Turk J Chem
35 (2011) , 413 – 426.

doi:10.3906/kim-1011-805

Voltammetric behavior of indole-3-acetic acid and kinetin at pencil-lead graphite electrode and their simultaneous determination in the presence of anionic surfactant

Yavuz YARDIM*, Zühre ŞENTÜRK
Yüzüncü Yıl University, Faculty of Science, Department of Analytical Chemistry, 65080 Van-TURKEY
e-mail: yavuz@yyu.edu.tr

Received: 12.11.2010

A method was developed for the simultaneous determination of indole-3-acetic acid (IAA) and kinetin, based on the oxidation of both phytohormones at a pencil-lead graphite electrode. The electrochemical behaviors were examined by cyclic, linear sweep, and square-wave voltammetry in the pH range of 2-12. In Britton-Robinson buffer solution (pH 6), the oxidation peaks of IAA and kinetin are well separated by square-wave voltammetry. By adding an anionic surfactant (sodium dodecylsulfate) in a concentration of 50 μM, the sensitivity of kinetin was increased significantly without reducing the resolution. The detection limits were 0.14 and 0.11 μM (24.53 and 23.67 ng mL^{-1}) for IAA and kinetin, respectively. Applicability to assays of maize plant extracts was illustrated.

Key Words: Phytohormones, indole-3-acetic acid, kinetin, pencil-lead graphite electrode, square-wave voltammetry, anionic surfactant, maize seed

Introduction

Plant hormones (phytohormones), or plant growth regulators (PGRs), are organic substances that, in very small amounts, regulate numerous aspects of plant growth, development, and response to stress.[1] Changes in phytohormone concentration not only influence the adaptive response but also affect the normal growth of the harvestable organs and thus influence economic productivity.[2] To date, several types of phytohormones have

*Corresponding author

been characterized, mainly including auxins, cytokinins, gibberellins, abscisic acid, and ethylene.[3] In addition to natural phytohormones, there are less expensive and more stable synthetic analogs on the market for use in horticulture. They can influence the life processes of plants, destroy the undesired parts of plants, or control or prevent the undesired growth of plants.

Auxins are a class of phytohormones that are involved in many aspects of the growth and development of plants. In this group, indole-3-acetic acid (IAA), the first plant hormone, is regarded as the principal native hormone, and is known to regulate processes such as division, elongation, and differentiation of cells.[4] Kinetin (N^6-furfuryladenine) is a synthetic substance belonging to the cytokinin family.[5] Although this phytohormone is a synthetic one, it was the first cytokinin discovered. Cytokinins (N^6-substituted adenine derivatives) are an important group of plant growth regulatory substances. They promote, in the presence of auxins, cell division in plant tissue cultures, and affect a wide range of biological processes, including seed germination, bud differentiation, branching, chlorophyll and starch production, plant-pathogen resistance, apical dominance, and leaf senescence.[6] Kinetin is used in studies related to germination and control of plant growth. Most importantly, it also exerts antiaging effects in plants[7] as well as in human skin cells and fruitflies.[8,9] The structures of IAA and kinetin are shown in Figure 1.

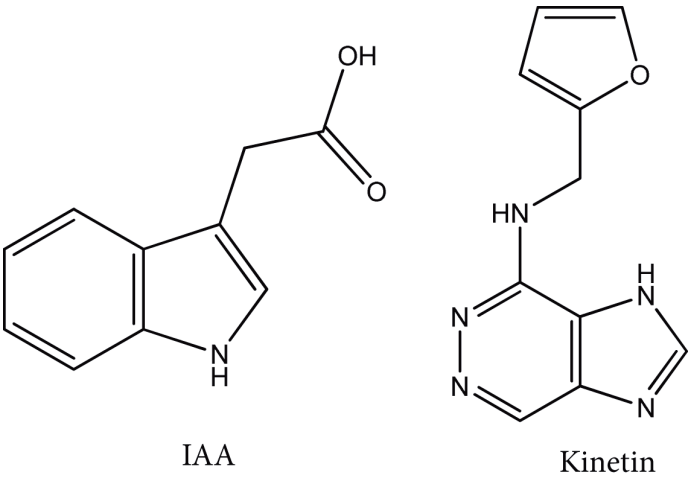

Figure 1. Chemical structures of IAA and kinetin.

Different phytohormones act very locally but are highly interconnected in a complex dynamic network; therefore, a change in one component of the hormonal system may affect other parts of the network. The morphogenetic abilities of plants may depend on plant growth modifications caused by changes in the ratio of auxins to other phytohormones, e.g. cytokinins.[10] Multianalyte techniques for hormone quantification allow a far more comprehensive analysis of the changes in plant hormone status. Traditional methods such as gas chromatography,[11–15] liquid chromatography,[16–22] enzyme-linked immunosorbent assays (ELISA),[23–25] and radioimmunoassay (RIA)[26,27] have been used for the individual or multicomponent analysis of IAA or kinetin in a variety of plants. Capillary electrophoresis has also been applied for their determination.[28–30] Although these methods have excellent selectivity and accuracy and high sensitivity, some of them require sophisticated instrumentation or radioactive chemicals, or are time-consuming. Electroanalytical methods, particularly the voltammetric ones, have distinct advantages compared to the abovementioned techniques. They could meet

these requirements since they are relatively simple to apply and fast and reasonably cheap, providing good sensitivity and selectivity to electroactive analytes. To date, some electrochemical techniques have been developed for the individual determination of IAA and kinetin using a carbon paste electrode modified with OV-17,[31,32] carbon fiber microelectrode,[33,34] carbon nanotube film modified electrode,[35] mercury electrode,[36] carbon paste electrode modified with sodium dodecylsulfate,[37] and graphite-polyurethane composite electrode[38] in plant extracts or in soil samples. Recently, an electrochemiluminescent method was described, based on a tri(2,2'-bipyridyl)ruthenium(II) complex system at a carbon nanotube/Nafion composite modified electrode for the low potential detection of kinetin.[39] In a very recent work by our research group, the electrochemical behavior and voltammetric determination of IAA at a boron-doped diamond electrode was also described.[40] These results are of important theoretical and practical significance in plant physiology. In the revised bibliography, however, no voltammetric method was found in which different classes of phytohormones were determined simultaneously.

Bearing in mind the regulatory role of the auxin/cytokinin balance[41] and the effects of exogenous kinetin application[42] in the growth and morphogenesis of plants, the aims of this study were, first, to demonstrate the usefulness of a pencil-lead graphite electrode in the presence of an anionic surfactant on the electrochemical behaviors of endogenous IAA and exogenously applied kinetin, and, second, to develop a simple and sensitive voltammetric method for the simultaneous determination of the 2 analytes for model samples. Finally, the practical utility of the proposed method was investigated in plant tissue extracts.

Experimental

Reagent and chemicals

IAA and kinetin were purchased from Sigma and were used as received. Their stock solutions (5×10^{-3} M) were prepared in methanol due to their low solubility in aqueous media. All stock solutions were preserved at 4 °C when not in use and were protected from daylight during use in the laboratory. On the day of the experiment, working solutions were prepared by diluting the stock solution with 1 of 4 selected supporting electrolytes, namely acetate (0.1 M, pH 4.8), phosphate (0.1 M; pH 3, 4, 5, or 12), Tris (0.1 M, pH 7), or Britton-Robinson (0.1 M; pH 2, 6, or 9) buffer solutions. Sodium dodecylsulfate (SDS) was prepared by dissolving the necessary quantity of reagent in water. SDS and all other chemicals (analytical grade) were obtained from Sigma or Merck. Aqueous solutions were prepared with deionized water further purified via a Milli-Q unit (Millipore).

Apparatus

Cyclic (CV), linear sweep (LSV), and square-wave (SWV) voltammetric measurements were carried out using a μAutolab type III potentiostat (EcoChemie, The Netherlands) controlled by GPES 4.9 software. The raw SWV voltammograms were treated by using the Savitzky-Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a peak width of 0.01 V.

In all measurements, the reference electrode was Ag/AgCl (3 M NaCl) (Model RE-1, BAS, USA) and the auxiliary electrode was a platinum wire. The pH values of solutions were measured using a WTW inoLab pH 720 meter with a combined electrode (glass-reference electrodes).

A pencil-lead graphite (PG) electrode served as the working electrode. For the PG electrode, a mechanical

pencil, Model T 0.5 (Rotring, Germany), was used as a holder for pencil-lead (Tombo, Japan), which was purchased from a local bookstore. The details of the preparation of the PG electrode were described in our previous study.[43] Before use, the PG surface was pretreated by applying a potential of +1.30 V for 30 s in the blank supporting electrolyte without stirring, in order to increase the hydrophilic properties of the electrode surface through introduction of oxygenated functionalities, accomplished with an oxidative cleaning. The measurements were carried out in a standard homemade 5-mL glass voltammetric cell at laboratory temperature (20-25 °C). The potential for the voltammetric experiments was recorded from +0.30 V to +1.30 V.

Preparation of plant extracts

The seed samples of maize plant (*Zea mays* L.) were provided by the Department of General Biology, Faculty of Science, Yüzüncü Yıl University, Van, Turkey. The collected materials were weighed, powdered by pestle and mortar in liquid nitrogen, and kept in a deep freezer until analysis. Extraction of plant material was carried out as described previously.[44] Briefly, 1 g of frozen powder was placed in cold 70% (v/v) aqueous methanol and stored in a refrigerator for 24 h. The sample was then homogenized in an Ultra Tissue Lysis and filtered through filter paper (Whatman No. 1). The supernatant was transferred into a clean vial. The residues were reprocessed as described above and combined with the former in order to minimize the loss of phytohormone. The supernatant was again filtered through PTFE filters (0.45 μm), and methanol was removed at 35 °C under reduced pressure. The extract was further dissolved in 0.1 M phosphate buffer (pH 8.5) and centrifuged at 10,000 rpm for 1 h at 4 °C. The supernatant was then put into a flask (25 mL) containing 1 g of polyvinylpolypyrolidone, mixed well, and filtered through Whatman filter paper (No. 1). The filtrate was passed through a C_{18} Sep-Pak cartridge to remove interfering lipids and pigments. The hormone adsorbed by the cartridge was eluated with 80% methanol-water (v/v) and collected in vials.

Results and discussion

Electrochemical characterization of IAA and kinetin using PG electrode

Without SDS in solution

The oxidation behaviors of IAA and kinetin were first studied by CV at the PG electrode. Figure 2 shows the CV curves of single components of IAA (curve a) and kinetin (curve b) recorded between +0.3 and +1.4 V at a scan rate of 100 mV s^{-1} in Britton-Robinson buffer (pH 6). IAA was oxidized yielding 1 (a_1) or 2 (a_1 and a_2) anodic steps, depending on its concentration. At an IAA concentration of 100 μM, the potentials of peaks a_1 and a_2 were +0.79 and +1.05 V, respectively. With decreasing concentrations of IAA, peak a_2 became less distinct and almost disappeared at $\leq$50 μM (Figure 2, inset). This voltammetric behavior of IAA at the PG electrode was similar to that reported previously at coated glassy carbon,[35] carbon paste,[37] and pyrolytic graphite[45] electrodes. Therefore, peak a_1 was chosen as the analytical signal for the determination of IAA. The CVs of IAA at scan rates as high as 500 mV s^{-1} exhibited no reduction peaks reversibly coupled to oxidation peaks a_1 or a_2. Kinetin was oxidized producing only 1 anodic peak at +1.02 V under the selected condition. No reduction peak could be observed, which showed that its electrochemical reaction was irreversible.

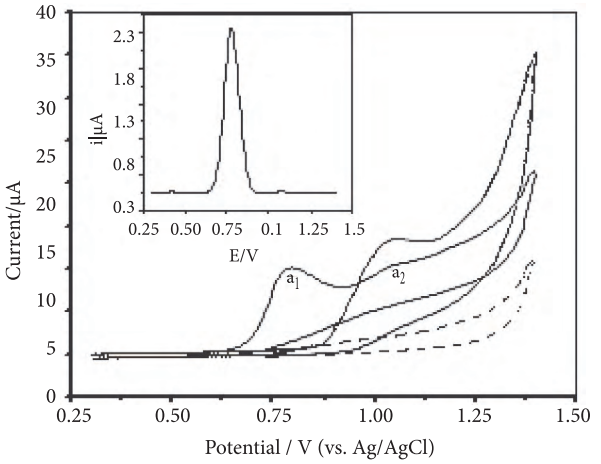

Figure 2. CV curves of 100 μM IAA (A) and 100 μM kinetin (B) at PG electrode. Inset: SW voltammogram of 10 μM IAA. Electrode: PG; supporting electrolyte: Britton-Robinson buffer (pH 6); CV condition: 100 mV s^{-1}; SWV conditions: pulse amplitude of 25 mV, frequency of 25 Hz, scan increment of 8 mV. Dashed lines represent background current.

Solutions of IAA and kinetin with a concentration of 100 μM at pH 6 were tested by LSV at different scan rates. In the range of 50-500 mV s^{-1} (n = 6), the peak currents increased linearly with the scan rate. The linear equations were i_p (μA) = 0.0294 ν (mV s^{-1}) + 2.8318 for IAA and i_p (μA) = 0.0335 ν (mV s^{-1}) + 1.4915 for kinetin, with correlation coefficients (r) of 0.998 and 0.996. This suggests that the electrooxidation of both phytohormones was an adsorption-controlled process at the PG surface. On the other hand, the voltammograms of IAA revealed a linear relationship between peak intensity and the square root of the scan rate: i_p (μA) = 0.8703 $v^{1/2}$ (mV s^{-1}) + 2.7568, r = 0.996. This demonstrates that, in spite of there being an adsorptive process, there was also a diffusion component in the electrochemical process.

The effect of the pH of the supporting electrolyte on anodic peak currents and potentials was studied to obtain the best peak resolution (ΔE_p) and maximum sensitivity in mixture solutions of IAA and kinetin (Figure 3). Since CV was not sensitive for the determination of low contents of compounds, SWV studies were performed in various buffered solutions in the pH range of 2-12. For both phytohormones, the increase in pH produced a shift in their voltammograms to less positive potentials, implying the involvement of protons in the oxidation process (Figure 3A). On plotting the peak potentials of IAA and kinetin against the pH value, linear variation was observed with negative slopes of 0.051 V (r = 0.992) and 0.067 V (r = 0.994) per pH unit, respectively, which suggests that an equal number of electrons and protons were involved in the electrode reactions. Analyzing the evolution of peak current (Figure 3B), it can be seen that this parameter was also affected by the pH of the buffer. The pK_a value of IAA was 4.75, which is attributed to the deprotonation of the carboxyl group,[46,47] whereas kinetin, a weak, diacidic base, had 2 pKa values, pK_{a1} = 9.8 and pK_{a2} = 3.8.[48] In the case of kinetin, clear changes at about pH 3 and 9 were observed, corresponding to minimum pHs, which were in concordance with the pK_a values of this molecule. According to the presented results, both the peak currents of IAA and kinetin attained their maximum at pH 12.0. However, in a solution with pH 6.0, the best peak separation (ΔE_p = 220 mV) was achieved and reasonable peak currents for each phytohormone

resulted (Figure 4). Therefore, Britton-Robinson buffer of pH 6 was chosen as the supporting electrolyte to realize the simultaneous determination of an IAA and kinetin mixture.

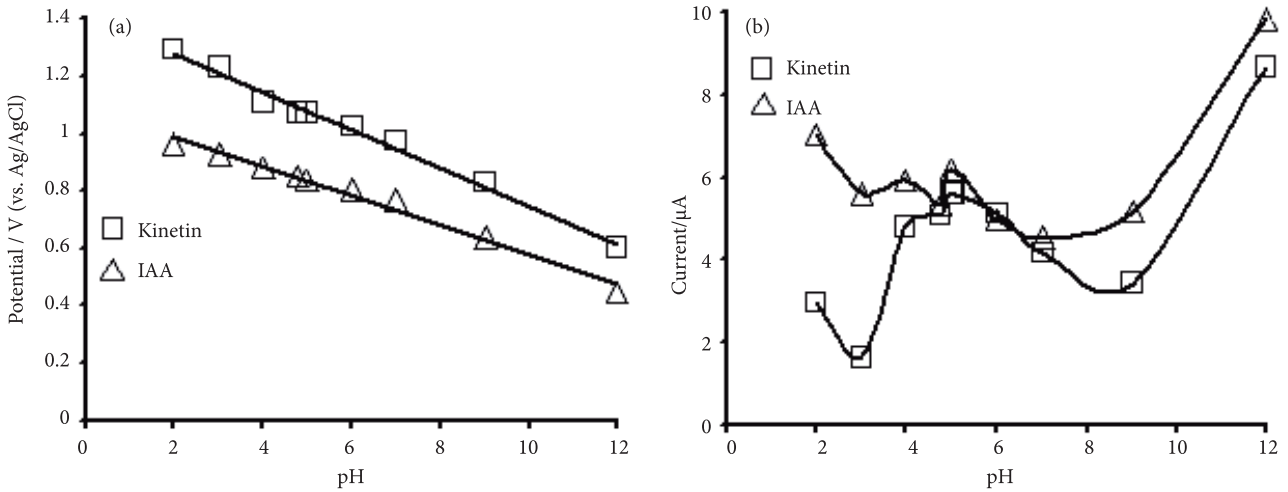

Figure 3. Effect of pH on the peak potentials and peak currents of IAA and kinetin mixtures. Each phytohormone, 50 μM; electrode, PG; SWV conditions as indicated in Figure 2.

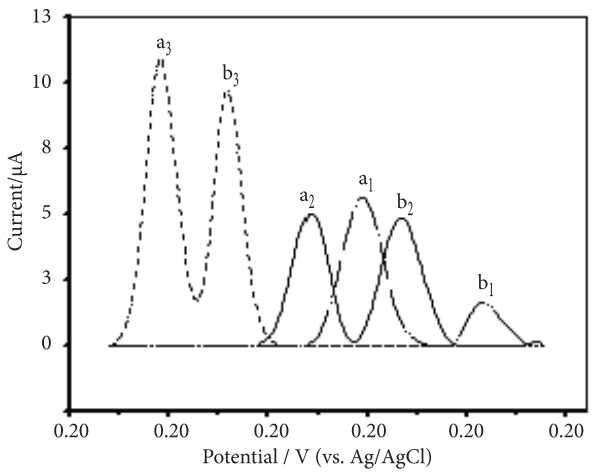

Figure 4. SW voltammograms of IAA (A) and kinetin (B) mixtures at different pH levels. Each phytohormone, 50 μM; electrode, PG. pH values: a_1 and b_1, 3; a_2 and b_2, 6; a_3 and b_3, 12. SWV conditions as indicated in Figure 2.

With SDS in solution

Surface-active substances (surfactants), a kind of amphiphilic molecule with a hydrophilic head on one side and a hydrophobic tail on the other side, have been widely applied in electrochemistry to improve the sensitivity and selectivity of voltammetric measurements.[49–53] The experimental procedure for the modification of carbon-based electrodes can be simplified if the modifying agent is added to the background electrolyte, which is termed

in situ modification. In situ modification has the advantage of shortening or eliminating the preparation steps before the analysis.

Taking into account that phytohormones occur in very low amounts in plant extracts, to improve the sensitivity of the voltammetric method described above, SDS (anionic surfactant), using its concentrations below the critical micelle concentration (CMC: 8.2×10^{-3} M),[54] was added to the sample solution. When the pH is higher than about 7, IAA ($pK_a = 4.75$) will be almost completely ionized (anionic form). Under the studied pH condition (pH = 6), the negatively charged anionic form exists predominately, with a small amount of the protonated (nonionized) form. Thus, treatment with SDS resulted in a nonsignificant increase in the peak current of IAA. In contrast, based on the discussion of the 2 pK_a values for kinetin described above, at pH 6, the molecule existed in only the positively charged monoprotonated form. As a result, in the presence of 50 μM SDS, the peak sensitivity of kinetin was enhanced by approximately 48% when compared to that in surfactant-free medium, indicating that the rate of electron transfer had increased. These behaviors can be explained by the acid/base character of the phytohormones and the fact that there is an electrostatic interaction between the protonated phytohormones and the ionized sulfate group of SDS at the electrode surface. The peak potentials shifted slightly to less positive values, but the differences among them were unaltered.

The effect of SDS concentration on the separation and peak currents were investigated within the range of 5-500 μM (n = 7) (data not shown) for a mixture of IAA and kinetin. It was found that the selectivity of the separation was not enhanced by increasing the SDS concentration. At the beginning, the oxidation peak currents increased with the increase of the concentration of SDS. When the concentration exceeded 50 μM, the peak intensities almost leveled off. This is attributed to the adsorption of the surfactant molecules on the electrode surface, which could be followed by the formation of micelle aggregates as the distance from the electrode surface increased.[55] Hence, this value of 50 μM was chosen for further experiments.

Figure 5A depicts the multisweep cyclic voltammogram obtained for the mixture of IAA and kinetin, each having a concentration of 50 μM in the presence of SDS. As is seen, the anodic peaks for IAA (peak a) and kinetin (peak b) were well separated, being of paramount importance for simultaneous determination of these species and, of course, for the development of an analytical method. When the cyclic voltammogram was recorded at repeated potential cycles, the oxidation peaks of IAA and kinetin were shifted to more a positive potential with a simultaneous decrease in the oxidation peak currents. This may have been due to the covering (adsorption) of the mixture of the oxidation products of IAA and kinetin to the electrode surface, which leads to the fouling of the working electrode.

Various linear sweep voltammograms were run at various scan rates, from 25 to 300 mV s^{-1} (n = 8) in the presence of surfactant. As displayed in Figure 5B, the oxidation peak currents linearly varied with the scan rate and can be expressed as follows: i_p (μA) = 0.0088 ν (mV s^{-1}) + 1.1337, r = 0.995 (for IAA); i_p (μA) = 0.0298 ν (mV s^{-1}) + 1.5214, r = 0.989 (for kinetin). Thus, the electrochemical reactions were an adsorption-controlled step rather than a diffusion-controlled process. The plot of the logarithm of peak current versus the logarithm of scan rate also showed straight lines with slopes of 0.64 (r = 0.998) and 0.66 (r = 0.979) for IAA and kinetin, respectively. These facts suggest a predominantly adsorption-controlled mass transfer.

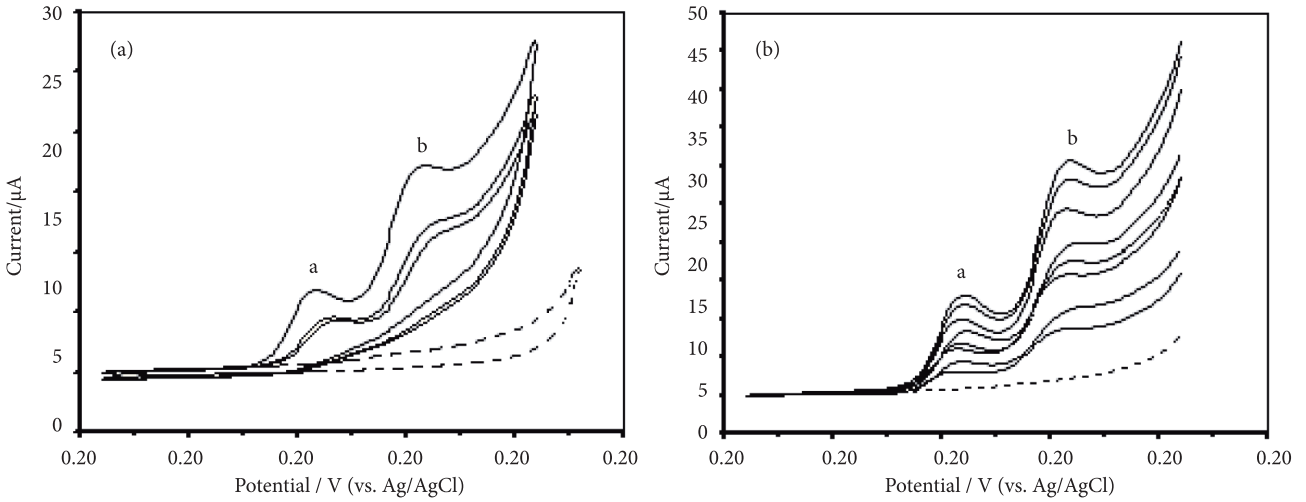

Figure 5. Multisweep CV curves at scan rate of 100 mV s^{-1} (a) and LSV curves at different scan rates between 25 and 300 mV s^{-1} (b) for IAA and kinetin mixtures in Britton-Robinson buffer (pH 6) containing 50 μM SDS. Each phytohormone, 50 μM; electrode, PG. Dashed lines represent background current.

The electrochemical oxidation mechanisms of IAA and kinetin in aqueous and aqueous/surfactant solutions are beyond the scope of the present work, which only shows how the compounds are oxidized in all above media at a PG electrode. However, from our experimental findings, and considering the voltammetric behaviors of IAA and kinetin at different carbon-based electrodes, some comments can be made. A mechanism for the complex nature of the oxidation process of IAA at pyrolytic graphite electrodes has been proposed by Hu and Dryhurst,[56] who carried out the cyclic voltammetry of the compound in an acidic aqueous solution. As reported in the literature data, the initial oxidation step of IAA could be due to the 2-electron 1-proton transfer that corresponds to the formation of the cation of 3-methyleneindolenine carboxylic acid. A very rapid decarboxylation of the initial oxidation product of this compound is followed by a series of chemical and additional electrochemical steps, including dimerization, which is further oxidized. On the other hand, the anodic voltammetric behavior of kinetin at a modified carbon paste electrode has been previously discussed.[32] Taking into account the studies based on the oxidation chemistry of structurally related adeninic compounds, the authors proposed that the oxidation of the kinetin involves the total 4-electron and 4-proton transfer process through the formation of 2-hydroxyadenine, which is rapidly oxidized to 2,8-hydroxyadenine. As the 2 consecutive electron-transfer steps of the purine ring occur simultaneously, only 1 oxidation wave may be observed as a voltammetric response.

SWV was chosen for quantifying each phytohormone in a sample due to its speed and higher sensitivity relative to other pulse voltammetric techniques. In order to obtain the best analytical signal, conditions for each of the parameters of the chosen technique were studied (figures are not given). The effect of frequency was studied in the range of 15-100 Hz. The peak currents increased with frequency due to the increase in the effective scan rate, but the peak shape and baseline were distorted at frequencies higher than 75 Hz. This was attributed to the greater contribution of the capacitive current at higher frequencies. The influence of scan increment was investigated between 4 and 18 mV. The peak height increased up to 8 mV because the effective scan rate was increased, but at higher values of scan increment, the peak heights decreased. The analytical signal was

dependent on the pulse amplitude even if this parameter seemed to be less important than the frequency. Pulse amplitude was examined in the range of 5-35 mV. Peak heights increased upon increase of the pulse amplitude. However, the peak shape became wider above 25 mV. Thus, the frequency of 75 Hz, the scan increment of 8 mV, and the pulse amplitude of 25 mV were selected for analytical determination.

Analytical application

In order to verify the performance of the method for the separation of the peak potentials of IAA and kinetin, it was recorded by SWV voltammograms (frequency: 75 Hz, scan increment: 8 mV, pulse amplitude: 25 mV) at different concentrations in Britton-Robinson buffer at pH 6.0 containing 50 μM SDS (Figure 6). As observed, a remarkable separation was clear with the increase of phytohormone concentrations, even at lower and higher concentrations. Hence, peak currents at potentials of +0.80 V and +0.99 V increased proportionally with the IAA and kinetin concentrations, respectively (Figure 6, inset). The calibration plots of both of them were linear in the 2 concentration ranges of 0.5-2.0 and 2.5-10 μM. Linear equations over these ranges were expressed as $i_p(\mu\text{A}) = 0.6359$ C (μM) – 0.3177 (r = 0.933) and $i_p(\mu\text{A}) = 0.4733$ C (μM) + 0.3351 (r = 0.996) for IAA, and $i_p(\mu\text{A}) = 0.8712$ C (μM) – 0.2224 (r = 0.999) and $i_p(\mu\text{A}) = 0.6017$ C (μM) – 1.2629 (r = 0.995) for kinetin, respectively (Figures 6A and 6B).

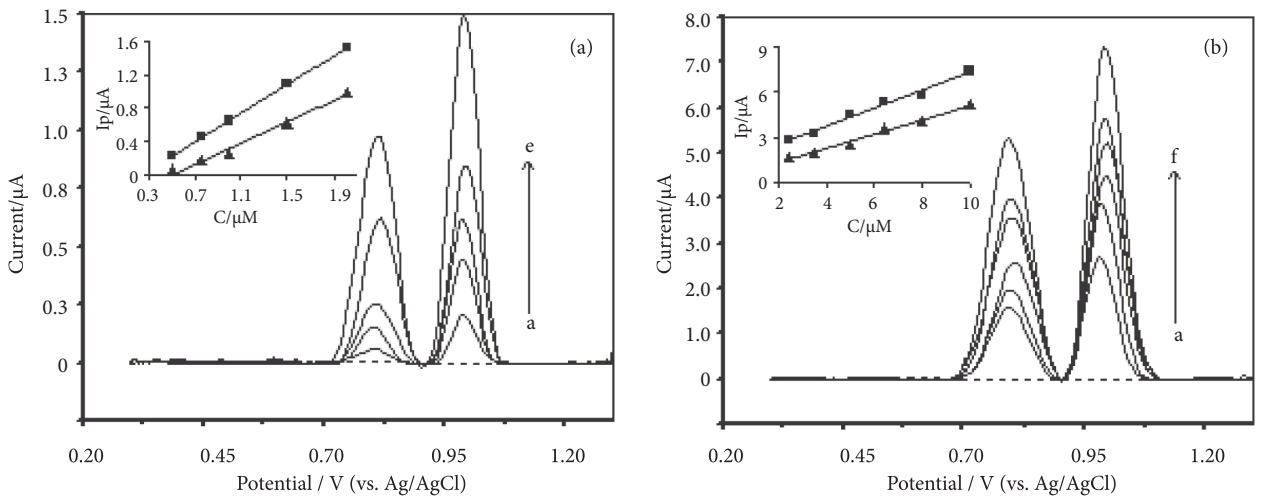

Figure 6. SWV voltammograms for a mixture of IAA and kinetin in Britton-Robinson buffer (pH 6) containing 50 μM SDS. Concentrations of both phytohormones were equally changed: (a) 0.5 to 2 μM (a-e), (b) 2.5 to 10 μM (a-f). SWV conditions: frequency of 75 Hz, pulse amplitude of 25 mV, scan increment of 8 mV. Inset depicts corresponding calibration plots for the quantitation of IAA and kinetin.

The sensitivity of the proposed method was evaluated with both the limit of detection (LOD) and quantification (LOQ) values. The LOD and LOQ were calculated using the following equations:[57] LOD = $3s/m$ and LOQ = $10s/m$, where s is the standard deviation of the response (blank) (7 runs) and m is the slope of the related calibration equation. The LOD and LOQ were calculated as 0.14 and 0.47 μM for IAA, while they were found to be 0.11 and 0.37 μM for kinetin, respectively. The precision of the proposed method was realized in terms of the relative standard deviation (RSD) for 9 identical measurements carried out at a

concentration level of 5 μM for each of the phytohormones. The RSD values were found to be 6.26% and 6.95% for IAA and kinetin, respectively. This suggests that the PG electrode has good sensitivity and reproducibility.

In order to evaluate the selectivity of the proposed method, increasing concentrations of the possible interfering agents, such as some ions and common phytohormone compounds that are usually present in the plants, were added to a solution mixture with a fixed amount (5 μM) of IAA and kinetin, and the corresponding voltammograms were recorded. The tolerance limit was defined as the maximum concentration of potential interfering substance that caused a change in the signal of $\pm$5%. At about a 100-fold excess, Cu^{2+}, Fe^{3+}, Mg^{2+}, K^{+}, NO_3^{-}, Cl^{-}, gibberellic acid (phytohormone belonging to the gibberellin family), abscisic acid (phytohormone), and methyl jasmonate (phytohormone) did not significantly interfere with the current response. When determining IAA and kinetin, interference from salicylic acid (phytohormone) is often a problem, because this substance is usually oxidized in the same potential window. It was found that salicylic acid was oxidized at the potential of +0.94 V at experimental pH levels, and this substance had a strong peak current. On the other hand, zeatin, a native phytohormone of the cytokinin group, had a similar oxidation with kinetin, resulting in an overlapped voltammetric response.

It is clear that the new voltammetric methodology can be used to determine IAA and kinetin simultaneously in ideal laboratory samples. In order to testify to its practical application, this voltammetric method was applied to the determination of endogenous IAA alone in seed samples of maize plant (*Zea mays* L.). After the extraction and purification process (not spiked previously with kinetin), an appropriate volume (100 μL) of the final extract was transferred to a voltammetric cell containing 4 mL of Britton-Robinson buffer (pH 6.0) and the voltammetric procedure was followed. A typical voltammogram of a seed sample is shown in Figure 7. It is clearly shown that the observed peak at about +0.80 V is assigned to the oxidation of IAA, since this peak height increased with the adding of IAA standard solution. In order to find the IAA concentration contained in the extract, quantification was performed by means of the calibration curve method. A concentration of 1.25 μM of IAA was found in the measurement cell. Taking into account the successive dilutions of the sample, it was calculated that the amount of IAA present in the original extract was 50.0 μM, i.e. 1.75 μg of IAA per gram of seed. The obtained IAA content in maize seed samples is in agreement with a previously published work.[58] To check the validity of the proposed method, spike/recovery experiments were also performed. Recovery of IAA was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure IAA. In the Table, the results of the analysis of spiked samples of maize seeds are shown. It was found that the IAA amount could be quantitatively recovered by the proposed method, being thus a guarantee of the accuracy of the voltammetric determination of IAA in seeds of the plant sample.

Table. Results of the recovery analysis of IAA in the extracts of maize plant seeds.

IAA added (μM)	Found (μM)	Recovery (%) $\pm$ RSD (%)
-	1.25	-
0.75	1.88	94.00 $\pm$ 7.91
1.00	2.24	99.56 $\pm$ 5.44
1.50	2.77	100.73 $\pm$ 2.60

[a] Values reported are the average of 3 independent analyses of each spiked sample.

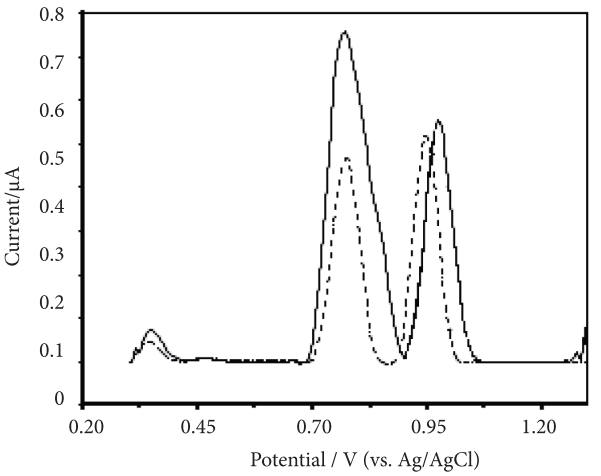

Figure 7. SWV voltammograms obtained for the determination of IAA in seed samples. Seed extracts of maize plant diluted 40× (dashed line) and extract spiked with 0.75 μM IAA (solid line). Other operating conditions as indicated in Figure 6.

Keeping in mind that kinetin is not a natural substance, the peak at about +0.97 V most likely corresponded to the oxidation of zeatin or other chemically related cytokinins. Thus, this method may be used if its selectivity is improved with an appropriate purification procedure before the quantification of kinetin.

Conclusions

As explained in the introduction, new analytical methods are needed to verify both the absence of added synthetic hormones and the presence of effective biostimulant activity in plant samples. This study reported on the development and evaluation of a novel SWV procedure for the simultaneous analysis of 2 different classes of phytohormones (auxins and cytokinins) in bulk aqueous forms with relatively high detection sensitivity. Well-separated peaks of IAA and kinetin could be achieved at a PG electrode. By adding an anionic surfactant (SDS) to the sample solution, the sensitivity of kinetin was especially remarkably enhanced without reducing the resolution. For the developed procedure, which is rapid, requiring less than 5 min to run a sample after the sample pretreatment, the detection limits reached levels as low as 0.14 and 0.11 μM (corresponding to 24.53 and 23.67 ng mL^{-1}) for IAA and kinetin, respectively. This method has an almost similar sensitivity as that of a graphite-polyurethane composite electrode.[38] It presents slightly higher sensitivity compared with the carbon paste electrode modified with OV-17,[31,32] while its sensitivity is approximately 8 times less than that of the carbon fiber microelectrode[33,34], carbon nanotube film modified electrode,[35] and carbon paste electrode modified with sodium dodecylsulfate.[37] The reported electrochemical methods for the individual determination of both phytohormones were mainly developed on the basis of the modified electrode. However, it is well known that the preparation of modified electrodes is not convenient and may sometimes be complicated. Considering the analytical features of the proposed method, it is possible to conclude that it presents advantageous characteristics when compared with other works, mainly due to simplicity, since it uses a PG electrode, a relatively new type of carbon electrode, without modification, as well as due to the low analytical cost considering the

negligible cost of the surfactant.

Indole derivatives are well known for their abilities to foul electrodes, and the tarry deposit formed on the electrodes during their oxidation is attributed to polymerization products,[59,60] which prevent the reproducibility of the subsequent current measurements at the same surface. However, in the case of our study, the PG electrode, disposable after one-time use, offered the advantage of eliminating the memory effects of most solid electrodes and showed good accuracy and reproducibility. In particular, the addition of surfactants as antifouling agents minimized the possibility of the fouling of the working electrode by products of the electrochemical reaction of the analytes.

On the other hand, keeping in mind the very important role of surfactants in increasing the solubility of organic substances that are sparingly soluble in water, and taking into account that many of the main hormones in plants are hydrophobic, the further advantage of using surfactants in this study is that it makes the proposed method more environmentally friendly than the most common reference methods for eliminating or at least minimizing the organic solvent.

The proposed approach was found to be a convenient and efficient method for the assay of IAA individually and in the presence of kinetin and/or natural cytokinins such as zeatin for practical applications in plant samples. However, because cytokinins are present in most plant tissues, when kinetin and natural cytokinins are extracted and purified from plant material by common techniques, they very often end up in the same fraction. In general, poor selectivity is a well-known disadvantage of electrochemical detection. To improve selectivity and to achieve the detection of kinetin in the presence of other cytokinins, the samples must be purified by means of preparative thin layer chromatography or a prepurification column.[32,61] Due to the low/no concentration level of endogenous salicylic acid in maize plants,[62] it cannot affect the accuracy of IAA and kinetin determination at the sensitivity level of voltammetric measurements.

Finally, it should be mentioned once again that as far as we know, this paper is the first report describing the usefulness of the voltammetric method for simultaneous monitoring of different groups of phytohormones. Considering the above knowledge, more work on the other forms of cytokinins and auxins is now being carried out in our laboratory to further apply the method to analyze different kinds of plant samples with high detection sensitivity together with selectivity.

Acknowledgements

The authors are thankful to M. Emre Erez, PhD, from Yüzüncü Yıl University, Faculty of Science, Department of General Biology, Van, Turkey, for supplying the plant samples and helping to carry out the plant extraction procedure.

References

1. Davies, P. J. *Plant Hormones: Physiology, Biochemistry and Molecular Biology,* 2nd ed., Kluwer, Dordrecht, Netherlands, 1995.
2. Albacete, A.; Ghanem, M. E.; Martinez-Andujar, C.; Acosta, M.; Sanchez-Bravo, J.; Martinez, V.; Lutts, S.; Dodd, I. C.; Perez-Alfocea, F. *J. Exp. Bot.* **2008**, *59*, 4119-4131.

3. Rivier, L.; Crozier, A. *Principles and Practice of Plant Hormone Analysis*, Academic Press, London, 1987.
4. Schneider, E. A.; Wightman, F. *Annu. Rev. Plant Physiol.* **1974**, *25*, 487-513.
5. Amasino, R. *Plant Physiol.* **2005**, *138*, 1177-1184.
6. Haberer, G.; Kieber, J. J. *Plant Physiol.* **2002**, *128*, 354-362.
7. Gan, S.; Amasino, R. M. *BioEssays* **1996**, *18*, 557-565.
8. Rattan, S. I. S.; Clark, B. F. C. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 665-672.
9. Sharma, S. P.; Kaur, P.; Rattan, S. I. S. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 1067-1071.
10. Posmyk, M. M.; Baabusta, M.; Wieczorek, M.; Sliwinska, E.; Janas, K. M. *J. Pineal Res.* **2009**, *46*, 214-223.
11. Schaerer, S.; Pilet, P. E. *Planta* **1993**, *189*, 55-59.
12. Yamaguchi, M.; Yoshida, H.; Nohta, H. *J. Chromatogr. A* **2002**, *950*, 1-19.
13. Zhang, F. J.; Jin, Y. J.; Xu, X. Y.; Lu, R. C.; Chen, H. J. *Phytochem. Anal.* **2008**, *19*, 560-567.
14. Gutierrez, C. K.; Matsui, G. Y.; Lincoln, D. E.; Lovell, C. R. *Appl. Environ. Microbiol.* **2009**, *75*, 2253-2258.
15. Affonso, V. R.; Bizzo, H. R.; Lage, C. L. S.; Sato, A. *J. Agric. Food Chem.* **2009**, *57*, 6392-6395.
16. García Sánchez, F.; Navas Díaz, A.; García Pareja, A. *J. Chromatogr. A* **1996**, *723*, 227-233.
17. Mattivi, F.; Vrhovšek, U.; Versini, G. *J. Chromatogr. A* **1999**, *855*, 227-235.
18. Ma, Z.; Ge, L.; Lee, A. S. Y.; Yong, J. W. H.; Tan, S. N.; Ong, E. S. *Anal. Chim. Acta* **2008**, *610*, 274-281.
19. Pan, X.; Welti, R.; Wang, X. *Phytochemistry* **2008**, *69*, 1773-1781.
20. Hou, S. J.; Zhu, J.; Ding, M. Y.; Lv, G. H. *Talanta* **2008**, *76*, 798-802.
21. Xi, Z.; Zhang, Z.; Sun, Y.; Shi, Z.; Tian, W. *Talanta* **2009**, *79*, 216-221.
22. Ge, L.; Hong Yong, J. W.; Goh, N. K.; Chia, L. S.; Tan, S. N.; Ong, E. S. *J. Chromatogr. B* **2005**, *829*, 26-34.
23. Chiwocha, S.; Von Aderkas, P. *Plant Growth Regul.* **2002**, *36*, 191-200.
24. Wu, T.; Zhang, J.; Cao, Y. *Sci. Hort. Amsterdam* **2008**, *116*, 27-33.
25. Hauserová, E.; Swaczynová, J.; Doležal, K.; Lenobel, R.; Popa, I.; Hajdúch, M.; Vydra, D.; Fuksová, K.; Strnad, M. *J. Chromatogr. A* **2005**, *1100*, 116-125.
26. Jiménez, V. M.; Bangerth, F. *Plant Cell Tiss. Org. Cult.* **2001**, *67*, 37-46.
27. Axelos, M.; Barbet, J.; Péaud-Lenoël, C. *Plant Sci. Lett.* **1984**, *33*, 201-212.
28. Yin, X. B.; Liu, D. Y. *J. Chromatogr. A* **2008**, *1212*, 130-136.
29. Olsson, J.; Claeson, K.; Karlberg, B.; Nordström, A. C. *J. Chromatogr. A* **1998**, *824*, 231-239.
30. Ge, L.; Yong, J. W. H.; Tan, S. N.; Ong, E. S. *Electrophoresis* **2006**, *27*, 2171-2181.
31. Hernández, P.; Galan, F.; Nieto, O.; Hernández, L. *Electroanalysis* **1994**, *6*, 577-583.
32. Ballesteros, Y.; Gonzalez de la Huebra, M. J.; Quintana, M. C.; Hernández, P.; Hernández, L. *Microchem. J.* **2003**, *74*, 193-202.
33. Hernández, L.; Hernández, P.; Patón, F. *Anal. Chim. Acta* **1996**, *327*, 117-123.
34. Hernández, P.; Patón, F.; Hernández, L. *Electroanalysis* **1997**, *9*, 1372-1374.
35. Wu, K.; Sun, Y.; Hu, S. *Sensor Actuat. B-Chem.* **2003**, *96*, 658-662.

36. Huskova, R.; Pechova, D.; Kotoucek, M.; Lemr, K.; Dolezal, K. *Chem. Listy II* **2000**, *94*, 1004-1009.
37. Zhang, S.; Wu, K. *Bull. Korean Chem. Soc.* **2004**, *25*, 1321-1325.
38. De Toledo, R. A.; Vaz, C. M. P. *Microchem. J.* **2007**, *86*, 161-165.
39. Guo, Z. H.; Tang, L. J.; Zhang, Z. J. *Chinese J. Anal. Chem.* **2009**, *37*, 13-18.
40. Yardım, Y.; Erez, M. E. *Electroanalysis* **2011**, *23*, 667–673.
41. Yokoya, N. S.; West, J. A.; Luchi, A. E. *Phycol. Res.* **2004**, *52*, 244-254.
42. Gadallah, M. A. A.; El-Enany, A. E. *J. Plant Growth Regul.* **1999**, *29*, 151-160.
43. Levent, A.; Yardım, Y.; Şentürk, Z. *Electrochim. Acta* **2009**, *55*, 190-195.
44. Battal, P.; Erez, M. E.; Türker, M.; Türker, İ. *Ann. Bot. Fennici* **2008**, *45*, 173-185.
45. Hu, T.; Dryhurst, G. *J. Electroanal. Chem.* **1997**, *432*, 7-18.
46. *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*, 12th ed., Merck & Co., Whitehouse Station, NJ, 1996.
47. Kelen, M.; Sanli, N. *J. Brazil Chem. Soc.* **2009**, *20*, 133-140.
48. Huang, Y.; Xue, S. F.; Tao, Z.; Zhu, Q. J.; Zhang, H.; Lin, J. X.; Yu, D. H. *J. Incl. Phenom. Macro.* **2008**, *61*, 171-177.
49. Hu, S. S.; Wu, K. B.; Yi, H. C.; Cui, D. F. *Anal. Chim. Acta* **2002**, *464*, 209-216.
50. Wang, X. G.; Wu, Q. S.; Liu, W. Z. *Electrochim. Acta* **2006**, *52*, 589-594.
51. Blanco-Lopez, M. C.; Lobo-Castanon, M. J.; Miranda-Ordieres, A. J.; Tunon-Blanco, P. *Electroanalysis* **2007**, *19*, 207-213.
52. Dos Reis, A. P.; Tarley, C. R. T.; Mello, L. D.; Kubota, L. T. *Anal. Sci.* **2008**, *24*, 1569-1574.
53. Chowdappa, N.; Kumara Swamy, B. E.; Niranjana, E.; Sherigara, B. S. *Int. J. Electrochem. Sci.* **2009**, *4*, 425-434.
54. Nakamura, H.; Sano, A.; Matsuura, K. *Anal. Sci.* **1998**, *14*, 379-382.
55. Rusling, J. F. *Colloid. Surface. A* **1997**, *123*, 81-88.
56. Hu, T.; Dryhurst, G. *J. Electroanal. Chem.* **1993**, *362*, 237-248.
57. Christian, G. D. *Analytical Chemistry*, 6th ed., John Wiley & Sons, New York, 2004.
58. Bandurski, R. S.; Schulze, A. *Plant Physiol.* **1977**, *60*, 211-213.
59. Tüken, T.; Yazıcı, B.; Erbil, M. *Surf. Coat. Technol.* **2005**, *200*, 2301-2309.
60. Deletioğlu, D.; Hasdemir, E.; Solak, A. O.; Üstündağ, Z.; Güzel, R. *Thin Solid Films* **2010**, *519*, 784-789.
61. Hernández, P.; Paton, F.; Ballesteros, Y.; Hernández, L. *Electroanalysis* **1997**, *9*, 235-238.
62. Janda, T.; Szalai, G.; Tari, I.; Paldi, E. *Planta* **1999**, *208*, 175-180.