-101.7	1.7
peak 2	1.2	1.198±0.048	95.0-105.0	6.1

3. 3. 3 Determination of 1-naphtylacetic acid in mixture with other stimulators

Because of the relatively low biological activity [37, 38], NAA often occurs in commercial available formulations in a mixture with other phytohormones, most often IAA and IBA. When the mixture of NAA and IAA was analyzed under the above described optimized conditions, DP voltammograms depicted in Fig. 6 were recorded. Dashed line represents the analysis of IAA alone in the absence of NAA. The maximum of IAA peak, corresponding with the oxidation of its dimer creating in acidic medium [39], lies about the potential of +1380 mV, which is very close to the potential of the first signal of NAA (+1450 mV). After the addition of NAA into the voltammetric cell, the observed peak increased, widened and shifted to more positive potential value (+1410 mV). Simultaneously, the second anodic peak of NAA was recorded. Therefore, a second oxidation signal of NAA (+1630 mV) should be used to its quantitative evaluation in the presence of IAA. Furthermore, it was experimentally found that the presence of IBA did not interfere with the NAA determination under these experimental conditions. As it was previously published [30], IBA provides oxidation peak at less positive potential of +900 mV in acidic media. IAA can be determined in a less acidic environment (pH 3) where it is not subjected to dimerization and it provides an oxidation peak at a less positive potential of +1000 mV. Therefore, there is no interference with the NAA signal. These results have been previously published [40].

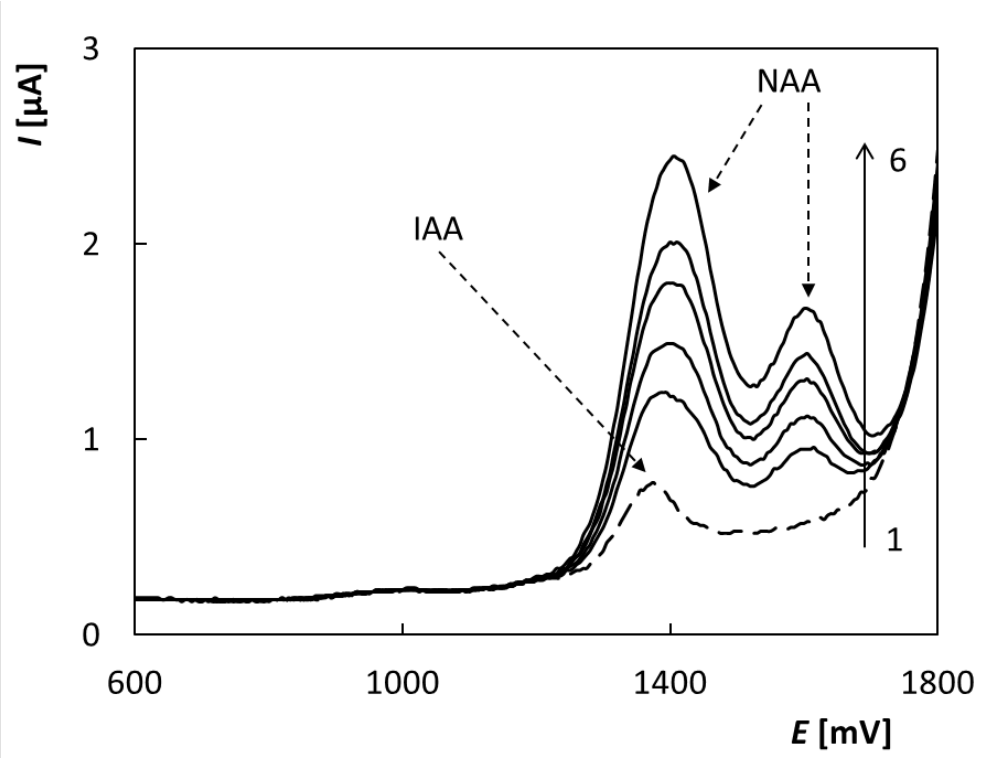


Figure 6 DP voltammograms of the mixture of IAA and NAA in dependence on increasing NAA concentration obtained on BDDE; supporting electrolyte – 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH, E_{in} = −200 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse amplitude = 50 mV, pulse wide = 40 ms, curve 1 – c_{IAA} = 4.67 µg mL^{-1}, curve 2-6 – c_{IAA} = 4.67, µg mL^{-1}, c_{NAA} = 4.96-14.9 µg mL^{-1}.

3. 3. 4 Determination of 1-naphtylacetic acid in real sample

Developed voltammetric method was finally applied for determination of NAA in the commercially available plant stimulators preparation STIMULAX I. It is mixed herbicidal preparation with the declared content of NAA 0.06 % (60 mg/100 g), of IAA 0.06 % (60 mg/100 g), and of IBA 0.05 % (50 mg/100 g). It was found by repeated voltammetric analysis (n = 5) that this analyzed product contains only NAA with the concentration closed to those declared by producer (63.1±2.8 mg/100g, this value corresponds to the NAA concentration of 1.43 µg mL^{-1} in polarographic vessel during the sample analysis).

An example of the obtained voltammograms is shown in Fig. 7. The other two active substances were not found in the sample. These results were verified by application of independent analytical method, namely isotachophoresis. Measurement conditions are summarized in detail in experimental part and the obtained results are placed in Table 4. Determined NAA amount is similar to that obtained with DPV. IAA and IBA were not found even using CITP method. It can be probably caused by instability of these substances [41].

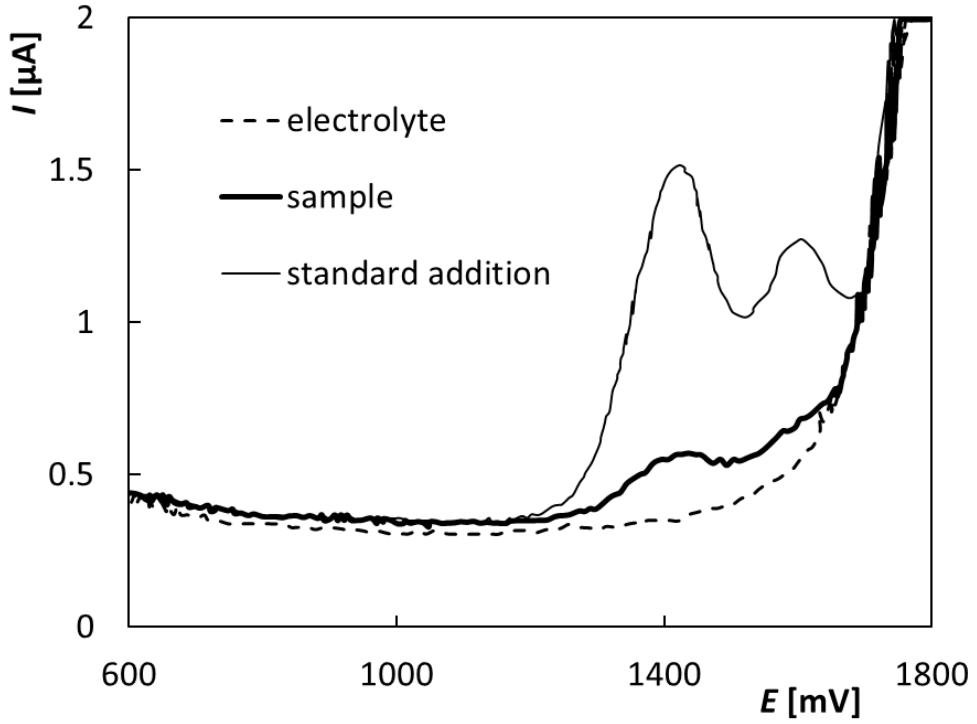


Figure 7 DP voltammograms of the analysis of real sample STIMULAXI using standard addition method; supporting electrolyte – 0.6 mol L^{-1} H_2SO_4 + 30 % EtOH, E_{in} = −200 mV, E_{fin} = +1800 mV, v = 40 mV s^{-1}, pulse amplitude = 50 mV, pulse wide = 40 ms, standard addition of NAA – 6.2 µg mL^{-1}.

Table 4 Results of the analysis of herbicide preparation using DPV and using CITP

	declared	found DPV	found CITP

	[mg/100 g]	[mg/100 g]	[mg/100 g]
NAA	60.0	63.1±2.8	65.0±3.6
IAA	60.0	not found	not found
IBA	50.0	not found	not found

4 Conclusion

Electrochemical behavior of the plant growth stimulator 1-naphthaleneacetic acid (NAA) was firstly studied using BDDE in the present paper. It was found that NAA provides two irreversible anodic signals about the potential values of +1450 and +1630 mV. Based on pH study, the solution of 0.6 mol L^{-1} H_2SO_4 was used as a supporting electrolyte. Due to the worse solubility of NAA in water medium, all measurements were carried out in 30 % EtOH.

For elucidation of the oxidation mechanism, products of NAA electrolysis were analyzed by GC-MS and ^{1}H NMR techniques. These experiments suggests following oxidation sequence: NAA → alcohol → aldehyde → insoluble polymeric products. First obtained anodic peak corresponds to oxidation of NAA to alcohol naphtalene-1-ylmethanol and oxidation of this alcohol to aldehyde naphthalene-1-carbaldehyde. Second anodic peak corresponds to the oxidation of aldehyde to polymeric products.

Subsequently, voltammetric method for NAA determination was developed using DPV in connection with BDDE. Basic parameters of this method were optimized and the procedure of BDDE surface pretreatment leading to electrode activation and suppression of passivation was inserted (RSD_{11} = 2.1 (10.8 μg mL^{-1})). Under the optimized parameters very low value of *LOD* (0.09 μg mL^{-1} (4.8×10^{-7} mol L^{-1})) for NAA determination was

obtained. Finally, this new method was successfully applied for NAA determination in model solutions as well as in commercially available herbicide preparation.

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