

Application of Copper Solid Amalgam Electrode for Determination of Fungicide Tebuconazole

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Tebuconazole (Teb) as a systemic fungicide amounts a potential risk for environment even in very low concentrations. The voltammetric behavior of Teb was investigated using the newly developed mercury meniscus-modified copper solid amalgam electrode (inner diameter 1.5 mm). Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were utilized for these purposes. The reaction mechanism was investigated using CV and elimination voltammetry with linear scan (EVLS). The optimum conditions for DPV determination of Teb were found in Britton-Robinson buffer/methanol (1:1, v/v) of pH 6.4, initial potential and accumulation potential $E_{in}=E_{acc}=+400$ mV vs. Ag/AgCl/3M KCl, scan rate 20 mV s⁻¹. Applying the prolonged accumulation time (60 s), the limit of detection 0.2 $\mu\text{mol L}^{-1}$ was reached. The applicability of the developed method for determination of Teb in two samples of real soil solutions was verified.

Keywords: Tebuconazole; Fungicide; Copper solid amalgam electrode; Voltammetry; Elimination voltammetry with linear scan; Soil solution

1. INTRODUCTION

The use of pesticides is a common practice in modern agriculture. At the present time, the trend is to reduce exposure to pesticides and to use safer alternatives. Several studies have estimated that less than 0.3% of the pesticide reaches its target pest. The remaining 99.7 % is released to the environment, representing a potential hazard for non-target organisms including humans. A significant proportion of

the pesticide frequently ends up in the soil where it undergoes biological and physicochemical transformations [1,2].

Azole fungicides (imidazoles and triazoles) are wide-spectrum antifungals effective in both preventing and curing fungal infections. Azole fungicides have the potential to affect the endocrine system of various species by interacting with steroidogenesis [3].

Tebuconazole (Teb), (RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)-pentan-3-ol) is a commonly used triazolic fungicide (Fig. 1). It is used to treat pathogenic fungi on plant in agriculture and on crops such as barley, wheat, and peanuts. Teb has a relatively high soil organic carbon-water binding coefficient and a half-life in soil of 49 – 610 days under aerobic conditions [1]. Major degradation products in soils include triazolic acid, hydroxytriazole, and triazolalanine [4]. Teb inhibits the sterol C-14 alpha-demethylation of 24-methylendihydrolanosterol, a cell membrane component in fungi, and decreases ergosterol biosynthesis, a key component of fungal cell membranes [5]. Frequent application of Teb leads to its accumulation in soils [6]. Teb may interact also with essential elements in soil (by this way can change its stability, toxicity, etc.), such as copper [7], and/or with hazardous metals such as cadmium [8]. There is a variety of copper intakes: from rainwater, volcanic activity, soil erosion, from human activities as agriculture with the use fungicides, metallurgical industry, and mining. After application, Teb can subsequently cause risks for soil ecosystems, groundwater, and surface water [8,9].

Various types of solid electrodes have been developed to replace the electrodes containing the liquid mercury, e.g., carbon paste electrodes [10,11], boron-doped diamond electrodes [11,12], solid composite electrodes [13-16]. Solid amalgam electrodes represent an intermediate step between mercury and solid working electrodes, e.g., [11,17-23]. They were first presented by Novotný and Yosypchuk in 2000 [24,25] and independently by Mikkelsen and Schroder in the same year [26]. The type presented by Mikkelsen et al. is based on dental amalgam [26] and the type introduced by Novotný et al. [24,25] is made from amalgam which is prepared by mixing metallic powders (Ag, Au, Cu, Ir etc.) with liquid mercury. The copper solid amalgam electrodes (CuSAEs) can be classified, according to the state of their surface, as polished (p-CuSAE; solid amalgam electrode which contains no liquid mercury and its surface was mechanically polished), film modified (MF-CuSAE, i.e., the p-CuSAE covered by mercury film) and mercury meniscus modified (m-CuSAE, i.e., the p-CuSAE covered by mercury meniscus) [27,28]. These electrodes combine advantages of mercury and solid working electrodes like high hydrogen overvoltage, wide range of working potentials, mechanical stability, simple preparation for analysis and possibility of electrochemical regeneration of the working surface [29,30]. The amalgam, mostly silver solid electrodes have been already successfully used in electrochemical analysis of many inorganic [17,31,32], organic [18-21,33,34] and bioactive compounds (e.g., peptides [22], DNA [35], folates [36-39] etc.). However, the copper amalgam electrodes have found their field of application as well (e.g., determination of inorganic ions (Cd^{2+} , Pb^{2+} , Mn^{2+}), DNA analysis [28], determination of triazines-based herbicides [29,30], cysteine and phytochelatin [40,41], etc.). The results achieved using amalgam electrodes are fully comparable with those achieved using other electrodes or techniques (e.g. [17]).

The application of m-CuSAE and p-CuSAE in voltammetric determination of tebuconazole has been discussed in the present paper. Furthermore, the optimum conditions of DPV determination of

Tebuconazole using both amalgam working electrodes, have been tested. This method has been developed to enable the environmentally important studies of transporting processes across the phospholipid (PL) cell membranes [42-45], i.e., it must enable determination of tebuconazole in extracellular as well as intracellular solutions. The electrochemical methods seem to be highly suitable for determination of plenty elements, inorganic as well as organic compounds under physiological conditions (e.g., [2,14,15,19,20,46-64]). Such transport is necessary, to start its role in plants or in human body. Generally, each particle, molecule, compound, etc. (electrically charged or uncharged) participating further in metabolic processes must be transported across the cell membranes [42,43,45,65-77]. Similar processes are realized into and out of any sub cellular structure, which are in some cases formed from bilayers too. Detail elucidation of membrane transport mechanisms plays a key role and is prerequisite for understanding the distribution of pollutants in real cells of more complex organisms (leaves, roots or the whole plants, animals or men) and for their possible control in the future [43]. Simultaneously, the development of highly sophisticated analytical devices for these purposes must be realized (e.g., [78-80]).

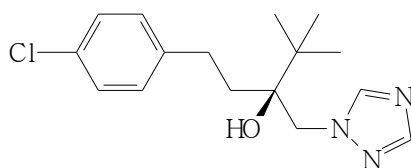


Figure 1. Structure of tebuconazole.

2. EXPERIMENTAL PART

2.1. Reagents

The stock solution of Teb (Sigma-Aldrich, Czech Republic) with concentration of $0.0162 \text{ mol L}^{-1}$ was prepared by dissolving 260 mg in 50 ml methanol (Penta-Svec, Czech Republic) and then stored in dark and cold. The analyzed solutions were prepared daily by its dilution. Britton–Robinson (B–R) buffers of pH values from 2 to 12 were prepared by mixing the proper amounts of alkaline component of 0.2 mol L^{-1} NaOH (Lachema, Czech Republic) and of acidic components consisting of 0.04 mol L^{-1} H_3PO_4 , 0.04 mol L^{-1} H_3BO_3 and 0.04 mol L^{-1} CH_3COOH (all Lachema, Czech Republic). All solutions were prepared in methanol. All the other chemicals used were of analytical grade. For all the measurements, deionized water from Milli-Q-Gradient, Millipore, Prague, Czech Republic (conductivity $< 0.05 \text{ }\mu\text{S.cm}^{-1}$) was used.

2.2. Instrumentation

Voltammetric measurements were performed with the computer controlled Eco-Tribo Polarograph (Polaro-Sensors, Prague, Czech Republic), equipped by MultiElChem 2.3 software for

Windows 7 (J. Heyrovský Institute of Physical Chemistry of the AS CR, v.v.i., Czech Republic). The working surface of the used m-CuSAE amounted to 2.1 mm² (the repeatability < ±5 %).

Ag/AgCl/3M KCl was used as a reference and platinum wire as an auxiliary electrode (both from Elektrochemické detektory, Turnov, Czech Republic) in the described experiments. The measurements were performed at laboratory temperature (23 ± 2 °C). Oxygen was removed from the measured solutions by bubbling nitrogen (purity class 4.6; Messer Technogas, Prague, Czech Republic) for 5 minutes. The values of pH were measured using pH-meter Jenway 3505 (Bibby Scientific Limited, UK).

2.3. Procedures

2.3.1. Voltammetric Measurements

Cyclic voltammetry (CV) and direct current voltammetry (DCV) were used in the first set of studies of voltammetric behavior of Teb on the m-CuSAE. Dependences of the obtained signals on pH and on scan rate have been recorded. Differential pulse voltammetry (DPV) was applied for most measurements with the initial potential $E_{in} = +400$ mV and the final potential $E_{fin} = -2000$ mV. The potential of accumulation was optimized as $E_{acc} = +400$ mV. The BR buffer/methanol (1:1, v/v) of pH 6.4 were used as supporting electrolyte. Nitrogen was passed through the bottle with methanol/distilled water (1:1, v/v), subsequently was passed through the solution with sample for a period of 10 min prior to the measurements and then nitrogen atmosphere was maintained above the solution in the cell. The heights of DPV peaks as well as of DCV waves (in the following text the term “peaks” was used for both techniques) were evaluated from the straight line connecting the minima before and after the peak (tangent to the curve joining the beginning and the end of a given peak).

The minimum number of standard additions was 5. Each measurement of the current signals was realized in cycles composed from three times repeated records under identical conditions. In some cases, the first curve differed partly from others. Such curves were omitted from evaluations. The achieved results were evaluated according to [81] and using QC Expert software (Trilobyte, Czech Republic) and Excel (Microsoft Inc. Czech Republic). The limits of decision (LC), detection (LD) and quantification (LQ) and the parameters of the calibration curves (e.g., slope, intercept) were calculated as described in [81].

2.3.2. Preparation of mercury meniscus-modified copper solid amalgam electrode

The working electrode was m-CuSAE with the disc diameter 1.5 mm. The electrode consisted of drawn-out glass tube, the bore of which near the tip was filled with copper solid amalgam which was connected to an electric contact. Afterwards, it was immersed into a small volume of liquid mercury and agitated for 15 seconds to form mercury meniscus-modified copper solid amalgam electrode. Mercury meniscus is created on the solid amalgam surface by this process. The electrode surface was repeated once a week in case of daily measurements. Before starting the work as well as after every pause longer than one hour, the electrode surface was activated in the solution of BR buffer/methanol (1:1, v/v) by applying -2200 mV vs. Ag/AgCl/KCl (3 M) electrode for 300 s while the

solution was stirred. The regeneration step of the electrode surface was inserted into the “design” utility of particular measuring methods used in the computer software MultiElChem 2.3. It consisted in setting of the fixed regeneration potential $E_{reg} = -2200$ mV for 300 s.

2.3.3. Elimination voltammetry with linear scan

To gain more detailed information about the electrochemical processes on the electrode surface, to increase the current sensitivity, and to obtain the benefits of higher resolution, the elimination voltammetry with linear scan (EVLS) was applied. This method is based on the mathematical data processing (Linear Sweep Voltammetry (LSV)), and on the different dependence of some particular currents on various scan rates [82-86]. In EVLS, the fact is that the total current is the sum of particular current contributions, such as the charging current I_c , the diffusion controlled current I_d , the kinetic current I_k and the s.c. irreversible current I_{ir} .

$$I = \sum I_j = I_k + I_c + I_d + I_{ir} + \dots \quad (1)$$

Each particular current can be expressed as:

$$I_j = W_j(v) \cdot Y_j(E) = v^x Y_j(E), \quad (2)$$

where I_j is a particular current, $W_j(v)$ is a function of the scan rate, $Y_j(E)$ is a function of the potential, and v is the scan rate [39,87,88]. As all n particular currents exhibit their specific dependence on the scan rate, all measurements must be done with n different scan rates. One scan rate is selected as reference rate (v_{ref}), the others are chosen to be multiples this reference scan rate, and the others are compared with it (v/v_{ref}) [82-84,86]. On the basis of these postulates the so-called elimination coefficients for elimination equations were calculated while only one current was conserved and the others were eliminated [82,86]. EVLS gives us the possibility of rapid analytical determination and provides to us more information about the electrode processes and, therefore, we can look much deeper into the process with EVLS (e.g., [14,20,37-39,50,85,86,89-92]).

2.3.4. Preparation and analysis of real sample solutions

The soil solutions were obtained from pot experiments realized with 5 pieces of alpine penny-cresses which were grown in a pot with 5 kg of fluvisol soil from the Litavka River alluvium (Czech Republic). The soil solutions were sampled using nylon suction cups (DI Gottfried Wieshammer, Wien, Austria) after 24 h saturation by deionized water to 100 % of water holding capacity [93-95]. This process corresponded to the filtration on filter with porosity 0.2 μm . The soil solutions were spiked with tebuconazole to the final concentration 2.5 and 750 $\mu\text{mol L}^{-1}$. These levels correspond with concentrations of Teb in soil samples under real conditions. It was stored in dark and cold (4 °C) until analysis.

The samples of soil solutions were filtrated on the suction cup. 2 mL of the filtrate was added to the 18 mL of the BR buffer/methanol (1:1, v/v) mixture of pH 6.4 and this solution was analyzed under identical optimized conditions as the above mentioned model samples.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behavior of tebuconazole

3.1.1. Dependence on pH

Firstly, the optimum composition of the supporting electrolyte had to be investigated. Teb is partly soluble in water and very well soluble in alcohols. Because the realized experiments had been realized in broad concentration range, the compound was dissolved in methanol and water was added. The light opalescence and precipitate were observed in stock solution in the case of 10 % (v/v) methanol solution. On the other hand, the current signals were partly deformed and decreased in case of more than 90% (v/v) concentration of methanol. Finally, the 50 % (v/v) concentration of methanol in supporting electrolyte was found as the most suitable.

The dependence of voltammetric behavior of Teb on pH was studied using CV on the m-CuSAE. The used concentration of Teb was $32 \mu\text{mol L}^{-1}$. The tested pH range was from pH=2 to pH=12. The peak which represented complex of Teb and copper was not observed either in strongly alkaline pH or in strongly acidic pH. The signals in acidic as well as basic in solutions were not so pronounced, their reproducibility was very poor, and the peak currents decreased in time.

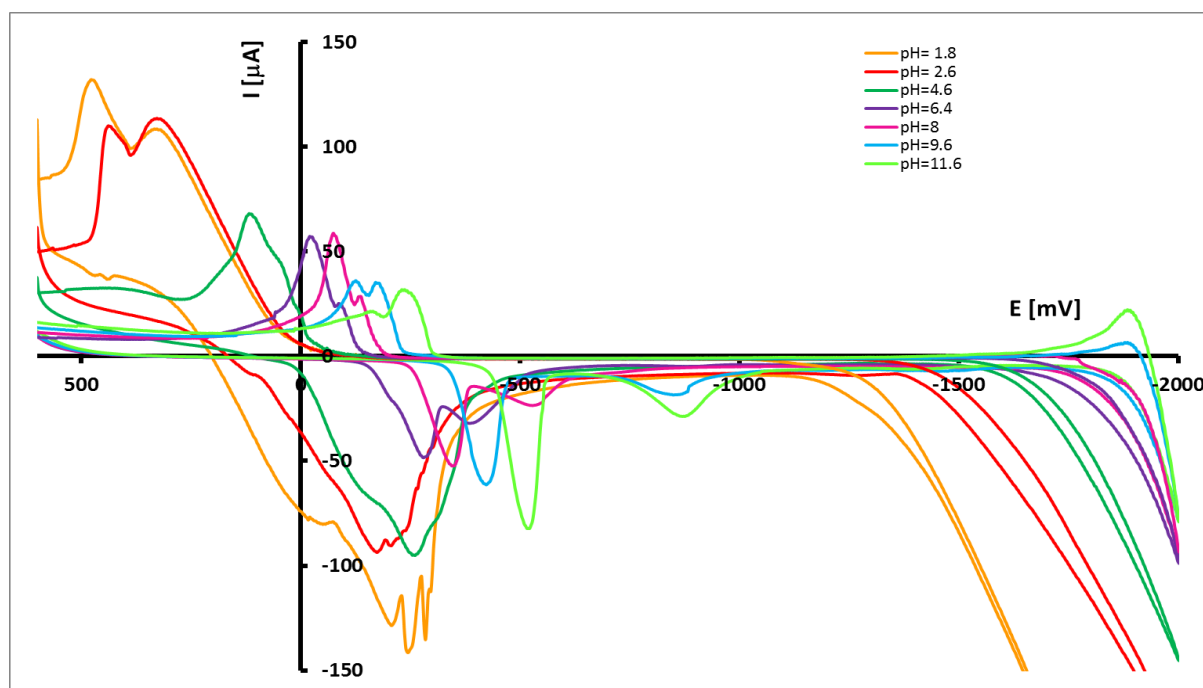


Figure 2. Dependences on pH. Cyclic voltammetry measurements on m-CuSAE in BR buffer/methanol (1:1, v/v), concentration of Teb (c_{Teb}) $32 \mu\text{mol L}^{-1}$; $E_{in} = 600 \text{ mV}$, $E_{fin} = -2000 \text{ mV}$, $v = 100 \text{ mV s}^{-1}$.

The best pronounced and the best reproducible cathodic signals were registered in media of pH about 6.4 and therefore, the BR buffer/methanol (1:1, v/v) of this pH was used for all following measurements on this electrode. Two overlapping reduction (cathodic) peaks were observed in

medium of pH 6.4 ($E_{P1} = -292$ mV, $E_{P2} = -484$ mV) without addition of Teb (i.e. in supporting electrolyte only). These peaks demonstrate different forms of copper reduction. After addition of Teb, three reduction peaks were registered ($E_{P1}' = -206$ mV, $E_{P2}' = -281$ mV, $E_{P3}' = -385$ mV, Fig. 2). A complex of Teb with copper was represented as the third peak ($E_{P1}' = -206$ mV). Positions of peaks were shifted to more negative potentials with increasing pH values.

Two oxidation (anodic) peaks were registered in presence as well as in absence of Teb ($E_{P1}'' = -21$ mV, $E_{P2}'' = -101$ mV) at pH 6.4. These peaks demonstrate different forms of copper oxidation and no any signal belonged to Teb presence. Positions of all peaks were shifted to more positive potentials with increasing value of pH.

The peaks were not separated completely at any pH. Therefore, it seemed to be more suitable to evaluate the peak area (charge) instead of peak current.

3.1.2. Dependence on scan rate

The dependences of current responses of Teb on scan rate obtained with m-CuSAE were investigated using cyclic voltammetry (CV). These dependences are shown in Fig. 3. The tested concentration of Teb was $47 \mu\text{mol L}^{-1}$. The dependences on the scan rate were linear for oxidation as well as for reduction peaks in the range of scan rates from 10 to 160 mV s^{-1} . The fact that the dependence of the cathodic peak current (I_c) on the scan rate was linear (Eq. 3) confirms that the process is controlled by adsorption.

$$I_c = (98.3 \pm 2.2) \cdot v [\text{mV s}^{-1}]; r = 0.9999 \quad (3)$$

The value of scan rate, which was selected as the most suitable for DPV experiments, was 20 mV s^{-1} .

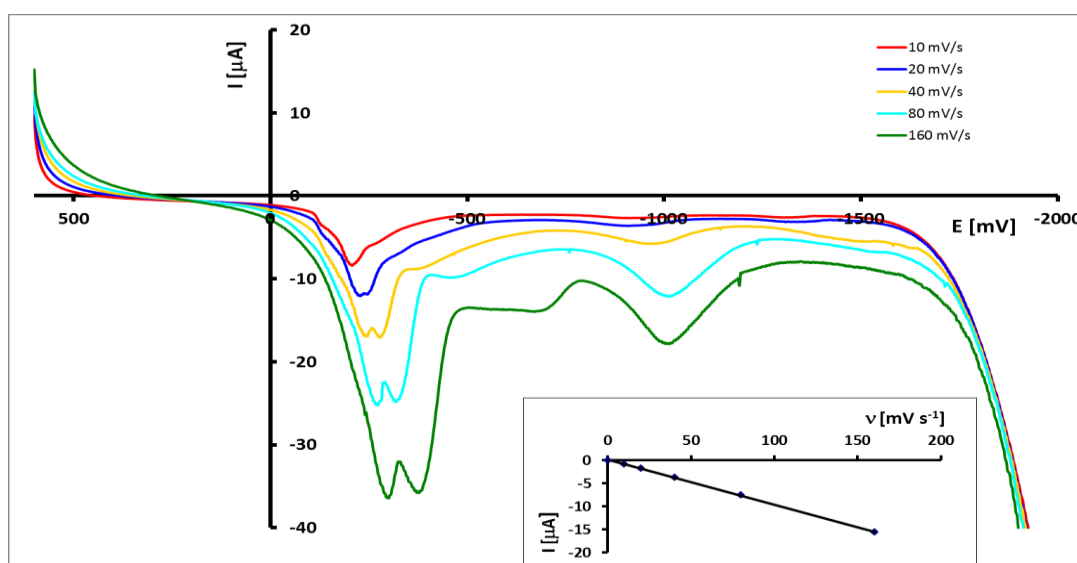


Figure 3. Dependences on scan rate. CV measurements on m-CuSAE in BR buffer/methanol (1:1, v/v), pH = 6.4, $c_{Teb} = 47 \mu\text{mol L}^{-1}$; $E_{in} = 600$ mV, $E_{fin} = -2000$ mV. Inset: Dependence of the cathodic peak currents on the scan rates under identical conditions.

3.1.3. The effect of scan rate and EVLS

EVLS was applied for analysis of processes occurring on the surface of the m-CuSAE. This technique enabled us to characterize the particular processes occurring during the cathodic and anodic scans. We evaluated the curves recorded with scan rates from 10 mV s^{-1} to 160 mV s^{-1} (divided in two sets: from 10 mV s^{-1} to 80 mV s^{-1} and from 20 mV s^{-1} to 160 mV s^{-1}) using this technique.

We separated at least six signals belonging to the particular processes, which were covered in wide peaks in DC voltammograms in the cathodic polarization (Fig. 4). Because the Cu^{2+} are released from copper amalgam at positive potentials, we can suppose that the two most positive signals (at about -90 mV and about -170 mV) belonged to the formation of either two different complexes of Cu^{2+} ions with Teb or of complexes of Cu^{2+} ions and Cu^+ ions with this ligand. In correspondence with this hypothesis, two most negative signals (at about -400 mV and about -500 mV – peaks E and F) belonged to the decomposition of these complexes (the copper cations are reduced back to the metallic copper and copper amalgam is formed). The signals at about -250 mV and about -290 mV which could be registered in the supporting electrolyte can be explained as peaks corresponding to the reduction of Cu^{2+} to Cu^+ and of Cu^+ to Cu^0 .

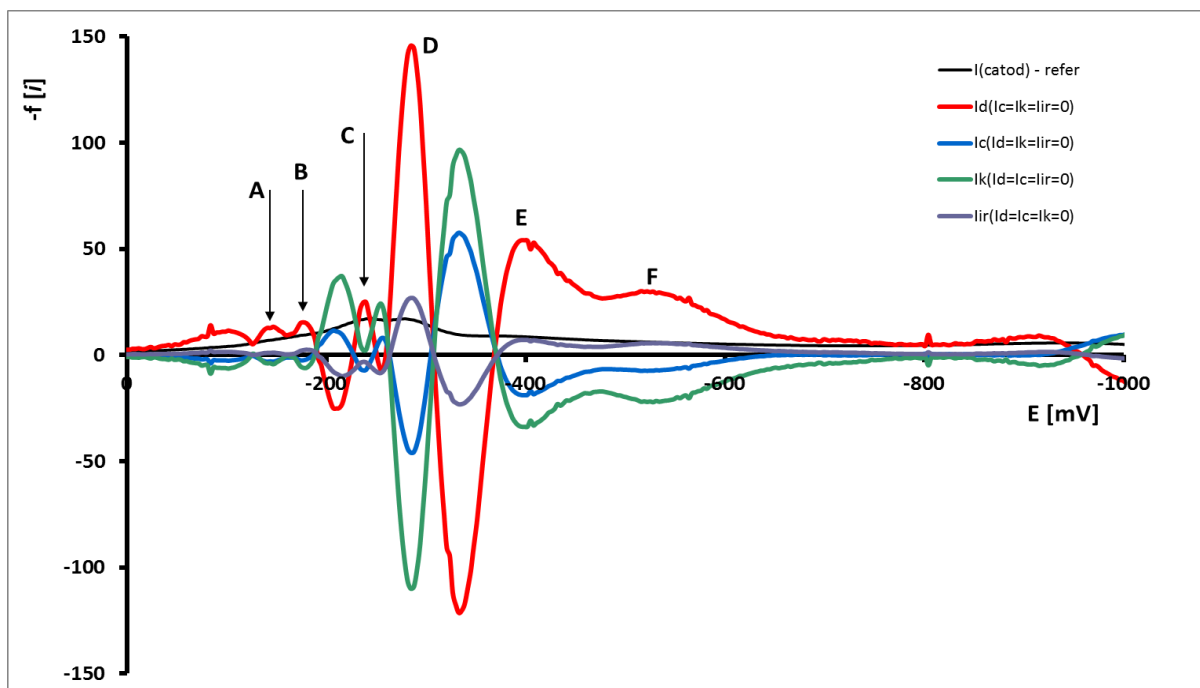


Figure 4. EVLS on m-CuSAE in BR buffer/methanol (1:1, v/v), $\text{pH} = 6.4$, $c_{\text{Teb}} = 47 \mu\text{mol L}^{-1}$; $E_{\text{in}} = 600 \text{ mV}$, $E_{\text{fin}} = -1000 \text{ mV}$. *Curves:* Black: Cathodic current recorded under reference scan rate (40 mV s^{-1}). Red: Diffusion elimination current function; Green: Kinetic elimination current function; Blue: Charging (Capacitance) elimination current function; Violet: Irreversible elimination current function. *Peaks:* A: B: elimination peaks belonging to the formation of two different complexes of $\text{Cu}^{2+}/\text{Cu}^+$ ions with Teb, C: elimination reduction peak $\text{Cu}^{2+} \rightarrow \text{Cu}^+$; D: elimination reduction peak of $\text{Cu}^+ \rightarrow \text{Cu}^0$, E, F: elimination peaks belonging to the decomposition of Teb complexes with $\text{Cu}^{2+}/\text{Cu}^+$.

According to the shapes of the elimination curves conserving particularly the processes controlled by diffusion, adsorption, kinetically controlled, and irreversible currents [83,86], and according to the mutual positions of peaks and counter-peaks, it is possible to conclude that both reduction steps, i.e., $\text{Cu}^{2+} \rightarrow \text{Cu}^+$ and $\text{Cu}^+ \rightarrow \text{Cu}^0$, are diffusion controlled processes in adsorbed state. Similarly, both diffusion controlled processes of formation of Teb complexes and the decompositions of Teb complexes can be characterized as diffusion controlled in adsorbed state too. These conclusions are in fair correspondence with those achieved using evaluation of scan rate dependences mentioned above. The counter-peaks were more pronounced by application of higher set of scan rates.

The peak positions were mutually too close to evaluate charge transfer coefficients and number of transfer coefficients (according to [82]) reliably.

3.2. Determination of tebuconazole in model samples

The optimum parameters for DPV determinations of Teb were found on m-CuSAE. Influence of three parameters of DPV on the recorded responses of Teb was tested: initial potential (E_{in}), potential of accumulation (E_{acc}) and time of accumulation (t_{acc}). It was observed that the initial potential did not affect the peak height substantially. In all following experiments, the value of $E_{in} + 400$ mV was chosen. The optimum scan rate 20 mV s^{-1} was applied.

In correspondence with the above described results, Teb can be adsorbed on the electrode surface and therefore it can be assumed that the increasing t_{acc} can increase the recorded peak currents using m-CuSAE. The influence of accumulation potential was tested from +800 to 0 mV vs. Ag/AgCl(3M KCl). Similarly as in the case of E_{in} , it can be concluded that the value of E_{acc} does not affect the observed voltammetric signal significantly. In spite of it, the highest current responses were registered for m-CuSAE at $E_{acc} = +400$ mV.

Therefore, these values of E_{acc} were applied for the analyte accumulation on the electrode surface before signal registrations. The suitable time of accumulation depended on concentration of Teb in the analyzed medium. The range of t_{acc} in which the signal of Teb increased linearly, was tested on different concentration levels from $0.162 \mu\text{mol L}^{-1}$ to $16 \mu\text{mol L}^{-1}$ on m-CuSAE.

E.g., the time dependence of charge for $c_{Teb} = 4.85 \mu\text{mol L}^{-1}$ was linear from t_{acc} in the range from 0 to 60 s (Fig. 5) and can be described by Eq. 4 with correlation coefficient $R = 0.9952$.

$$Q [\mu\text{C}] = (57.71 \pm 0.42) t_{acc} [\text{s}] + (0.111 \pm 0.012) \quad (4)$$

The found optimum parameters of DPV were applied for Teb determination in a model solution. The relative standard deviations (RSD) of the 11 times repeated measurement of Teb solution (concentration $32 \mu\text{mol L}^{-1}$) amounted to 3.74 % on the same m-CuSAE.

The reproducibility of Teb determination using m-CuSAE was tested by five times repeated analysis of Teb at three different concentration levels. The method of standard addition was applied for their evaluation. The results obtained are summarized in Table 1. All calculated values of relative standard deviations ($\text{RSD}_D(5)$) are lower than 4.8 % which confirms high reproducibility of repeated determinations.

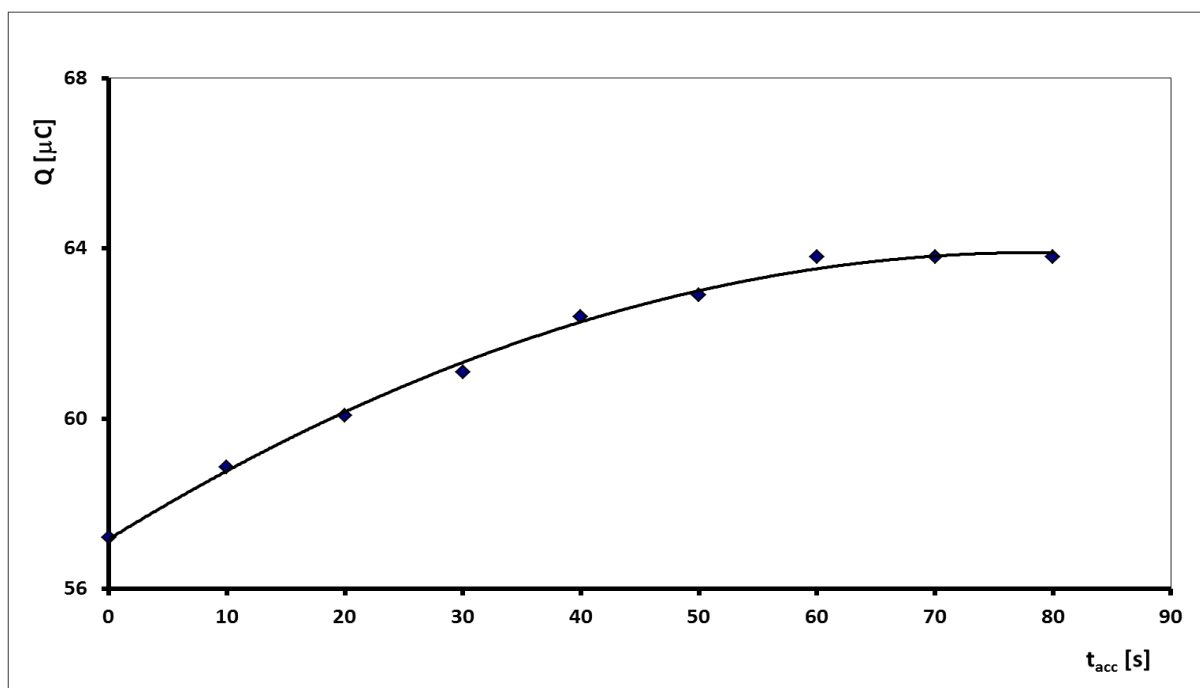


Figure 5. Dependence of the peak current on the time of accumulation. DPV on m-CuSAE in BR buffer/methanol (1:1, v/v), pH = 6.4, $c_{Teb} = 4.85 \mu\text{mol L}^{-1}$; $E_{in} = 400 \text{ mV}$, $E_{fin} = -2000 \text{ mV}$, $v = 20 \text{ mV s}^{-1}$, $E_{acc} = 400 \text{ mV}$.

Table 1. Comparison of added and found amounts of Teb determined using m-CuSAE.

Added [$\mu\text{mol L}^{-1}$]	Found* [$\mu\text{mol L}^{-1}$]	RSD _{D(5)} [%]
0.2	0.212±0.012	4.5 %
0.5	0.492±0.010	1.7 %
1.0	0.981±0.026	2.1 %
10.0	10.11±0.30	2.4 %

* average of 5 determinations

The linear dynamic range (LDR) corresponding to the Teb peak on m-CuSAE, achieved using $t_{acc} = 60 \text{ s}$ as the longest accumulation time, was recorded from 0.1 to 1.5 $\mu\text{mol L}^{-1}$ ($t_{acc} = 60 \text{ s}$), which can be described following Eq. 5 with $R = 0.992$, can serve as an example (Fig. 6).

$$Q [\mu\text{C}] = (16.7 \pm 1.4) c [\mu\text{mol.L}^{-1}] + (23.3 \pm 1.3) \quad (5)$$

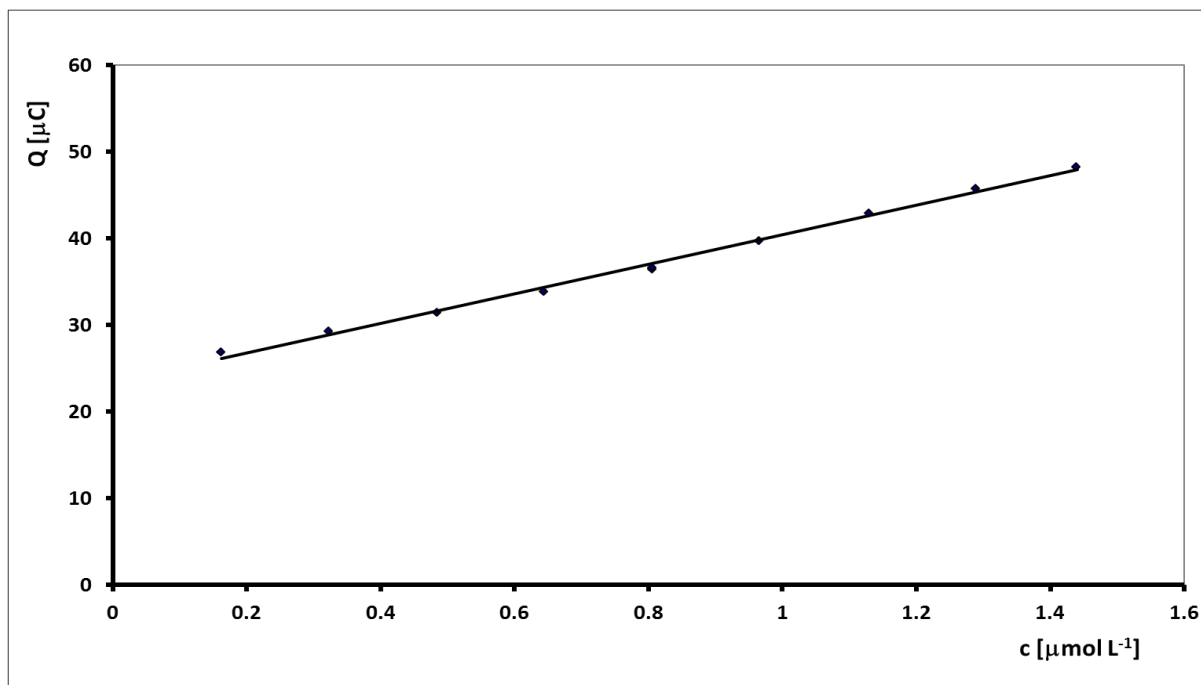


Figure 6. Dependence of Teb concentration on signal charge. DPV on m-CuSAE in BR buffer/methanol (1:1, v/v), pH = 6.4, $c_{Teb} = 4.85 \mu\text{mol L}^{-1}$; $E_{in} = 400 \text{ mV}$, $E_{fin} = -2000 \text{ mV}$, $v = 20 \text{ mV s}^{-1}$, $E_{acc} = 400 \text{ mV}$, $t_{acc} = 60 \text{ s}$.

Other chemometric parameters were calculated using direct method of signal according to IUPAC [81]: limit of decision (CC): $0.11 \mu\text{mol L}^{-1}$, LOD $0.21 \mu\text{mol L}^{-1}$, and limit of quantification (LOQ) $0.30 \mu\text{mol L}^{-1}$. These parameters were achieved after 60 s long accumulation time. Increase of accumulation time for amalgam electrodes caused decrease of linearity of concentration dependences. It is possible to suppose that these reached chemometric parameters in model samples seem to be sufficient for Teb determination in real samples (e.g., soil solutions).

The validation parameters, calculated from 5 repeated determinations realized within one day are shown in Table 2. The validation parameters, evaluated for ten repeated determinations realized within one week, are summarized in Table 2 too.

Table 2. Validation parameters of Teb determination (found vs. determined) using m-CuSAE calculated from 4 repeated determinations realized within one day and within one week ($\alpha = 0.05$).

	One day	S.D.	One week	S.D.
Intercept	i.s. (0.0012±0.0065)	0.00094	i.s. (0.02±0.18)	0.0036
Slope	0.9977±0.0035	0.0028	1.03±0.13	0.0024
Slope includes the value 1	Yes		Yes	
Correlation coefficient	0.988		0.986	
Calculated level of noise	0.0021		0.059	

i.s. – statistically insignificant, S.D. standard deviation

3.3. Real sample analysis

Two samples of real soil solutions were analyzed under identical optimized conditions as the model samples. To eliminate the interferences of surface active substances and of other compounds likely to be present in the analyzed material, the standard addition method was used for the analysis of Teb in real samples. Each curve was repeated 3 times and 5 standard additions were added. The determination was 5 times repeated.

It was found that the concentration of Teb in more concentrated real soil solution sample amounted to $721 \pm 41 \mu\text{mol L}^{-1}$ and the $\text{RSD}_D(5)$ 4.6 % was achieved (Table 3). By the EC/ESI-MS analysis of the same soil solution sample, the concentration $798 \pm 50 \mu\text{mol L}^{-1}$ with $\text{RSD}_D(5)$ 5.0 % was found [96]. Both values were in correspondence with the prepared concentration of the spiked soil solution $750 \mu\text{mol L}^{-1}$.

Similar equivalency was found among real concentration ($2.50 \mu\text{mol L}^{-1}$), voltammetrically determined concentration ($2.56 \pm 0.14 \mu\text{mol L}^{-1}$) and concentration achieved using ESI-MS ($2.44 \pm 0.15 \mu\text{mol L}^{-1}$) ($p \leq 0.05$). Similarly as in the case of more concentrated real sample, $\text{RSD}_D(5)$ was below 5 %.

Moreover, it is possible to conclude that the confidence intervals of all results in both cases overlapped on the significance level 0.05. Therefore, it is possible to conclude that the results achieved by both methods are equivalent (Table 3).

Table 3. Comparison of added and found amounts of Teb determined using m-CuSAE ($\alpha = 0.05$).

Soil solution [$\mu\text{mol L}^{-1}$]	Found m-CuSAE* [$\mu\text{mol L}^{-1}$]	$\text{RSD}_D(5)$ [%]	Found ESI-MS* [$\mu\text{mol L}^{-1}$]
750	721 ± 41	4.6 %	798 ± 50
2.50	2.56 ± 0.14	4.9 %	2.44 ± 0.15

* average of 5 determinations

4. CONCLUSIONS

The voltammetric behavior of Teb using m-CuSAE has been investigated and the analytical method for the determination of Teb in model solutions and afterwards in real sample was described in the present paper.

It can be supposed that Teb forms the complexes with Cu^+ as well as with Cu^{2+} at positive potential area and that these are decomposed in more negative potential area on the surface of m-CuSAE.

Britton-Robinson buffer/methanol (1:1, v/v) of pH 6.4 was found as the most suitable supporting electrolyte for Teb determination. DPV with optimized experimental conditions was first applied for determination of Teb in model solutions. LOD was calculated as $0.2 \mu\text{mol L}^{-1}$. The

acceptable reproducibility of repeated measurements ($RSD_{m-CuSAE} = 3.74\%$) as well as the reproducibility of complete determinations ($RSD_{m-CuSAE} < 4.5\%$) were achieved. The applicability of m-CuSAE for DPV determination of Teb was verified by analysis of two real samples of soil solutions. It was proved that the results achieved on tested amalgam electrode were equal to those achieved using EC/ESI-MS ($p \leq 0.05$).

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