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Electrochemical study of the fungicide acibenzolar-s-methyl and its voltammetric determination in environmental samples

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The electrochemical behavior of new generation fungicide acibenzolar-s-methyl (S-methyl 1,2,3-benzothiadiazole-7-carbothioate, ASM) on the hanging mercury drop electrode (HMDE) was investigated using square wave adsorptive stripping voltammetry. This method of determination is based on the irreversible reduction of ASM at the HMDE. The well-defined ASM peak was observed at ≈ 0.4 V (vs. Ag/AgCl) in BR buffer at pH 2.2. The reduction peak current was proportional to concentration of ASM from 1.0 × 10⁻⁸ to 6.0 × 10⁻⁸ mol L⁻¹ with detection and quantification limit 3.0 × 10⁻⁹ and 1.0 × 10⁻⁸ mol L⁻¹, respectively. The applicability of the developed method for analysis of spiked samples of tap water, river water, and soil is illustrated. The effect of adsorption on the mercury electrode was studied in detail using the AC impedance method. Possible interferences with other common pesticides and heavy metal ions were examined. Clarification of the electrode mechanism was made using cyclic voltammetry (CV) technique.

Keywords: Acibenzolar-s-methyl, environmental samples, fungicide, hanging mercury drop electrode (HMDE), square wave adsorptive stripping voltammetry (SWAdSV).

Introduction

Acibenzolar-s-methyl (s-methyl 1,2,3-benzothiadiazole-7-carbothioate, ASM, inset in Fig. 1) is a new fungicide belonging to the group of plant activators. Acibenzolar is a selective systemic compound which induces host plant resistance with no direct effect on the target pests. This is a unique mode of action which mimics the natural systemic activated resistance (SAR) response found in most plant species.[1] ASM is applied for the suppression of bacterial spot (Xanthomonas campestris pv. vesicatoria) and bacterial speck (Pseudomonas syringae pv. tomato) on tomatoes, and for the control of blue mold (Peronospora tabacina) on tobacco.[2]

ASM is practically non-toxic to birds or mammals both on an acute and chronic basis. It is moderately to highly toxic to freshwater fish, moderately toxic to freshwater invertebrates and estuarine fish, and highly toxic to estuarine/marine animals and other aquatic plants.[3] The lowest LC₅₀ (0.4 mg L⁻¹) was found for the rainbow trout, and the 48 h LD₅₀ per bee was 128 μg for oral toxicity.[4] Therefore, it is crucial to monitor pesticide residues in the environment as their accumulation can cause severe consequences, similar to those related to the controversial use of DDT.[5,6] Results can be sometimes unpredictable, e.g., the spread of colony collapse disorder among bees following the use of potentially safe neonicotinoid pesticides, such as imidacloprid.[7]

Due to the fact that ASM can be harmful to living organisms, it is important to develop analytical methods for the determination of this pesticide with the lowest possible concentration range. This can be attained by a combination of square wave voltammetry (SWV) and a hanging mercury drop electrode (HMDE). Such electrochemical setups have been used to determine many pesticides[8–12] Frequently, electrochemical methods of determination, e.g., of blasticide,[13] offer lower limits of detection than those of HPLC or high performance capillary electrophoresis. The benefits and limitations of electroanalytical procedures have already been described by Zuman.[14,15] Recently, we have conducted studies on ASM with the use of a renewable silver amalgam film electrode,[16] which better fits the current fashion of green chemistry, but offers a higher limit of detection and a narrower linear concentration range.
Moreover, SWV affords not only a sensitive and cheap pesticide analytical methodology but also the possibility to study the electrode kinetics\cite{17,18} of the electrode reaction.\cite{19} In combination with cyclic voltammetry (CV) and constant potential electrolysis, SWV can elucidate the nature of the investigated peak current. Furthermore, a detailed study of adsorption can provide additional information on the electrochemical processes occurring on the electrode surface. Possible interferences with common pesticides and metal ions are analyzed and the developed electroanalytical procedures are applied for the analysis of spiked tap water, river water, and soil samples.

**Materials and methods**

**Instrumentation**

Square wave voltammetric measurements were performed using a \textmu{}Autolab type II/GPES (General Purpose Electrochemical System Version 4.9, Eco Chemie, Utrecht, The Netherlands) computer-controlled electrochemical system. Experiments were performed on a three-electrode system consisting of HMDE (electrode area 0.0102 cm$^2$, mtm-anko instruments, Cracow, Poland) as a working electrode, Ag/AgCl (3 M KCl) as a reference electrode, and Pt wire as a counter electrode. Measurements of pH were performed using a CP-315M pH-meter (Elmetron, Zabrze, Poland) with a conjugated glass membrane electrode.

**Chemicals**

Acibenzolar-s-methyl (99.5%), metam, cyromazine, aldicarb, methidathion thiophanate-methyl, acephate, clothianidin, dodine and methamidophos) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Ethanol, sodium hydroxide, and acids: hydrochloric, boric, citric and phosphoric (85%) were of analytical grade and purchased from POCh (Gliwice, Poland). Acetic acid (99.5%) and acetone were purchased from Merek (Warsaw, Germany) and Labscan (Gliwice, Poland), respectively. Citrate and Britton–Robinson buffers were used as the supporting electrolyte. Their concentration was 0.04 and 0.1 mol L$^{-1}$, respectively. Doubly distilled and deionized water was used throughout the experiments.

**General voltammetric procedure**

Ten milliliters of the supporting electrolyte (1:1 mixture of buffer and water, \textit{v:v}) was placed in a cell and deoxygenated by bubbling argon for 10 min to remove any oxygen interferences in the cathodic potential window. After growth of a subsequent electrode drop, the accumulation stage at a desired potential was applied with vigorous mixing of the analyzed solution, followed by a waiting time of 10 s. Following the equilibrium time, a potential scan was applied towards negative values. The solution was purged with argon for another 30 s if any reagents were subsequently added. The recorded peaks were measured after subtracting the blank solution voltammogram. The optimal results were obtained in Britton–Robinson (BR) buffer at pH 2.2, with an accumulation potential of $-0.10$ V, accumulation time of 90 s, amplitude of 130 mV, frequency of 150 Hz, and step potential of 8 mV. All measurements were performed at the ambient temperature of laboratory.

**Adsorption measurements**

Adsorption measurements were performed using another voltammetric set, different from the one used in SW voltammetric analysis. These results were normalized with respect to working electrode area to avoid misleading comparison. The studies were performed in thermostated cells at 298 K with an Autolab/GPES electrochemical analyzer (Version 4.9). Investigations were conducted in a three-electrode cell with a CGMDE (controlled growth mercury drop electrode, mtm-anko instruments, Cracow, Poland) as the working electrode, Ag/AgCl (3 M KCl) as a reference electrode, and Pt wire as a counter electrode. The reference electrode was connected to the electrolytic cell via an intermediate vessel filled with the solution to be investigated.

The general procedure was as follows: 10 mL of the supporting electrolyte (Britton–Robinson buffer, pH 2.2) was placed in the electrochemical cell and the solution was deoxygenated using high-purity nitrogen for 5 min. If any reagents were subsequently added, the solution was purged with nitrogen for a further 30 s.
Preparation of solutions and sample treatment

A fresh $1 \times 10^{-3}$ mol L$^{-1}$ stock solution of ASM was prepared by dissolution of accurate mass of the pesticide in the water/ethanol mixture (1:1, v/v) with the aid of an ultrasonic bath (5 min). Lower concentrations of ASM were obtained by proper dilution of stock ASM solution. Tap water and river water, collected from a local water supplier and the Bzura River, were tested as samples. These samples were spiked with the ASM without any pretreatment. Soil samples were collected from nearby fields. The samples (2,000 g) were vigorously mixed for 15 min with 5 mL of BR buffer (pH 2.2) and 5 mL of ethanol. After centrifugation at 10,000 rpm, supernatant was transferred to a 25 mL volumetric flask; subsequently 0.25 mL of $5 \times 10^{-4}$ mol L$^{-1}$ ASM solution was added, and the flask was filled up to the volume with deionized water. Next, 40 μL of this solution, and 9.96 mL of supporting electrolyte (BR buffer: water, 1:1, v:v) were placed in the voltammetric cell ($C_{ASM} = 2 \times 10^{-8}$ mol L$^{-1}$).

Voltammograms of the samples were recorded at the same parameters as for pure pesticide analysis. The recovery of the pesticide was calculated with six repetitive measurements. Quantifications were performed by means of the standard addition method.

Results and discussion

Selection of experimental and instrumental conditions

With the goal of achieving optimal instrumental and experimental conditions for quantitative determination of aclonoprob-s-methyl, an extensive study was performed using HMDE. The effect of the medium was examined using Britton-Robinson buffer (pH 2.0 to 9.0), citrate buffer (pH 1.5 to 4.0) and HNO$_3$ solution (pH 1.4 to 2.3). The best sensitivity and the highest well-shaped ASM signals were observed in BR buffer. The peak height decreased substantially with increasing pH, indicating the involvement of a proton in the reaction mechanism (Fig. 1). Since the acidic medium caused such a sharp maximum, caution must be taken to stabilize the pH of the supporting electrolyte during determination experiments. The ASM voltammetric response was stable over a week. The voltammetric response of that pesticide was recorded as a single peak at about $-0.4$ V in pH 2.2 BR buffer.

As it was observed ASM signals were strongly dependent to the accumulation factor (Fig. 2). For accumulation time 30 s ($t_{acc}$) accumulation potential ($E_{acc}$) was changed in the range from 0.25 to $-0.3$ V. As $E_{acc}$ decreased, an increase in the ASM peak current was observed with a maximum between at about $-0.1$ V. A further decrease of $E_{acc}$ caused a slight reduction in response as it approached $-0.3$ V. An accumulation potential of $-0.1$ V was applied in further experiments. Additionally, ASM signal increased with accumulation time up to 90 s. A subsequent increase in $t_{acc}$ caused a considerable decrease in the ASM signal. Due to that, accumulation time 90 s was chosen for further studies.

The range of square wave amplitude values was between 10 and 200 mV. The ASM signal was investigated with respect to its height $I_p$ as well as the ratio of peak height to half peak width $\Delta E_{p/2}$. Both peak current and half peak width depends on the applied amplitude value. The ratio $\Delta I_p/E_{sw}$ continuously decreases with amplitude increase while the gradient $\Delta E_{p/2}/E_{sw}$ increases in the same time. In other word, although the height of the signal is the most important criterion the broadening of the signal is unfavorable and therefore $\Delta I_p/\Delta E_{p/2}$ ratio is the criterion for choosing the best amplitude value for analytical measurement.[20] $\Delta I_p$ increased as the amplitude approached 130 mV and then started to decrease up to $E_{sw} = 200$ mV. The $\Delta I_p/\Delta E_{p/2}$ ratio increased to $E_{sw} = 130$ mV and later decreased with higher amplitude values. An amplitude of 130 mV was chosen for further studies.

The scan rate applied to the HMDE (as in CV) may be tested by alteration of step height and frequency. The highest signal was apparent with a step height of 8 mV. A further increase of this parameter caused no change in the response and, additionally, led to an ill-defined shape of the ASM peak. The influence of frequency shows a proportional increase in the pesticide voltammetric response with an increasing frequency value. Such a pattern is consistent with an irreversible electrode reaction and immobilization of the electroactive reagent.[20] In subsequent studies, 150 Hz was used since at that frequency recorded ASM signal was high and had good peak shape (with respect to $\Delta E_{p/2}$ and the $\Delta I_p/\Delta E_{p/2}$ ratio), and low background noise. Higher frequencies were avoided due to uncompensated ohmic drop resistance.[21]
Mechanistic and adsorption studies

The reduction peak at about −0.4 V was registered without any anodic peak on the reverse scan which clearly suggests reaction irreversibility. The peak potential shifts linearly toward a more negative potential according to a slope equal to 72.8 mV ($R^2 = 0.985$) with increasing pH. This suggests that an equal number of electrons and protons takes part in the electrode process. Also a constant potential electrolysis experiment was performed in order to confirm the number of electrons exchanged during the process, which amounted to two. The influence of the scan rate was studied to explain the nature of the electrode process. The relationship between the recorded peak current and the scan rate ($\nu$) was linear and the dependence $\Delta I_p$ vs. the square root of the scan rate was nonlinear. To confirm that the electrode mechanism was adsorption-controlled we checked the regression line of the logarithmic dependence between the peak current and the scan rate. The slope was equal to 0.829 with a correlation coefficient of $R^2 = 0.999$ (the range of investigated $\nu$ was 25 to 1000 mV). These results suggest that the reduction process is of adsorptive nature. All these results imply the reduc-tation of the molecule.\[24\] In our opinion, thiadiazole moiety is the place where positive charge can be accumulated. It is also worth noting that an increase of ASM concentration increased proportionately to the supporting electrolyte in that region. The peak potentials were slightly shifted to less negative potentials with an increasing concentration of ASM. A similar course of differential capacity curves was noticed in the potential range $E < −1100$ mV. It is also worth noting that for $−600$ mV $> E > −1100$ mV potentials the situation was opposite; in the presence of ASM the differential capacity decreased in proportion to the supporting electrolyte, which was related to the strong adsorption of ASM molecules on the mercury surface.

As can be seen from Table 1, the values of $E_z$ shifted to less negative potentials together with increasing ASM concentration. These results suggest that the positive end of the ASM molecule is strongly adsorbed on the mercury electrode surface and causes a non-planar surface organization of the molecule.\[27,28\] In our opinion, thiadiazole moiety is the place where positive charge can be accumulated. It is also worth noting that an increase of ASM

<table>
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<th>$C_{ASM}$/mol L$^{-1}$</th>
<th>$E_z$/mV</th>
<th>$\gamma_z$/mN m$^{-1}$</th>
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<td>5×10$^{-7}$</td>
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<tr>
<td>3×10$^{-5}$</td>
<td>396.4</td>
<td>412.9</td>
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concentration in the supporting electrolyte (BR buffer, pH 2.2) led to decreased surface tension at zero charge potential \( \gamma_z \) (Table 1). This behavior confirms the adsorption of ASM molecules on the mercury surface.

Analytical determination of ASM

The analytical performance of pesticide voltammetric determination was investigated on the basis of experimental conditions described in section Selection of experimental and instrumental conditions. SW voltammograms recorded during calibration curve examination are shown on Figure 4. They indicate a linear relationship between signal height and ASM concentration in the range of \( 1 \times 10^{-8} - 6 \times 10^{-8} \) mol L\(^{-1}\). The relatively improved sensitivity (as compared to Hg(Ag)FE) could be due to the size of the working electrode. A further deviation from linearity can be justified by saturation of the adsorbed pesticide layer.

The mathematical relationship between the analytical signal (\( \mu A \)) and the concentration of ASM (\( \mu \)mol L\(^{-1}\)) was \( \Delta I_p = (53.6 \pm 2.2) C_{ASM} + (0.42 \pm 0.06) \), with 95% confidence limit, correlation coefficient of 0.989 and an average RSD 5.1%, respectively. Limit of detection and quantification, estimated on equations \( LOD = 3 s/m \) and \( LOQ = 10 s/m \), where \( s \) was the standard deviation of the peak current (six runs) and \( m \) represents the slope of the calibration curve\(^{[29]} \) were \( 3 \times 10^{-9} \) and \( 1 \times 10^{-8} \) mol L\(^{-1}\), respectively. Intra-day repeatability of the developed method was checked by six experiments with measuring peak current for one chosen ASM concentration (RSD was found to be in the range from 3.1% to 4.5%). Accuracy and precision of the method were established by calculating ASM concentrations on the basis of measured peak current and calibration curve equation (Table 2). The validation parameters\(^{[30]} \) show a satisfactory performance of the presented method.

Effect of interferences

To check selectivity of the developed method influence of other commonly used pesticides (thiophanate-methyl, acephate, methidathion, aldicarb, metam, clothianidin, cyromazine, methamidophos and dodine) on ASM \( (C_{ASM} = 5 \times 10^{-8} \) mol L\(^{-1}\) peak current and potential was examined. The presence of clothianidin, dodine, methamidophos, and methidathion always hindered the peak related to the presence of investigated compound in the solution. Cyromazine, metam and thiophanate-methyl caused no interference up to concentrations 20-fold higher than the ASM concentration. The presence of acephate and aldicarb caused no change in the peak current at any investigated concentration level. We also studied the effect of the presence of common heavy metal ions. Zinc and nickel ions did not interfere. On the other hand, cadmium, copper, and lead cations caused distortions in the recorded peak current at concentrations 10-fold higher than the ASM concentration.

Analysis of river water, tap water and soil samples

The developed method for quantitative determination of ASM was successfully applied for ASM determination in spiked samples (river water, tap water and soil). Samples were prepared according to descriptions in the section “Preparation of solutions and sample treatment.” All the samples were investigated on the basis of standard addition method. The concentrations of standard additions were in the linear range of the recorded pesticide peak current. Some exemplary from all recorded voltammograms in the interference investigation are presented in Figure 5. In all the examined samples (river water, tap water and soil) interference or matrix influence was not observed. The data given in Table 3 show very good results of acibenzolar-s-methyl determination in the investigated samples of tap water and Bzura River water. Thereby,

![Fig. 4. SWAdSV curves of ASM, recorded in B-R buffer pH 2.2: C\( _{ASM} \) = 0 (1); 0.010 (2); 0.020 (3); 0.030 (4); 0.040 (5); 0.050 (6); and 0.060 \( \mu \)mol L\(^{-1}\) (7). The inset shows the corresponding calibration graph. Parameters: \( E_{acc} = -0.10 \) V, \( t_{acc} = 90 \) s, \( f = 150 \) Hz, \( E_{sw} = 130 \) mV, \( \Delta E = 8 \) mV, and equilibrium time \( t_{eq} = 10 \) s.](image-url)
developed method is sufficiently precise and accurate to determine ASM in investigated real samples.

**Conclusion**

The electrochemical study of ASM was described. Acibenzolar-s-methyl is electrochemically active not only at the renewable silver amalgam film electrode. It can be also more sensitively determined using HMDE, which was the basis for its quantitative determination conducted in this work. Verifying the analytical parameters of both acibenzolar determination methods, it can be seen that the one presented herein extends the linear concentration range down to $1 \times 10^{-8}$ mol L$^{-1}$. While the accuracy and precision remain at the same level. When the method was applied to natural spiked samples no significant interferences were observed.

The electrochemical behavior of ASM at HMDE assures a possibility to detect and analyze the pesticide content in real samples. It can also afford an insight in the adsorption processes occurring at the electrode surface. This paper demonstrates that ASM can be determined with square wave voltammetric technique in tap water, river water, and soil samples. The analysis procedure in natural samples requires only simple stages of sample preparation. With these experiments we have shown that the presented method offers a great opportunity for monitoring and is useful for environmental and food control of ASM using different types of working electrodes. The developed method has a better linear range for ASM determination ($1 \times 10^{-8} – 6 \times 10^{-8}$ mol L$^{-1}$) than the previous method employing a renewable silver amalgam electrode ($5 \times 10^{-8} – 3 \times 10^{-7}$ mol L$^{-1}$).

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**References**


