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Voltammetric behavior of indole-3-butyric acid and a novel approach for its determination in mixture with indole-3-acetic acid using boron-doped diamond electrode as a sensor

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In the present paper, the voltammetric behavior of indole-3-butyric acid (IBA) is described employing a boron-doped diamond electrode (BDDE). In acidic media, IBA exhibited one well-developed oxidation signal. Two voltammetric methods, linear-sweep (LSV) and differential pulse voltammetry (DPV), were optimized and low limits of quantification obtained (0.79 µg mL^{-1} for LSV and 0.52 µg mL^{-1} for DPV). The latter technique with optimized parameters was successfully applied to the determination of IBA in growth stimulation formulas. Moreover, simultaneous voltammetric determination of IBA and other auxin phytohormone, indole-3-acetic acid (IAA), employing BDDE was also examined. Despite their similar structures, a simple method based on DPV and derivatization of a selected part of the obtained curves was developed. It has been proven that simultaneous determination of IBA and IAA is possible at various concentration ratios with satisfactory results. Applicability of the proposed method was verified by successful analysis of the growth stimulation preparation containing IBA or a mixture of the auxins studied.

Keywords: Phytohormone; Auxin; Indole-3-acetic acid; Indole-3-butyric acid; Voltammetry; Boron-doped diamond electrode.

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Introduction

Indole-3-acetic acid (IAA, Fig. 1A), the best known phytohormone belonging to a group called auxins, is being found as an essential plant hormone and its biology belongs to the oldest and the widest fields of the experimental plant research. This substance regulates diverse processes in plants, such as a tropic response to light and gravity, root and shoot growth, rate of cell expansion and division, or fruit maturation [1–5]. Besides IAA, the group of naturally occurring auxins also includes indole-3-butyric acid (IBA, Fig. 1B), 4-chloroindole-3-acetic acid and phenylacetic acid [1, 5–7]. On the other hand, synthetic plant growth regulators with auxin-like action, such as 1-naphthaleneacetic acid and 2,4-D derivatives (2,4-dichlorophenoxyacetic acid *etc.*) are often employed as well [1]. IBA is an exogenous plant hormone, which promotes rooting even more effectively than IAA, mainly due to its higher stability in solutions [7]. IBA alone or in combination with IAA is commercially available in various growth regulation formula.

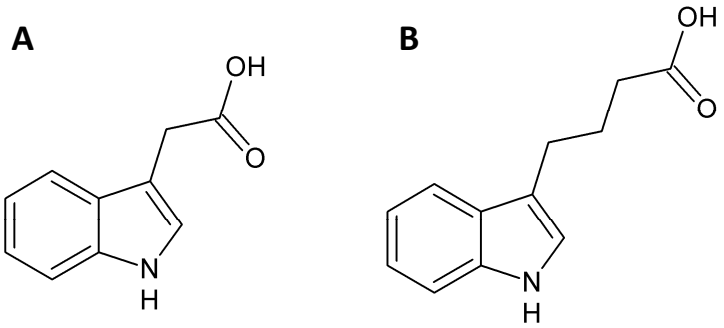

Fig. 1 The structure formula of IAA (**A**) and IBA (**B**)

Due to the significant biological activity of the auxins, various analytical methods have already been introduced for their determination, mainly those based on liquid chromatography [8–12], gas chromatography [8,13], electrokinetic capillary chromatography [8,14], or immunoassay detection [8,15]. Modern electroanalytical techniques represent fast, reliable, sensitive, and cheap alternative to the above-mentioned instrumentation. It was found out that IAA is electrochemically oxidizable compound and its voltammetric behavior was widely examined by employing the working electrodes from various carbon materials, including boron-doped diamond electrode BDDE [16–25]. Two anodic signals about +930 mV and +1375 mV *vs.* Ag | AgCl | NaCl (3 mol L^{-1}) were recorded in an acidic media (pH 2) on BDDE. They correspond to the $2e^-/1H^+$ oxidation and the formation of a particular cation, which undergoes rapid decarboxylation followed by sequences of chemical and electrochemical steps including dimerization [16,19]. The electroanalytical methods were also employed for determination of IAA in plant samples like seed [19] or extracts [20–25].

So far, however, the electrochemical behavior of IBA has been examined only briefly. Enache and Oliveira-Brett were interested in the voltammetric behavior of indole compounds with C3 substituent including IBA on the glassy carbon electrode (GCE) [18]. Two oxidation signals were recorded, and the first peak was ascribed to the oxidation of the pyrrole ring followed by the oxidation of the benzene moiety in a neutral medium [18]. The only paper had dealt with electrochemical determination of IBA and the respective procedure was based on the recording of particular tensametric signals at the adsorption potential of IBA when using the hanging mercury drop electrode [26].

Due to the lack of information about IBA electrochemical behaviour and its voltammetric determination too, our paper mainly targets at the description of the voltammetric behaviour of IBA with the working electrode from boron-doped diamond. The second aim of our work was to develop a simple voltammetric method for simultaneous determination of two phytohormones IBA and IAA, especially in growth stimulator formulas. To the best of our knowledge, any paper concerning the voltammetric behavior of IBA on BDDE and/or simultaneous determination of these two auxins has not been yet published.

Materials and methods

Chemicals

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

Standard solutions of 1.752 g L^{-1} IAA (0.01 mol L^{-1}) and 2.032 g L^{-1} IBA (0.01 mol L^{-1}), respectively, were prepared by dissolution of the calculated amount of the IAA or IBA powder (purity higher than 99%, Carl Roth, Karlsruhe, Germany) in ethanol (96%, v/v). The standard solutions were prepared fresh weekly. The standard solutions were diluted with distilled water (in case of further dilution) or with the supporting electrolyte (in case of the direct analysis) to obtain the desired concentration. As a supporting electrolyte, 0.1 mol L^{-1} acetate buffer (pH 5.0), 0.1 mol L^{-1} NaOH, 0.1 mol L^{-1} H_2SO_4 or 0.1 mol L^{-1} H_2SO_4 combined with ethanol (30, 60 and 90%), acetonitrile (20 and 40%) and isopropanol (45, 65 and 95%) were tested. A mixture of 0.1 mol L^{-1} H_2SO_4 and 30% ethanol was selected for the subsequent analysis as the supporting electrolyte besides analysis of the mixture of IBA and IAA as well as real samples, when 90% ethanol in combination with 0.1 mol L^{-1} H_2SO_4 was employed due to a better solubility of the analytes.

The proposed voltammetric method was applied to the analysis of two growth stimulation formulas - RHIZOPON A (Rhizopon B.V., Hazerswoude-Rijndijk,

Netherlands) containing only IBA and GELSTIM B (EXPLANTEX Vondruš, České Budějovice, Czech Republic) consisting of a mixture of IAA, IBA, 1-naphthaleneacetic acid and chinosol.

Instrumentation

Electrochemical analyzer EP 100VA (HSC Servis, Bratislava, Slovak Republic) was employed for all voltammetric analyses. The measuring cell was in three-electrode set-up, where the BDDE with the active surface of 7.07 mm^2 and B/C of 1000 ppm in the gas phase (declared by the producer Windsor Scientific, UK) served as the working electrode, Ag │ AgCl │ KCl (*sat.*) as a reference and platinum wire as an auxiliary electrode (both from Monokrystaly, Turnov, Czech Republic).

The pH values were measured by pH-meter MV 870 Präcitronic (VEB Präcitronic, Dresden, Germany). Homogenization of the solutions was performed employing an ultrasonic bath Bandelin Sonorex (Schalltec, Allmendingen, Germany). Weighing was carried out by means of a balance Denver TB 124 A (Denver Instruments, Bohemia, NY, USA). All the measurements were performed at the laboratory temperature (23 ± 2 °C).

Measurement procedures

Cyclic voltammetry (CV) was selected for the basic study of the voltammetric behaviour. The measurements were carried out from the initial potential (E_{in}) −300 mV to the switch potential (E_{switch}) +1700 mV and backward to E_{in} with a scan rate (v) of 250 mV s^{-1}, if not stated otherwise. Linear-sweep voltammetry (LSV) in the potential range from +600 to +1800 mV was utilized for the next studies. The scan rate study was realized applying the following values of this parameter: 25, 40, 80, 125, and 250 mV s^{-1}. Using LSV with v = 40 mV s^{-1}, the effect of the supporting electrolyte was examined and various concentration dependencies of IBA and IAA as well recorded. Differential pulse voltammetry (DPV) in the potential range from +600 to +1800 mV was employed for further measurements. The effect of the following parameters were tested in the mentioned ranges: v of 10, 25, 40, and 50 mV s^{-1}, applied pulse amplitude (A_{pulse}): 30 mV, duration of pulse (t_{pulse}): 60 ms, A_{pulse} of 10, 20, 30, 40, 50 and 60 mV (v = 40 mV s^{-1}, t_{pulse} = 60 ms) and t_{pulse} of 40, 60, 80 and 100 ms (v = 40 mV s^{-1}, A_{pulse} = 40 ms). The value of 40 mV for A_{pulse}, 40 ms for t_{pulse} and 40 mV s^{-1} for v, respectively, were selected for further measurements. The pre-treatment step consisting of stirring and application of the regeneration potential (E_{reg1} = +2000 mV) for the regeneration time (t_{reg1} = 10 s) followed by the second regeneration potential (E_{reg2} = −200 mV) set for the same regeneration time (t_{reg2} = 10 s), was inserted before every scan.

Preparation of samples

RHIZOPON A was in a tablet form. One half of tablet was dissolved in 25 mL of 96% ethanol. Three solutions were prepared in the same way and then analyzed by the proposed method. 100 μL of the prepared sample solution was added to the polarographic cell with 15 mL of the supporting electrolyte and analyzed. 20 μL of the diluted IBA standard solution (c = 0.976 g L^{-1}) was added at least in five replicates. GELSTIM B was in a gel form and analyzed only after simple dilution without any preparation step. Thus, 5 mL and 10 mL of the gel was directly added to 10 mL and 5 mL of the ethanolic solution of 0.1 mol L^{-1} H_2SO_4 in case of IBA and IAA analysis, respectively. 10 μL (2.032 g L^{-1} IBA) was added as the standard addition for the determination of IBA and 20 μL (1.752 g L^{-1} IAA) was used for quantification of IAA. The determination was made in three replicates for each analyte. Amount of the respective plant hormone was calculated using a Nelinear program (Assoc. prof. V. Jehlička, Faculty of Transport Engineering, University of Pardubice) employing again the standard addition method [27].

Results and discussion

Cyclic voltammetric behavior of IBA and electrode treatment optimization

As mentioned above, only a single paper was published dealing with the voltammetric behavior of various indole compounds including IBA on GCE [18]. On the other hand, electrochemistry of IAA is well known, and it has been described on various solid and paste working electrodes [16–25] including a detailed study with BDDE [19]. Due to the lack of papers concerning voltammetric behavior of IBA at BDDE, we focused on this task first. Considering the similar structure of IBA to IAA, it was supposed, that IBA could be anodically oxidized at BDDE. One wide anodic signal with maximum of about +1000 mV was recorded in the supporting electrolyte used after addition of 13.3 μg mL^{-1}, which is apparent from the Fig. 2. Any corresponding cathodic peak could not be registered in the recorded potential range and thus, the process could be found as electrochemically irreversible. The signal could be described, based on the data from literature [16,18,19], as the $2e^-/1H^+$ oxidation of the pyrrole ring and the formation of a particular cation. Such a cationic species formed should be rapidly decarboxylated and the sequence of chemical and additional electro-chemical steps followed. The second anodic step, which was observed with GCE [18], could not be recorded under the conditions used.

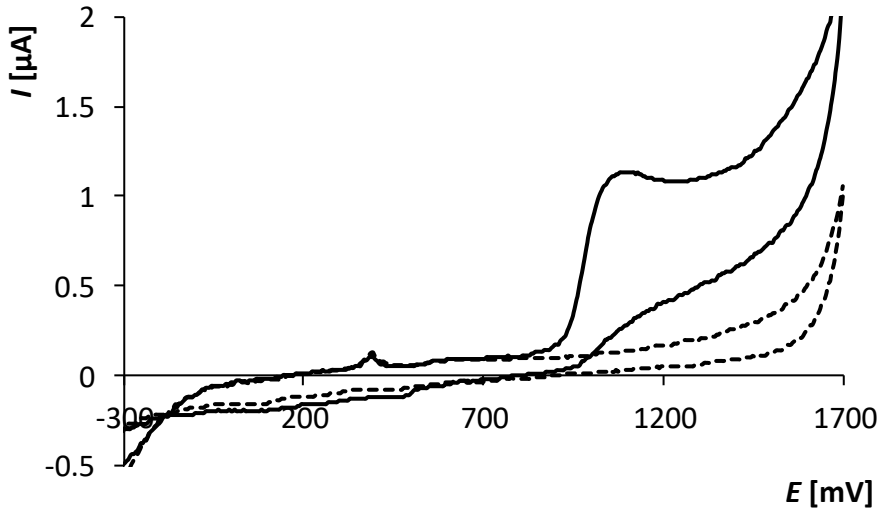

Fig. 2 Cyclic voltammogram in absence (dashed line) and in presence (solid line) of IBA recorded at BDDE in acidic medium

Conditions of analysis: c(IBA) = 13.3 µg mL^{-1}; supporting electrolyte: 0.1 mol L^{-1} H_2SO_4 + 30% ethanol; CV parameters: E_{in} = E_{fin} = −300 mV; E_{switch} = +1700 mV; v = 250 mV s^{-1}

Repeated measurements had demonstrated that the IBA signal was not very stable and reproducible. A significant decrease of 13.3 µg mL^{-1} IBA peak was observed after repeated measurements without any treatment step, probably due to the passivation of the working electrode. Thus, some cleaning procedure was further investigated. The regeneration of the electrode surface was carried out directly in the analyzed solution under stirring. Insertion of the positive regeneration potential (+2000 mV) for the regeneration time of 10 s between every scan improved the peak shape and the repeatability as well. Moreover, this procedure ensured the increase of the peak recorded. A prolongation of the t_{reg} led to only a minor increase of the signal and did not influence the repeatability at all. Simple insertion of the negative potential of −2000 mV for 50 s resulted in a dramatic decrease of the peak. Thus, less negative potentials as −1000 and −200 mV were tested. Application of −1000 mV for 50 s caused a slow increase and stabilization of the response. The same was observed also for the second negative potential examined. The best results (with fast stabilization of the response, good repeatability, and well-developed high signal) were obtained by a combination of the positive and negative potential. The highest and the most stable response was obtained after inclusion of the treatment consisting of E_{reg1} = +2000 mV (t_{reg1} = 10 s) and followed by E_{reg2} = −200 mV (t_{reg2} = 10 s) in combination with stirring. Above that, the stirring was favorable, too, when considering the homogenization of the solution analyzed.

Scan rate study

The effect of scan rate on the voltammetric response of the analyte could reveal some important information about the studied process. Thus, scan rates of 25, 40, 80, 125 and 250 mV s^{-1} were applied and the signal of 13.3 μg mL^{-1} IBA was recorded. The significant increase of the peak could be observed with growing v but the obtained dependence was non-linear. As expected, the linear course was obtained for the dependence of the peak height (I_p) and the square root of the scan rate ($v^{1/2}$), which is apparent from the equation (1). This result coincided with the diffusion-controlled electrode reaction, which is typical for BDDE. For further examination, log v-log I_p analysis was also constructed, and this linear dependence could be described by equation (2). The confidence interval of the slope (0.3720 ± 0.0023) does not contain the value 0.5 on the level of significance ≤0.05 and thus it could be assumed, that the electrode reaction is more complicated, and its kinetics plays also important role. This result corresponded to the relatively complex reaction pathways of the substituted indoles [16,18,19].

$$I_p\,[\mu\mathrm{A}] = (0.1280 \pm 0.0069) v^{1/2}\left[\mathrm{mV\ s^{-1}}\right]^{1/2} + (0.3510 \pm 0.0708); R = 0.9956 \quad (1)$$

$$\log I_p\,[\mu\mathrm{A}] = (0.3720 \pm 0.0232) \log v\left[\mathrm{mV\ s^{-1}}\right] - (0.5260 \pm 0.0443); R = 0.9942 \quad (2)$$

Voltammetric behavior of IBA in various media and choice of the supporting electrolyte

In general, the supporting electrolyte influences mechanism of the electrode reactions, stability of analytes, and behavior of the working electrode as well. Thus, the choice of the supporting media is further necessary step of the whole optimization process. The obtained response of the analyte should be reproducible and stable in the long-term view, which could also be strongly affected by the properly selected supporting electrolyte.

Influence of pH of the supporting medium on the IBA response was examined at first. The effect of the electrolyte composition was studied next. A solution of 13.3 μg mL^{-1} IBA was employed for this study. 0.1 mol L^{-1} H_2SO_4 was used for analysis in an acidic media; 0.1 mol L^{-1} acetate buffer for oxidation detected in slightly acidic or neutral electrolytes and 0.1 mol L^{-1} NaOH for presentation of the behavior of IBA in alkaline electrolytes. Various measurements were carried out and the most favorable voltammetric behavior of IBA was observed in the acidic medium. The peak was very stable, which was confirmed by a low relative standard deviation of 15 repeated measurements (RSD(15) ≤ 3%). Moreover, the signal was easily evaluable, which is illustrated

by Fig. 3A, where the dependence of 2.7–18.9 μg mL^{-1} IBA is depicted. It is obvious that the IBA signal increased with its addition but – as we found after evaluation of series of various measurements – the increase was non-linear. Equation 3 belonged to this dependence. Considering the used supporting electrolyte, the recorded signal was stable, well developed and easily evaluable, this medium was found suitable for further experiments. On the other hand, the oxidation of IBA could also be recorded in neutral and alkaline solutions as well, and one anodic signal was measured. Worse solubility of the analyte, especially at a higher concentration level, was observed in the neutral solution. Moreover, the recorded peak was less stable than in the acidic medium. The signal of IBA obtained in alkaline solution was low and poorly developed. Above that, any evaluable signal belonging to the determined analyte could not be recorded after few replicated scans in NaOH and the electrode had to be activated by application of a value of +2000 mV in acidic solution. Thus, acidic environment was selected for all further measurements of IBA.

$$I_p\left[\mu\mathrm{A}\right] = -0.0004\, c^2\left[\mu\mathrm{g\ mL^{-1}}\right]^2 + 0.0896\, c\left[\mu\mathrm{g\ mL^{-1}}\right] - 0.038 \qquad (3)$$

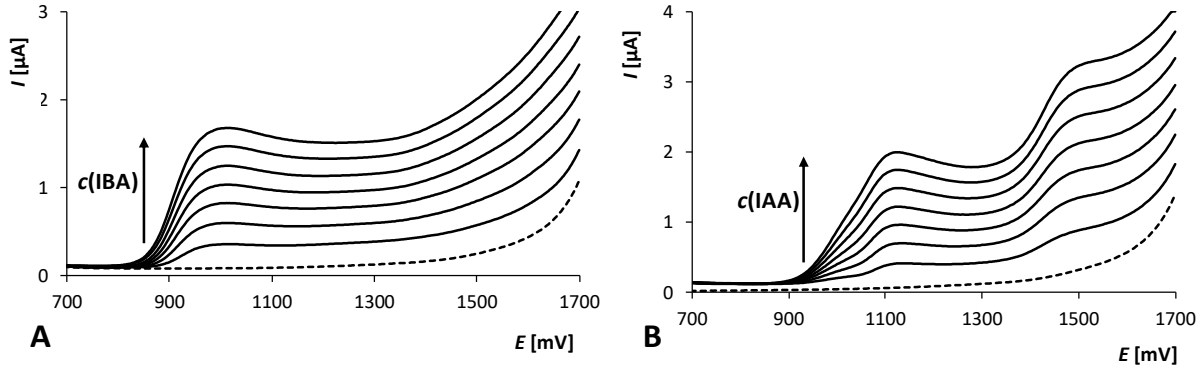

Fig. 3 Linear-sweep voltammograms in absence (dashed line) and in presence (solid line) of IBA (A) and IAA (B), respectively, at various concentration levels recorded at BDDE in acidic medium

Conditions of analysis: c(IBA) = 2.7–18.9 μg mL^{-1} (A); c(IAA) = 2.33–16.35 μg mL^{-1} (B); supporting electrolyte: 0.1 mol L^{-1} H_2SO_4 + 30% ethanol; LSV parameters: E_{in} = +600 mV, E_{fin} = +1700 mV, v = 40 mV s^{-1}, E_{reg1} = +2000 mV, t_{reg1} = 10 s, E_{reg2} = −200 mV, t_{reg2} = 10 s

The presence of an organic solvent in the chosen supporting electrolyte (0.1 mol L^{-1} H_2SO_4) was examined as the next step. Our target was to find the optimal mixture of sulfuric acid and organic solvent, which could result in a higher sensitivity or/and reaching of a linearity of the concentration dependencies. The dependence of IBA in the range from 12.9 to 90.3 μg mL^{-1} was selected for this set of experiments. The respective results (calibration equation, limit of detection (LOD) and limit of quantification (LOQ)) are

summarized in Table 1. The least-squares method was used for evaluation of LOD and LOQ. Software Nelin was applied to the evaluation of the concentration dependences [28,29].

Table 1 Results of the non-linear regression $I_p = f(c)$ in dependence of the composition of the supporting electrolyte*

Organic solvent	Equation $I_p = f(c)$ I_p [μA]; c [μg mL^{-1}]	LOQ, [μg mL^{-1}]	LOD, [μg mL^{-1}]
---	$I_p = -0.0004c^2 + 0.093c - 0.01$	0.52	0.15
30% ethanol	$\boldsymbol{I_p = -0.0001c^2 + 0.051c + 0.06}$	**0.79**	**0.24**
60% ethanol	$I_p = -0.00006c^2 + 0.043c - 0.02$	0.78	0.23
90% ethanol	$I_p = -0.0001c^2 + 0.034c - 0.05$	0.71	0.21
45% isopropanol	$I_p = -0.00008c^2 + 0.046c + 0.05$	1.1	0.53
65% isopropanol	$I_p = -0.00007c^2 + 0.045c + 0.07$	1.1	0.32
95% isopropanol	$I_p = -0.0001c^2 + 0.041c + 0.08$	0.77	0.23
20% acetonitrile	$I_p = -0.0002c^2 + 0.082c - 0.08$	1.2	0.37
40 % acetonitrile	$I_p = -0.00008c^2 + 0.068c + 0.09$	1.5	0.44

* supporting electrolyte consisted of 0.1 mol L^{-1} H_2SO_4 and selected solvent

It was found out that presence of ethanol (at all concentration levels under study) in the supporting electrolyte caused only a slight decrease of the recorded wave and insignificant increase of LOQ. Very similar results were received in the case of isopropanol. The presence of acetonitrile caused only gently increment of the recorded waves, but the obtained limits (LOD, LOQ) are almost identical to them calculated for ethanol or isopropanol. The desired linearity of the dependence studied have not been reached in any solution tested, which is confirmed by equations depicted in Table 1. Due to the results described and when taking into account the toxicity of acetonitrile, an electrolyte based on mixture of sulfuric acid with ethanol should be selected for all further analysis. Presence of ethanol ensured better solubility of the compounds studied mainly at higher concentration levels. The mixture of sulfuric acid with 30 % ethanol was then selected for all further analyses.

It was also proved that analysis of the second phytohormone IAA is possible in the proposed electrolyte. IAA provided two oxidation signals around potential of +1100 mV and of +1500 mV, which is consistent with the previous results published in [19]. Both peaks non-linearly increased (Figure 3B) with the added amount of IAA and the concentration dependence of the first signal could be described by equation in Table 2.

Choice of the evaluation model

Using software Nelin [28], linear and non-linear models of evaluation have been examined and the outcomes (equations, LOD and LOQ) are summarized in Table 2. It is evident that the non-linear evaluation mode resulted in about 3.3× lower LOD and LOQ, respectively, which confirmed the properly chosen way of evaluation. Thus, the non-linear model was chosen for subsequent analysis. The same results were obtained for both signals of IAA. The non-linear mode led again to about 3.0× (signal 1) and 4.2× (signal 2), respectively, lower LOD and LOQ, which was also convenient for further experiments.

Table 2 Comparison of the linear and non-linear evaluation models

Analyte	Concentration range [μg mL^{-1}]	Equation $I_p = f(c)$ I_p [μA]; c [μg mL^{-1}]	LOQ [μg mL^{-1}]	LOD, [μg mL^{-1}]
IBA	2.70–18.9	$I_p = 0.079c - 0.097$	1.8	0.54
		$\boldsymbol{I_p = -0.0005c^2 + 0.091c - 0.05}$	**0.55**	**0.16**
IAA (1)*	2.33–16.4	$I_p = 0.11c - 0.076$	1.1	0.32
		$\boldsymbol{I_p = -0.0006c^2 + 0.12c - 0.04}$	**0.35**	**0.11**
IAA (2)*		$I_p = 0.072c - 0.084$	2.4	0.72
		$\boldsymbol{I_p = -0.0009c^2 + 0.089c - 0.03}$	**0.56**	**0.17**

* IAA (1) – evaluation of the first signal of IAA; IAA (2) – evaluation of the second signal of IAA

Two concentration levels of IBA (1.29 and 12.93 μg mL^{-1}) and one selected concentration of IAA (4.53 μg mL^{-1}) were determined in five replicates using the standard addition method, when applying min. five successive standard additions. The resulting contents were calculated using software Nelinear described in our previous paper [27]. The obtained results, namely 1.3 ± 0.054 μg mL^{-1} and 13.3 ± 0.423 μg mL^{-1} for IBA and 4.7 ± 0.13 μg mL^{-1} for IAA, respectively, were found as correct and precise and the relative standard deviations of five repeated determinations (RSD(5) = 6.3 %, 3.2 %, and 4.3 %, respectively), confirmed repeatability of the proposed method and properly chosen evaluation mode as well.

Analytical performance

Optimization of DPV parameters

In general, pulse voltammetric techniques were introduced and soon proven as an effective and sensitive tool for analysis of various bioactive compounds. Therefore, we decided to compare the results obtained from LSV and DPV measurements; the latter representing such a pulse variant.

Only simple analysis with LSV is sufficient for determination of IBA or IAA alone with excellent results, which was discussed in the previous chapter. On the other hand, if a mixture of the studied compounds was to be analyzed, LSV was insufficiently sensitive. The corresponding signals were totally overlapped and the correct evaluation thus impossible. Due to this, we decided to apply the DPV technique, and the first step was the optimization of its operating parameters. Considering the lack of the information about the voltammetric behavior of IBA together with the previously published paper [19] focusing on voltammetric behavior of IAA on BDDE, the DPV parameters were examined only for IBA. A solution containing 1.35 μg mL^{-1} IBA was employed in this study and a single well-developed signal with a peak potential of +950 mV recorded. The scan rate was examined at first and values of 10, 25, 40 and 50 mV s^{-1} were consecutively tested. The highest signal was recorded from 30 to 40 mV s^{-1}, whereas further increase of the scan rate already led to a slight decrease of the response. Then, a scan rate of 40 mV s^{-1} was chosen for all subsequent experiments. The effect of another investigated parameter, the pulse amplitude, was recorded in the range from 10 to 60 mV with a step of 10 mV. As found, the signal significantly increased up to A_{pulse} = 40 mV and the following growth was much slower. Thus, the value of 40 mV was used further on. The last operational parameter under optimization was the duration of pulse and it was changed from 40 to 100 ms. An exponential decrease of the signal with the increasing value of t_{pulse} was recorded; hence, t_{pulse} = 40 ms was chosen.

DPV analysis of solutions with IBA and IAA separately

DPV with optimized parameters was applied to the voltammetric determination of IBA. Various concentration ranges were recorded and the concentration of 1.35 μg mL^{-1} IBA was found as the lowest reliably evaluable value. Thus, two concentration levels of 1.35 and 10.8 μg mL^{-1}, respectively, IBA was used for further study (Fig. 4A). Two evaluation modes, as in case of LSV, were applied and the equations summarized in Table 3. LOD and LOQ calculated for particular dependencies can be found in Table 3 as well. About three-times lower values of LOD and LOQ, respectively, were obtained for the non-linear evaluation mode and thus, this model was applied in all further measurements. Reproducibility of

the determination was defined from the five-times repeated analysis of the solution containing 1.35 μg mL^{-1} IBA. The excellent results obtained (1.290 ± 0.046 μg mL^{-1}, RSD(5) = 5.4%) confirmed properly chosen parameters of DPV, as well as the evaluation mode.

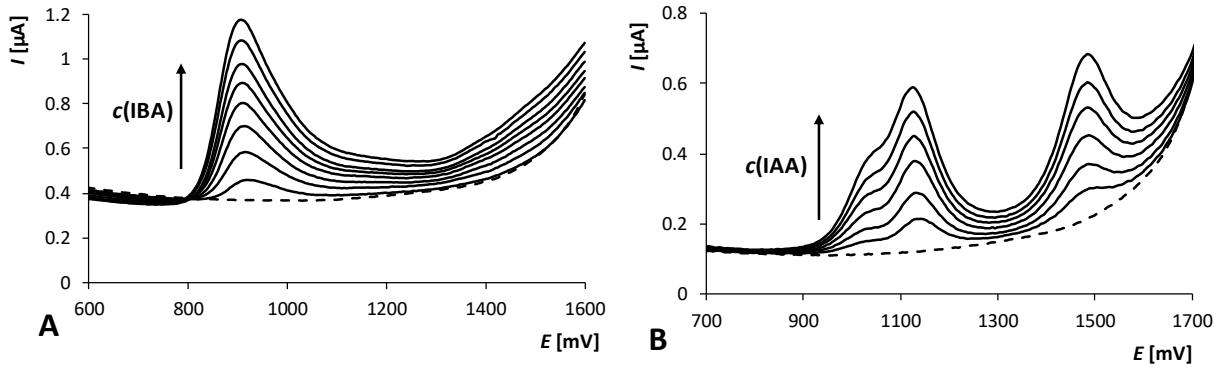

Fig. 4 Differential pulse voltammograms in absence (dashed line) and in presence (solid line) of IBA (A) and IAA (B), respectively, at various concentration levels recorded at BDDE

Experimental conditions: c(IBA) = 1.35–10.8 μg mL^{-1} (A); c(IAA) = 1.17–7.02 μg mL^{-1}; supporting electrolyte: 0.1 mol L^{-1} H_2SO_4 + 30% ethanol; DPV parameters: E_{in} = +600 mV, E_{fin} = +1700 mV, v = 40 mV s^{-1}, A_{pulse} = 40 mV, t_{pulse} = 40 ms, E_{reg1} = +2000 mV, t_{reg1} = 10 s, E_{reg2} = −200 mV, t_{reg2} = 10 s

Table 3 Comparison of the linear and non-linear evaluation model

Analyte	Concentration range [μg mL^{-1}]	Equation I_p = f(c) I_p [μA]; c [μg mL^{-1}]	LOQ [μg mL^{-1}]	LOD [μg mL^{-1}]
IBA	1.35–10.8	$I_p = 0.707c - 0.0012$	0.44	1.48
		$\boldsymbol{I_p = -0.0013c^2 + 0.086c - 0.036}$	**0.16**	**0.52**
IAA (1)*	1.17–7.02	$I_p = 0.56c - 0.022$	0.34	1.15
		$\boldsymbol{I_p = -0.0014c^2 + 0.067c - 0.0041}$	**0.16**	**0.52**
IAA (2)*		$I_p = 0.057c - 0.055$	0.15	0.51
		$\boldsymbol{I_p = -0.00054c^2 + 0.061c - 0.015}$	**0.097**	**0.32**

* IAA (1) – evaluation of the first signal of IAA; IAA (2) – evaluation of the second signal of IAA

Applicability of the proposed DPV method to the determination of IAA was examined only briefly. It was proven that this auxin hormone provided two peaks with potentials of about +1100 mV and +1500 mV (Fig. 4B). Unlike the LSV signals, as expected, the DPV peaks were better separated, well-shaped and developed. The less positively situated signal was wide and seemed to be divided

into two peaks. On the other hand, the second signal was easily evaluable and suitable for determination of IAA. Two evaluation modes have been employed as in case of IBA and the obtained equations, as well as LOD and LOQ, are summarized in Table 3. Considering both LOD and LOQ values, the non-linear evaluation way was chosen. Model solution containing 1.17 μg mL^{-1} was analyzed five-times and the obtained results (1.17 ± 0.029 μg mL^{-1}, RSD(5) = 3.6%) confirmed again appropriately chosen conditions of IAA determinations.

DPV analysis of the mixture of IBA and IAA

The main reason, why these two specific phytohormones were chosen for analysis, was their common presence in the growth stimulator preparations. Moreover, besides IAA, plant synthetize also three other endogenous auxins including IBA [5,6]. Based on various experiments, we found out that simple LSV could not be applied for their determination in the mixture due to the overlapping of the first IAA wave by the IBA signal. Considering their similar structures, this behavior was nevertheless expected. But the distinction of the signals was possible by utilizing DPV as a working technique (sufficient for determination of IAA) followed by a derivative step (essential for IBA analysis). Moreover, as discussed above, there were also lower LOD and LOQ and thus, one could expect a higher sensitivity of the respective method.

Fig. 5 illustrated DP voltammograms of a mixture containing 2.3 μg mL^{-1} IAA + 5.4 μg mL^{-1} IBA, respectively. Image A represents the curves after multiple additions of IAA to the mixture and indication of the signal suitable for the IAA determination. Compared to this, image B shows the voltammograms of a mixture with multiple additions of IBA and the labelling of the increasing peak is detailing a part which had to be subjected to the derivative step. This procedure and subsequent evaluation were enabled by the software of the electro-chemical analyzer employed. The curves seen in Fig. 6 had the peak shape and its height corresponding to the IBA content in the analyzed solution. By taking into account the non-linear course of the dependence between the peak height and concentration of the analyte, application of at least five standard additions was necessary for the correct evaluation. The proposed method was employed for analysis of various model solutions containing the mixture of the phytohormones and the results of five repeated determinations are summarized in Table 4. Due to the presence of these substances in the growth stimulation preparation at the same concentration level, this ratio was analyzed preferably.

The respective experiments have confirmed that the tested voltammetric method is convenient for correct simultaneous determination of IAA and IBA.

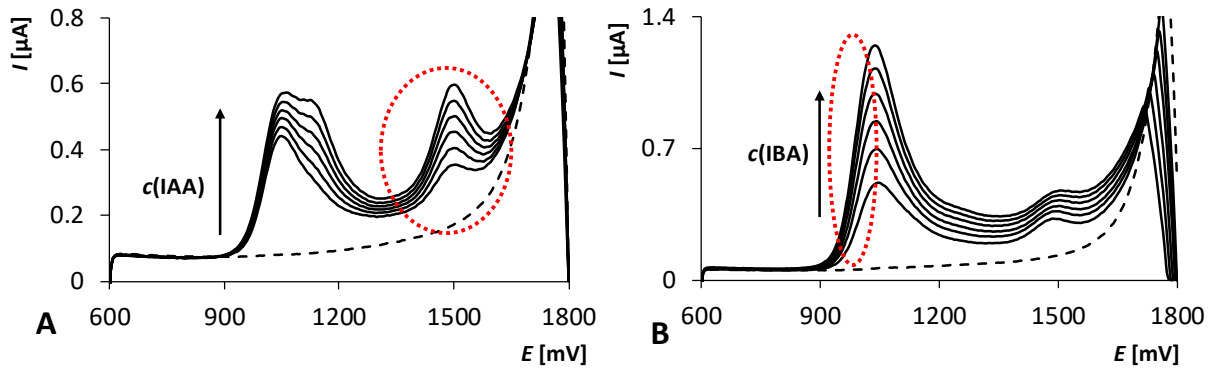

Fig. 5 Differential pulse voltammograms in the absence (dashed lines) and presence (solid line) of IAA and IBA in the mixture and after multiple additions of IAA (A) and IBA (B), respectively

The dotted ellipses labelled the signal suitable for IAA determination (A) and the increasing peak part which had to be subjected to the derivative step (B), respectively

Experimental conditions: c(IAA) = 2.33–8.18 µg mL^{-1} (A), c(IAA) = 2.33 µg mL^{-1} (B), c(IBA) = 5.40 µg mL^{-1} (A), c(IBA) = 5.40–18.9 µg mL^{-1} (B); supporting electrolyte: 0.1 mol L^{-1} H_2SO_4 + 30% ethanol; DPV parameters: same as in Fig. 4

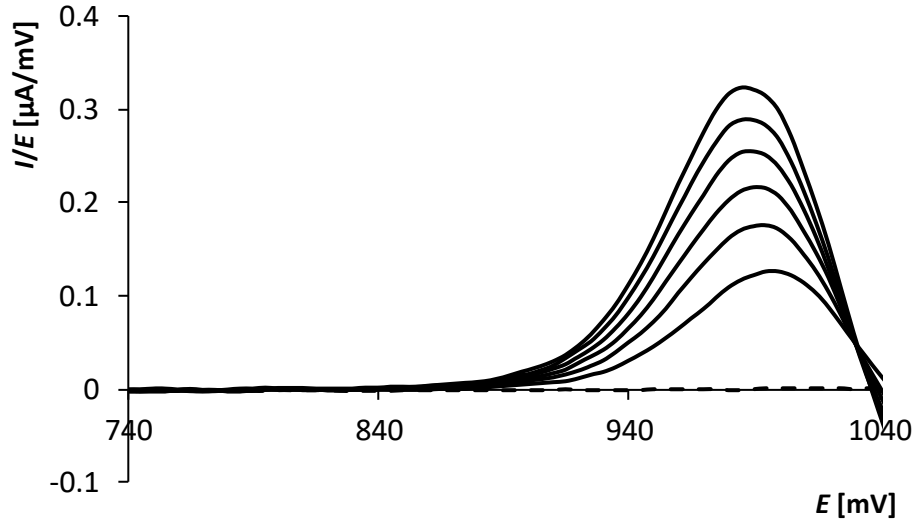

Fig. 6 Derivatized curves of 5.40–18.9 µg mL^{-1} IBA

Experimental conditions and parameters of DPV were the same as in Figure 5B

Table 4 Results of simultaneous voltammetric determination of IAA and IBA in model solutions

Mixture no.	Analyte	Added [μg mL^{-1}]	Found* [μg mL^{-1}]	Recovery [%]
1	IAA	2.30	2.45 ± 0.17	99.1-113.9
	IBA	2.70	2.50 ± 0.22	91.2-100.7
2	IAA	2.30	2.48 ± 0.20	99.1-116.5
	IBA	5.40	5.59 ± 0.29	98.1-108.9
3	IAA	0.900	0.910 ± 0.025	98.3-103.9
	IBA	1.04	1.09 ± 0.08	97.1-112.5

* average of five repeated determinations (n = 5)

Analysis of real samples

Applicability of the proposed DPV method was verified by analysis of two growth stimulator preparations commonly available on the Czech market. RHIZOPON A containing only IBA was analyzed first. As documented in Fig. 7, no interfering signal was recorded after addition to the sample. Moreover, the recorded peak had increased with IBA additions. The content of IBA was calculated using software Nelinear [27] and the results obtained from three replicate measurements are given in Table 5, showing that the average value is consistent with the declared amount. The second analyzed preparation – GELSTIM B contained both of the plant hormones in the mixture with 1-naphthaleneacetic acid and chinosol. It was proven that any of the compounds present did not interfere with the determination of IAA and IBA, which is depicted in Fig. 8. The signal of IBA (image A) was well-developed and it could simply be evaluated after the derivative step. The IAA responses could however be only poorly observed, which was probably caused by the matrix form with a gel-like character. Thus, double amount (10 mL instead of 5 mL as in case of IBA) of the sample had to be analyzed in the case of IAA. The proposed method of evaluation was applied (employing the positively situated signal of IAA) and the calculated contents (average from 3 results) of the compounds studied are summarized in Table 5. By considering the results, it can be concluded that the proposed method is suitable for analysis of the growth stimulation formula including preparations with complicated matrixes containing both compounds.

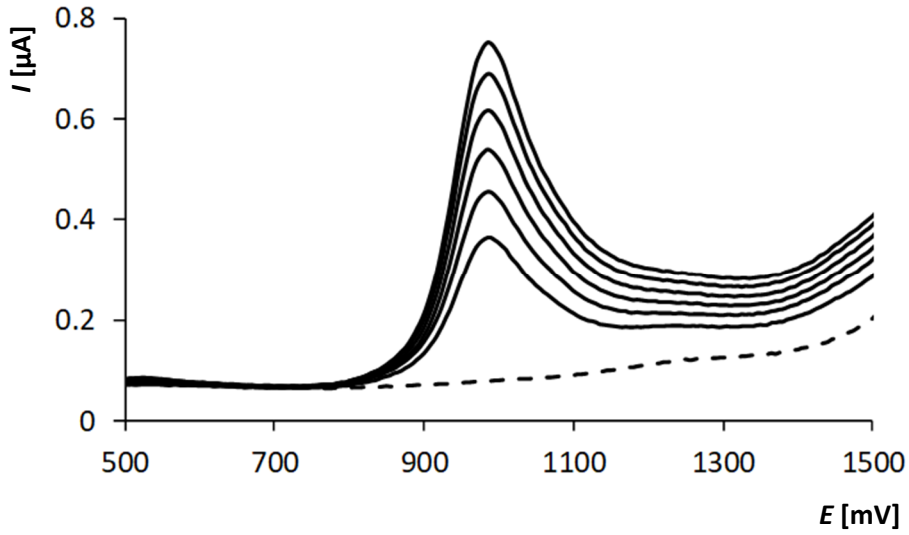

Fig. 7 Determination of IBA in the preparation RHIZOPON employing the proposed electrochemical method

Experimental conditions: V(sample) = 100 µL; additions of IBA c = 0.976 µg mL^{-1}, V = 20 µL; supporting electrolyte: 15 mL ethanolic solution of 0.1 mol L^{-1} H_2SO_4; DPV parameters: same as in Fig. 4

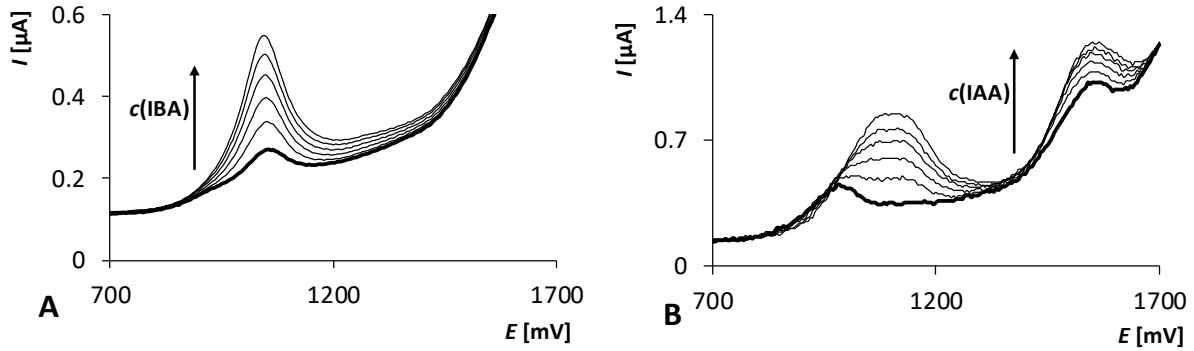

Fig. 8 Determination of IBA (A) and IAA (B) in the preparation GELSTIM B (bold line) employing the proposed electrochemical method

Experimental conditions: V(sample) = 5 mL (A) and 10 mL (B); additions of IBA (A): c = 2.032 g L^{-1}, V = 10 µL; additions of IAA (B): c = 1.752 g L^{-1}, V = 20 µL; supporting electrolyte: 10 mL (A) and 5 mL (B) ethanolic solution of 0.1 mol L^{-1} H_2SO_4; DPV parameters: same as in Fig. 4

Table 5 Analysis of the growth stimulator preparations – the results

RHIZOPON A			
Analyte	Declared [mg/tablet]	Found* [mg/tablet]	Recovery [%]
IBA	50.0	53.5 ± 4.1	98.8–115.2
GELSTIM B			
IAA	9.5	10.3 ± 0.9	98.9-117.9
IBA	8.0	8.5 ± 0.7	97.5-115.0

* average of three repeated determinations (n = 3)

Conclusions

The goal of the paper presented herein was to describe voltammetric behavior of the auxin phytohormone IBA on BDDE and to optimize working parameters for its simple determination. It was proven that the oxidation peak is suitable for IBA determination and both voltammetric techniques used (LSV and DPV) were successfully tested for its determination in model samples. The second aim of this work was to develop voltammetric method for simultaneous determination of the indole-based phytohormones, IAA and IBA, in real samples, especially the preparations with growth stimulators. DPV method proposed was optimized with respect to the IBA determination and it was confirmed that, after simple procedure, the simultaneous determination of the studied phytohormones is feasible despite their quite similar structures. Moreover, the novel approach proposed was found to be a suitable and reliable tool for analyzing the formulas of growth stimulators, including those with complicated matrixes.

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