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Trace Determination of *Carbendazim* Fungicide Using Adsorptive Stripping Voltammetry with a Carbon Paste Electrode Containing Tricresyl Phosphate

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In this study, a carbon paste electrode based on tricresyl phosphate (TCP-CPE) as a binder has been applied to the voltammetric characterization and determination of *Carbendazim* fungicide (methyl-*1H*-benzimidazol-2-yl-carbamate, MBC). The pH effect (in Britton-Robinson buffers, pH 2.0-8.0), as well as the presence of 2-hydroxypropyl-β-cyclodextrin (HPCD) on the electrochemical behavior of MBC were investigated. In the potential range of interest, the oxidation signal was observed with the overall shape strongly dependent upon pH and exhibiting the most favorable signal-to-noise ratio in mild acidic solutions (pH 4.0). This has indicated that also protons are involved in the electrode transformation of MBC. Furthermore, it was confirmed that addition of $3.6 \cdot 10^{-5}$ mol L^{-1} HPCD significantly enhanced the sensitivity towards the target analyte. The experimental conditions optimised for the determination of MBC in the differential pulse adsorptive stripping voltammetric mode (DPAdSV) were as follows: initial potential, -0.10 V vs. Ag/AgCl; final potential, +1.30 V; accumulation potential, -0.35 V; accumulation time, 120 s, and the scan rate, 100 mV s^{-1}. The method developed offers linearity in the concentration range of $5.0 \cdot 10^{-7}$ – $1.0 \cdot 10^{-5}$ mol L^{-1} MBC, with r = 0.995 and the limit of detection of about $3.0 \cdot 10^{-7}$ mol L^{-1}. In a model sample of spiked river water, the recovery rate achieved was 101.9 % (at the concentrations from $1.0 \cdot 10^{-6}$ to $3.0 \cdot 10^{-6}$ mol L^{-1} MBC), suggesting one that the procedure can be applied in analysis of real samples.

Keywords: Adsorptive stripping voltammetry, Carbon paste electrode, *Carbendazim*, Fungicide, Determination, River water

1. INTRODUCTION

Carbendazim (methyl-*1H*-benzo-[d]-imidazol-2-yl-carbamate, MBC), is a widely used broad-spectrum benzimidazole fungicide playing an important role in plant disease control as the systemic [1]. Typically, MBC is applied to control a broad spectrum of diseases on arable crops (e.g. cereals, oilseed rape), fruits and vegetables, usable is also in post-harvest food storage and as a seed pre-planting treatment [1]. Its function is inhibition of the fungi growth; reportedly [2], by interfering with spindle formation at mitosis (i.e. a cell division). MBC has extensive applications worldwide, with the global market worth over $200 million, equivalent to over 12,000 tons active ingredient [3]. Its half-life is 8-32 days in soils and 2-25 months in water [4]; depending on the temperature and pH. Finally, MBC is a metabolite of *Benomyl* and *Methilthiophanate*; both representing other systemic fungicides from the family of benzimidazoles [5]. Degradation and toxic effects of MBC in humans and animals have been recently reviewed [6]. As a widely used fungicide, it has demanded research on developing sensitive and rapid analytical methods for monitoring it in soil, water samples, marketed fruits, fruit juice concentrates, and vegetables. The ANVISA, a Brazilian regulatory agency, sets a limit of 0.02 mg kg^{-1} as the human acceptable daily intake of MBC [7].

A wide palette of analytical methods has already been applied to the analysis of MBC; most of them being based on chromatographic techniques [8-13]. In case of gas chromatography, benzimidazole-based fungicides cannot be analyzed directly due to their polar and thermolabile nature. Thus, HPLC is preferred; often, with diode-array (DA [8-10]), fluorimetric [11], or mass spectrometric detectors [12,13]. Also, capillary electrophoresis (CE) coupled with DAD was employed in the determination of MBC [14]. However, analyses by HPLC or CE usually require highly sophisticated instrumentation and the respective procedures may be time-consuming. Thus, one can choose some alternate determinations, such as those based on UV/Vis spectrophotometry [15], fluorimetry [16,17], immunoassays [18], or voltammetric measurements [19-27].

Regarding the last named technique, the corresponding methods — combinable with a large variety of electrodes or even special detection systems [28-31] — are often simpler, rapid, and inexpensive, offering still sufficient sensitivity and selectivity for a large-scale monitoring of electrochemically active environmental pollutants [30]. As found out, already with the mercury electrodes, MBC catalyzes the reduction of Co(II) in the mixture of sodium-barbital, nitric-acid, sodium-chloride media, giving rise to a catalytic effect utilizable for voltammetric determination of this fungicide [25]. Carbon based electrodes have been shown applicable as well; namely, the glassy carbon electrode modified with carbon nanotubes (CNTs-GCE, [23]), carbon fiber ultramicroelectrode [24], and carbon nanotubes-polymeric Methyl-red film modified electrodes [22]; all having been capable to oxidise MBC. Moreover, there were clay [26] and polypyrrole [27] modified GCEs employed in the electrochemical stripping analysis mode, as well as a carbon paste electrode (CPE) based on graphite powder and silicone-based pasting liquid applicable as a trace-level sensor for *Benomyl* [20] and MBC [21]. By using this electrode, the one-step separation employing specific adsorption of the target organic substance on the electrode surface had been involved, allowing one to analyze voltammetrically the trace level of *Benomyl* or MBC [20,21].

Among the electrodes of choice, the above-mentioned CPEs offer a number of advantageous features [28-32], such as simple preparation (often in labs in a wide palette of various configurations, including quite unusual mixtures), minimal cost, favorable signal-to-noise characteristics (in both faradic and non-faradic measurements), unique surface characteristics, and mainly – almost unlimited possibilities of being chemically and biologically modified. This is also the case of some "special" carbon pastes, such as the mixture of graphite powder with liquid tricresyl-phoshate, introduced into electrochemistry as the "TCP-CPE" type in the early 1990s (see e.g. [33,34]). Regarding organic environmental pollutants, this rather atypical CPE has already been tested for the determination of 6-benzylaminopurine (a plant hormone [35]), neonicotinoid insecticides [36-40], and *Linuron* (a herbicide from the family of phenylurea [41]).

With respect to MBC, the applicability of the TCP-CPE is for the first time reported herein and the aim of this work was to find the optimal type of CPE for the determination of MBC, when comparing three different carbon paste mixtures based on silicon oil, paraffin oil, and tricresyl-phosphate. In parallel, the effect of 2-hydroxypropyl-β-cyclodextrin (HPCD) as potentially effective modifier was studied in detail, revealing its particular adsorption capabilities as a ligand [42,43], which could be utilised for enhancing the sensitivity of the method developed. The resultant procedure, employing differential pulse adsorptive stripping voltammetry (DPAdSV), was then successfully tested in determinations of the target fungicide at the trace level in samples of spiked river water.

2. MATERIALS AND METHODS

2.1. Chemicals and Solutions

MBC (99% purity) was obtained from Fitofarmacija a.d. (Zemun, Serbia); all other chemicals being purchased from Merck unless stated otherwise. The MBC stock solution was made 0.01 mol L^{-1} in concentration by dissolving this fungicide in N,N-dimethylformamide (Sigma-Aldrich,) and kept in dark at -4 ^{o}C. As the supporting electrolytes, Britton-Robinson buffer solutions of different pH (between 2.0 and 8.0) were prepared by mixing solutions 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 and adjusting pH by adding suitable amounts of 0.2 mol L^{-1} NaOH. Acetate buffer pH 4.0 was made from 0.04 mol $L^{-1}CH_3COOH$ and 0.2 mol L^{-1} NaOH. All other reagents were of analytical reagent grade and solutions were prepared in doubly distilled water. For the preparation of the mobile phase in HPLC experiments, acetonitrile (Sigma-Aldrich) and doubly deionised water were used. The water sample was collected from Elbe river (in Pardubice, Czech Rep.) and stored at *ca.* 4 °C for one week before analysis. The agent tested and then used for modification, 2-hydroxypropyl-β-cyclodextrin (HPCD), was of analytical grade and purchased from Sigma-Aldrich.

2.2. Apparatus

Voltammetric experiments were performed using an Autolab electrochemical analyzer (model "PGSTAT-12", Ecochemie / Metrohm) operated *via* the GPES 4.9 software (the same manufacturer). The conventional three-electrode configuration with different types of the working CPE, based on

silicon oil (SO), paraffin/mineral oil (MO) or tricresyl-phosphate (TCP) liquid binders, was employed throughout the work. An Ag/AgCl/3 mol L^{-1} KCl electrode (Radelkis, Hungary) and a Pt-plate served as the reference and auxiliary electrode, respectively. In studies on the electrode oxidation of MBC, a Pt-disc in the rotating disc electrode configuration was also used (Pt-RDE, with diameter of 2 mm).

In electrochemical stripping measurements, a magnetic stirrer was employed agitated at approx. 300 rpm. All electrochemical experiments were carried out in a conventional voltammetric cell (with operating volume of 20 ml) at room temperature (23±1 ^{o}C).

The reference HPLC measurements were performed on a liquid chromatograph (model "1100"; Agilent Technologies Inc., USA), Zorbax Eclipse XDB-C18 (250 mm × 4.6 mm, 3.5 μm) column when using DAD as the detection unit of choice.

2.3. Procedures

Preparation of Carbon Paste Electrodes. For comparative purposes, three different carbon pastes were prepared by intimate hand-mixing of graphite powder (product "CR 5"; Lučební závody Kolín, Czech Republic) with (1) tricresyl-phosphate (TCP, mixture of isomers; Sigma-Aldrich), (2) paraffin oil (Sigma-Aldrich), or (3) highly viscous silicone oil ("Lukooil, MV 8,000"; Lučební Závody, Kolín, Czech Republic); the carbon-to-pasting liquid ratio being the same: 0.25 g graphite + 0.1 mL. All the pastes homogenized manually using a pestle and mortar were packed into piston-driven Teflon® holders of own design [44]. Whenever needed, the surface of carbon paste (with diameter of 2 mm) was mechanically renewed by extruding *ca*. 0.5 mm carbon paste out of the electrode holder and smoothing with a wet filter paper. Usually, this simple operation was made before starting a new set of experiments. For optimization of the tricresyl-phosphate content in TCP-CPE, the paste mixtures with 0.25 g graphite and 20%, 30% or 40% (w/w) TCP were additionally prepared by using identical preparation procedure, as well as the way of surface renewal.

Cyclic Voltammetry (CV) and Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV). To compare the behavior of CPEs with three different binders as well as three different variants of TCP-CPE, the respective electrodes were tested in model solutions containing Britton-Robinson buffer with the appropriate pH (diluted 1:1). These solutions were deaerated by passing argon gas through for *ca*. 10 min. and the voltammograms registered at ambient temperature. With all the electrodes, CV experiments were performed in the potential range from −0.1 to +1.2 V using a scan rate of 100 mV s^{-1} in the presence and absence of MBC ($5.2 \cdot 10^{-4}$ mol L^{-1}) in the same Britton-Robinson buffer (pH 8.0).

Both CV and DPAdSV were performed to characterize the MBC oxidation at the TCP-CPE, while the Britton-Robinson buffer-based supporting electrolyte covered the pH range from 2.0 to 8.0. For the E_p-pH correlation of oxidation peak at E_{pa} 0.8 V *vs*. ref., CV was performed, while the scan rate was 100 mV s^{-1}. Additionally, the same technique was run at scan rates from 20 to 100 mV s^{-1} to characterize the "I_p-v", and "I_{pa}/I_{pc}" dependences and the oxidation mechanism of MBC at pH 4.0. For the measurement of the number of exchanged electrons, voltammetry in the linear scan mode (LSV) was applied while the intensity of MBC oxidation signal was compared to that obtained with the standard solution of $[Fe(CN)_6]^{4-}$. In this case, the Pt-RDE was employed with rotation speed of 1500

rpm, using again the scan rate of 100 mV s^{-1}, when the test solutions were 0.005 mol L^{-1} $[Fe(CN)_6]^{4-}$ in 0.1 mol L^{-1} KCl, and $1.0 \cdot 10^{-4}$ mol L^{-1} MBC in Briton-Robinson buffer (pH 4.0). Other specification concerning each experiment is then given later – together with the corresponding commentary.

Analytical Procedure. The solutions were deaerated by passing argon gas for 5 min. Before each set of measurements, the buffer-immersed working electrode was electrochemically activated by potential cycling (with 50 cycles) in the range from −0.1 to +1.6 V at v = 250 mV s^{-1}. Before measuring MBC, the blank signal was always recorded first. In case of measurements in the presence of HPCD, its optimal concentration (see later) was added first, and then, the desired amount of MBC solution was injected. The optimal experimental conditions of the DPAdSV measurements for the determination of MBC were as follows: start potential, -0.10 V vs. ref.; end potential, +1.30 V; accumulation potential, E_{acc}, -0.35 V; accumulation time, t_{acc} 120 s, and the scan rate, 100 mV s^{-1}. In the case of real sample the river water sample was spiked with the standard solution of MBC to yield a concentration of $1.0 \cdot 10^{-6}$ mol L^{-1}.

The river water sample was kept in the fridge (at 4 °C) before analysis without any sample pretreatment. Before measurements, the aliquots of the water samples were diluted with Britton-Robinson buffer (pH 4.0) at a ratio of 1:1 (v/v). 10.0 mL of the buffered sample was spiked with 10.0 µl 0.001 mol L^{-1} MBC solution before two consecutive standard addition of two another 10.0 µl of MBC solution. The concentration of HPCD was made as that optimized in model measurements. The model solutions were measured without filtering, whilst the water sample from Elbe river (sampled at the banks in Pardubice town, Czech Rep.) had to be filtered. All experiments concerning the real samples were performed in triplicate.

The Reference Method (Employing HPLC). All aliquots were filtered through syringe filters (product "Millipore, 0.45 µm"; Millipore, USA). The mobile phase was a mixture of acetonitrile and doubly distilled water in ratio 3:7 (v/v). The separation was performed in the isocratic regime and the flow rate was 0.8 mL min^{-1}. The target analyte was detected with DAD, at a wavelength of 254 nm and with the retention time of 4.3 min.

3. RESULTS AND DISCUSSION

3.1. Choice of Carbon Paste Electrode for Voltammetry of Carbendazim

As mentioned at the end of the introductory part, three different binders — i.e., tricresyl-phosphate (TCP, curve **a**) mineral oil (MO, curve **b**), and silicon oil (SO, curve **c**) — were first compared for their suitability in the role of pasting liquids. The test of choice was a CV experiment with 0.01 mol L^{-1} MBC at pH 8.0 (see Fig. 1).

In all cases, the electrochemical response of MBC, accumulated/adsorbed on the electrode surface gave rise to a well-defined anodic peak at potentials near to 0.8 V vs. ref. within the first scan, the backward scanning then resulted in two cathodic peaks at potential near +0.4 V and +0.7 V with significantly lowered intensities.

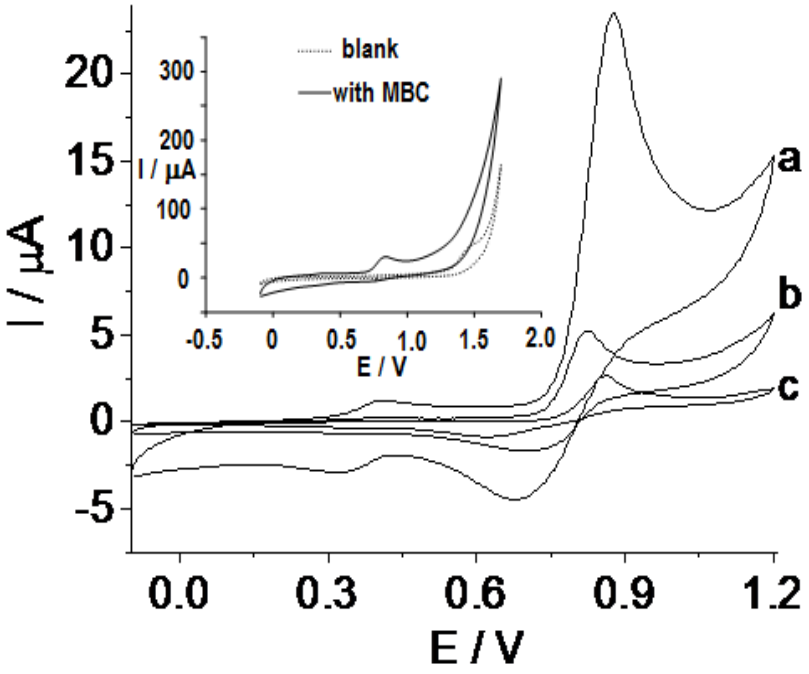

Figure 1. Cyclic voltammograms of *Carbendazim* at sub-millimolar level in Briton–Robinson buffer (pH 8.0) on CPEs based on different binders: a) tricresyl-phosphate, b) mineral oil, c) silicone oil. Inset: cyclic voltammograms of blank Britton-Robinson buffer pH 8.0 (dotted line) and 5.2 10^{-4} mol L^{-1} *Carbendazim* (full line) obtained by TCP-CPE. v =100 mV s^{-1}.

During the repeated scannings, a second anodic peak appeared at a potential near +0.3 V having yet less intensity than the signals observed previously. Moreover, this peak had gradually disappeared after repetitive scanning (up to n = 10), followed by ultimate vanishing together with the original reduction signal at *ca.* +0.4 V, which was observed for all the electrodes. Similar electrochemical behavior of MBC was reported for silicon oil-containing CPE [21], as well as for the GCE modified with a thin layer of carbon nanotubes [23].

As seen in the figure, the peak at ca. +0.8 V vs. ref., corresponding to the oxidation of MBC and its counterpart at +0.7 V, has shown a nearly reversible behavior at a scan rate of 50 mV s^{-1}. (Note: Regarding CPEs, however, a reversible behaviour is somewhat simplified characterisation. As repeatedly emphasised in literature [30, 45, 46], almost all CPEs exhibit specific electrode kinetics with notable moderation of the electrode process at the carbon paste surface caused by the present liquid binder and, in CV, evident as a certain shift between the parent anodic and cathodic peaks; typically, with an increment of 50-100 mV compared to carbon solid electrodes [30, 47].) Further, it can be noticed that the redox pair from the first cycle exhibits different intensities for both anodic and cathodic peaks; i.e., $I_{pa} \neq I_{pc}$ (at 50 mV s^{-1}).

The advantage of the TCP-CPE over SO-CPE and MO-CPE is evident if one compares the shape of voltammograms recorded, when the corresponding CV has exhibited the lowest residual currents together with the highest peak currents (compared to the other two CPEs). When considering the typical properties of each CPE tested, such a behavior of the TCP-CPE seems to be a result of the

polarity of tricresyl-phosphate at the central phosphorus atom, i.e. $(Ar–O–)_3P^{(+)}–O^{(–)}$ [34,35], thus facilitating the direct contact of MBC molecules with the electrode surface.
In contrast, similar mechanism cannot be assumed for neither MO-CPE nor SO-CPE that both contain nonpolar binders [30, 31, 47]. Finally, the oxidation peak of MBC at TCP-CPE is positioned slightly more positively than at SO-CPE and MO-CPE, which did not affect the determination. Comparing the shape and intensity of the oxidation and reduction peaks obtained by the TCP-CPE, the oxidation peak with E_{pa} at 0.80 V was chosen, for the determination of MBC (see later).

To optimize the amount of TCP in the carbon paste mixture, three types of TCP-CPE was prepared containing 20, 30, and 40% (w/w) of the binder. The respective DPV curves, i.e. measurements already directly associated with the optimization of the analytical procedure, obtained in the same solution of MBC (with $3.0 \cdot 10^{-6}$ mol L^{-1}) at pH 4.0 have proved clearly that the optimal signal-to-noise characteristics, as well as the proper consistency and good mechanical properties, were obtained with the TCP-CPE containing 30% TCP (not shown). This paste was thus chosen for further measurements.

As emphasized in our previous report [41], the DPV curves obtained in the series of Britton-Robinson buffers (with pH between 2.0 and 8.0) had contained the single irreversible oxidation signal of unclear origin whose shape and peak potential, E_p, strongly depended on pH. In acidic media, at pH 2.0, the E_p is positioned at +0.9 V [41] (not shown), while in slightly alkaline media, at pH 8.0 (see again Fig. 1 and the inset), the peak shifted towards highly positive potentials at ca. +1.5 V vs. ref.

Although this peak — likely due to the oxidation of the electrode surface — seemed to be sufficiently separated from the oxidation signal of MBC, its occurrence was evaluated as undesirable for analytical measurements because of its quite large absolute intensity, as well as high background around this signal. Thus, the TCP-CPE electrode was subjected to the electrochemical activation, which is a special pretreatment being helpful in such situations [41]. According to some previously recommended procedures (see e.g. [41, 47, 48]), the activation was accomplished by potential cycling in the potential range from –0.10 to +1.60 V, when using 10 cycles at a scan rate of 250 mV s^{-1}. It was found out that this treatment had indeed led to the desired benefit; the resultant improvement being triple: (i) predominant decrease of the oxidation peak, (i) certain widening of the potential window, and (iii) additional stabilisation of the signal. In addition, after this treatment, there was no notable change in sensitivity, and therefore, this way of conditioning the TCP-CPE was adopted for all subsequent measurements.

3.2. Carbendazim Oxidation Mechanism

The pH effect on the oxidation pathway of the substance of interest was investigated in two voltammetric modes: with the aid of (i) CVs (Fig. 2A) and via (ii) DPAdSV curves (Fig. 2B); both recorded at the TCP-CPE in the pH-range of 2.0-8.0 for a model concentration of $3.0 \cdot 10^{-6}$ mol L^{-1} MBC.

The sharpest and most favorably developed peak was obtained in slightly acidic solutions (at pH 4.0; see Fig. 2). Further, both E_{pa} and I_{pa} were found dependent upon pH of the supporting electrolyte. Similarly to the previously published data [20,21], the E_{pa}−pH resultant plot (shown in Fig.

2A) was linear with the regression equation, E_{pa} =-0.062 E' [V pH^{-1}] + 1.18 (with r = 0.995 and the slope, S = 62 mV pH^{-1}).

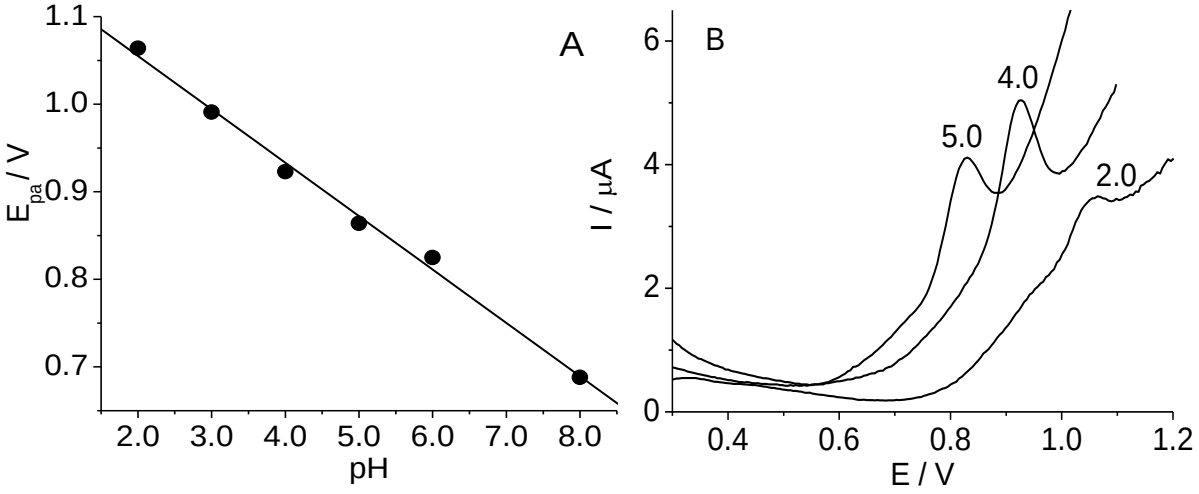

Figure 2. pH dependence of the *Carbendazim* oxidation signal recorded with TCP-CPE: A) E_{pa}-pH plot based on CV curves recorded in Britton-Robinson buffer from pH 2.0 to 8.0 while v = 100 mV s^{-1}, containing 3 · 10^{-5} mol L^{-1} MBC and B) Illustrative DPAdSV curves (deposition potential, -0.35 V; deposition time, 120 s; equilibrium time, 5 s; Britton-Robinson buffers (pH 2.0, 4.0, and 5.0).

The number of electrons involved in the reaction was evaluated from measurements with the linear voltammetric ramp (LSV mode) when using the same model solution of MBC at the Pt-RDE (see Experimental) and compared to that obtained with test solution containing $[Fe(CN)_6]^{4-}$ ions, representing maybe the most frequent model system for the investigation of redox electrode reactions with almost ideal reversible process and with the exchange of one single electron. The comparison of the limiting currents of both systems has indicated that the oxidation of MBC involves two electrons and, when using the E_{pa}–pH slope (obtained by TCP-CPE; see above), and the respective Nernst equation (expressed as 0.062 = (0.0592 · P)/n → P≅ 2), the number of protons involved could be calculated as well, resulting in the stoichiometry with 2 H^+.

Analysis of CV curves obtained by TCP-CPE at various scan rates (speeds, "v") between 50 and 1000 mV s^{-1} and at the same pH 4.0 (see Fig 3A) has shown that with the increasing scan rate, I_{pa} and I_{pc} increase both proportionally to the $v^{0.5}$. A diffusion process is responsible for the mass transfer towards the surface of the electrode, since either I_{pa} and I_{pc} are linear functions of $v^{0.5}$ according to the equations: I_{pa} = 6.07 $v^{0.5}$- 58.22 (r = 0.999) and I_{pc} = 4.43 $v^{0.5}$-23.61 (r = 0.999). It can be proposed that, at lower scan rates, the chemical reaction is sufficiently rapid to proceed and the resultant height of cathodic peak is lower, which is in contrast to the experiment at higher scan rates, where the electrode process gives rise to one-step reversible reaction (viewed as the single peak) and the corresponding ratio of I_{pa}/I_{pc} = 1. The E_{pa} then shifts to more positive potentials with the increasing scan rate, confirming thus E_rC_i mechanism [21, 24].

According to the obtained results the suggesting mechanism is shown in Fig. 4. By the way, the same mechanism was reported previously for the oxidation of MBC at silicone oil-based based CPE [21], as well as on an ultramicroelectrode array [24].

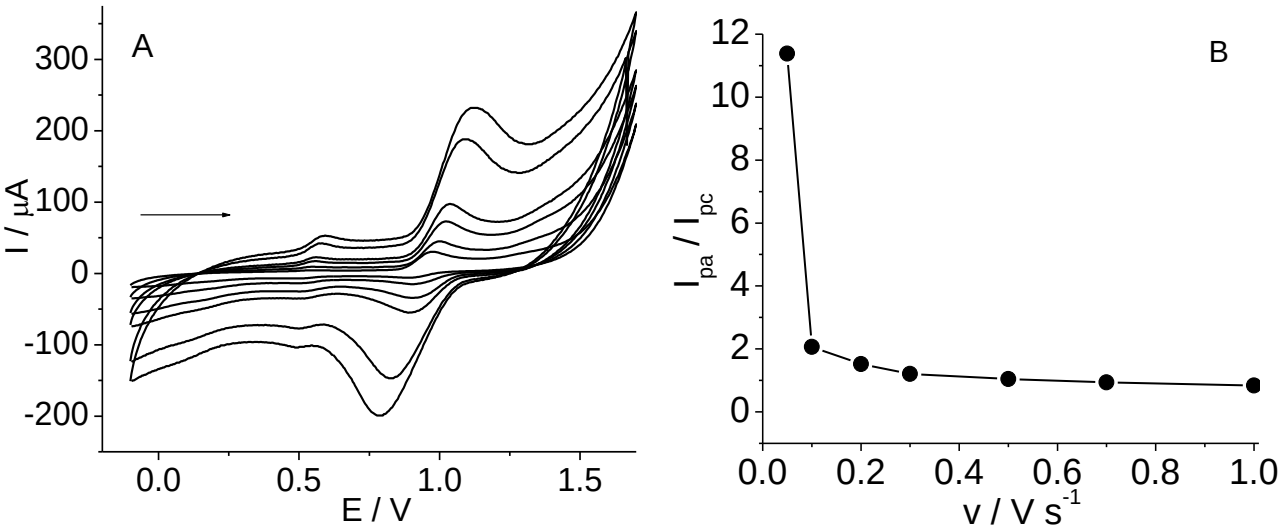

Figure 3. Effect of the CV scan rate for oxidation peak of *Carbendazim* ($5.2\ 10^{-4}$ mol L^{-1}) and its counterpart in Britton-Robinson buffer solution (pH 4.0) obtained with TCP-CPE: A) Scan rates: 50, 100, 200, 300, 500, 700 and 1000 mV s^{-1} and B) Ratio of I_{pa} and I_{pc} recorded at the appropriate different scan rates.

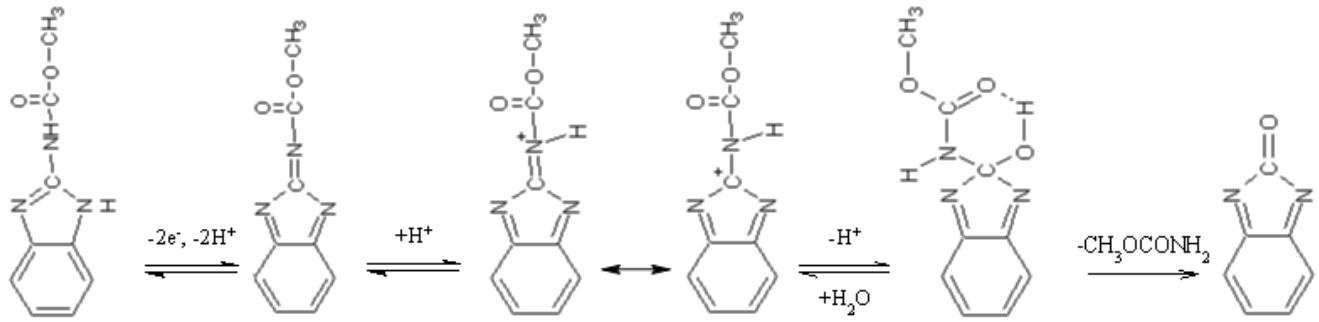

Figure 4. Oxidation mechanism of *Carbendazim* at TCP-CPE in Britton-Robinson buffer (pH 4.0).

3.3.2-Hydroxypropyl-β-Cyclodextrin Assisted Adsorptive Stripping Voltammetry of Carbendazim

Two supporting electrolytes, the acetate-based and Briton-Robinson buffers with the same acidity (pH 4.0) were tested as the media of choice for the determination of MBC. The results have indicated that better peak shape and higher peak maxima could be obtained in the Britton-Robinson buffer (not shown).

For the determination of MBC at the sub-micromolar level, the DPAdSV mode was tested and finally selected. The current intensity, I_{pa}, of the signal of interest was found to be dependent upon the

accumulation potential, E_{acc} between +0.20 and -0.70 V vs. ref. As shown in Fig. 5A, the peak current increased down to a potential of -0.35 V and then started to decrease; the optimal value being evident.

Another optimized parameter was the deposition / accumulation time, t_{acc}, when Fig. 5 B illustrates that the oxidation signal has increased with prolongation of the deposition time to 180 s, whereas yet another prolongation of deposition (up to 300 s) did not enhance the peak significantly. This seems to follow a pattern of typical saturation curve, confirming the adsorption at the TCP-CPE surface. As the optimum, a period of 120 s was selected to achieve down to the low ppb level.

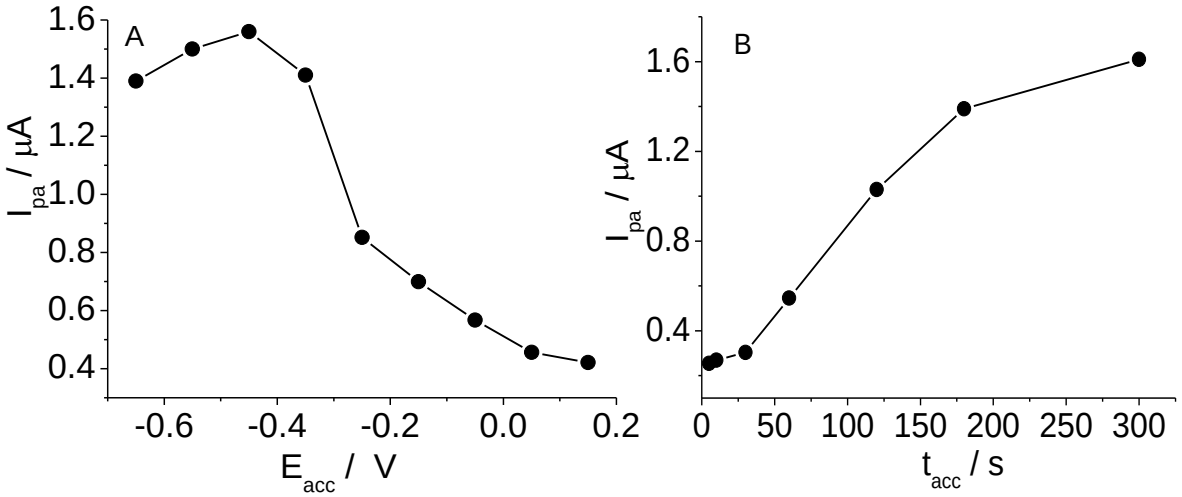

Figure 5. Key paremeter optimization for adsorptive striping differential pulse voltammetric determination of MBC ($3 \cdot 10^{-6}$ mol L^{-1}) in Britton –Robinson buffer pH 4.0: A) Influence of E_{acc} on I_{pa} values 120 s t_{acc} and B) Effect of t_{acc} at E_{acc} -0.35 V. All other DPAdSV variables are as those in Fig 2.

In a separate study, the presence of isopropyl-β-cyclodextrine in the sample solution was tested as a way for further sensitivity enhancement [42, 43]. As can be seen in Fig. 6, the addition of appropriate amount of this reagent has indeed resulted in the increase of sensitivity for the determination of MBC and the entire effect can be attributed to a specific interaction between HPCD and the target analyte MBC based on the inclusion of the latter.

Regarding the optimal concentration of the modifier, the result could be evaluated from the measurement depicted in Fig 6. Evidently, with higher concentrations of 2-hydroxypropyl-β-cyclodextrin, the peak current increased up to a maximum for $3.6 \cdot 10^{-5}$ mol L^{-1} HPCD and its further addition did not have any notable effect. (Herein, it is useful to quote that the 2-hydroxypropyl-β-cyclodextrin itself have had no oxidation signal in the potential range of the MBC electroactivity.)

3.4 The Final Optimization of the Method for Voltammetric Determination of Carbendazim

In the presence of 2-hydroxypropyl-β-cyclodextrin, the experimental conditions for the DPAdSV determination of MBC were as follows: initial potential, E_{init} = -0.10 V vs. ref., final potential, E_{fin} = +1.30 V, accumulation at E_{acc} = -0.35 V for 120 s and at a scan rate of 100 mV s^{-1}.

As found out, the analytical performance of the method with modifier was optimal in the concentration range of $5.0 \cdot 10^{-7}$ – $1.0 \cdot 10^{-5}$ mol L^{-1} MBC (Fig. 7, and inset); the individual parameters being gathered in Table 1.

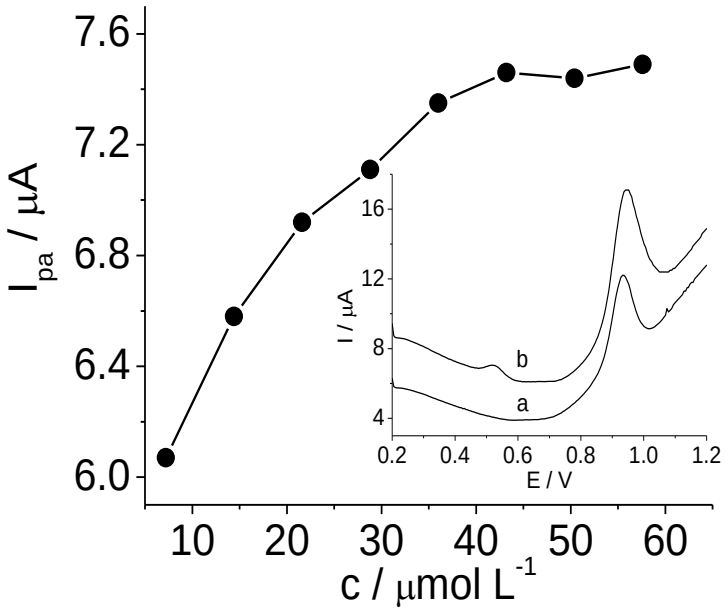

Figure 6. Dependence of I_{pa} height of MBC on the concetration of 2-hydroxypropyle-β-cyclodextrin in Britton-Robinson buffer pH 4.0. Inset: $1 \cdot 10^{-5}$ mol L^{-1} MBC (curve **a**), and $1 \cdot 10^{-5}$ mol L^{-1} MBC +3.6 $\cdot 10^{-5}$ mol L^{-1} (curve **b**). E_{acc}= -0.35V, t_{acc}= 120 s.

Table 1. Analytical parameters of the DPAdSV and HPLC/DAD determination of *Carbendazim*. LOD (limit of detection), LOQ (limit of quantification), *n* number of replicate measurements, r linear regression coefficient.

Parameter	Method	
	DPAdSV	HPLC/DAD
Concentration interval [mol L^{-1}]	$5.0 \cdot 10^{-7}$ – $1.0 \cdot 10^{-5}$	$4.7 \cdot 10^{-7}$ –$5.2 \cdot 10^{-5}$
Intercept [μA]	0.55 μA	1.10 mAU min
Slope	0.59 μA L mol^{-1}	40.32 mAU min L mol^{-1}
r	0.998	0.999
LOD [mol L^{-1}]	0.20	0.15
LOQ [mol L^{-1}]	0.50	0.47
RSD [%] (*n*=8)	1.9	1.7

The respective data show that MBC could be determined down to the trace concentration level, when the linear dependence corresponded to the regression equation: I_{pa} [μA] = 0.541 c_{MBC} + 0.530 (with r = 0.995) and the estimate of detection limit (3σ) as about $3.0 \cdot 10^{-7}$ mol L^{-1}.

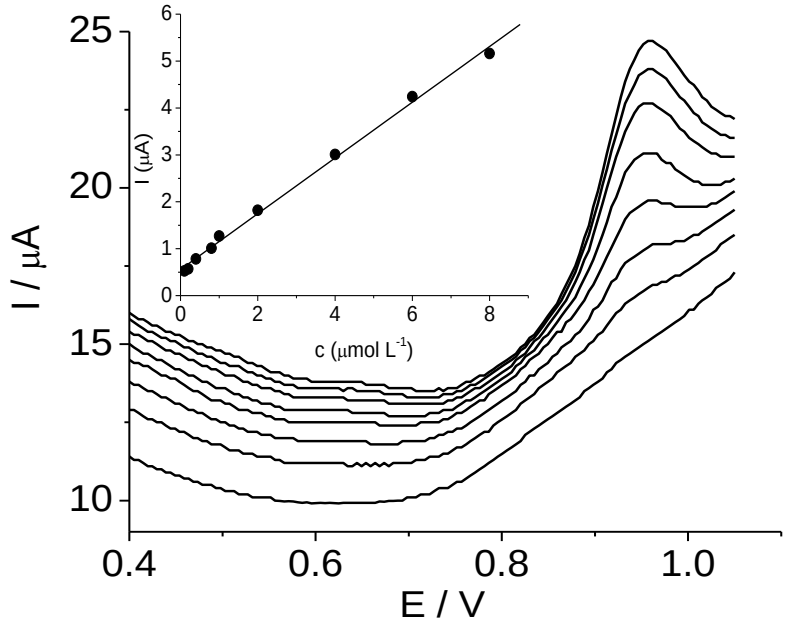

Figure 7. Adsorptive stripping differential pulse voltammograms recorded at TCP-CPE and in the presence of $3.6 \cdot 10^{-5}$ mol L^{-1} HPCD for different concentrations of *Carbendazim* in Britton-Robinson buffer (pH 4.0). The corresponding calibration plot is shown in the inset. E_{acc} -0.35 V, t_{acc} 120 s, t_{ep}, 5 s.

Inevitable assays on repeatability of the analytical signal and some basic studies on the effect of potentially interfering species has completed the optimization measurements, resulting in the RSD of *ca*. ±4.9% (for a model concentration of $2.0 \cdot 10^{-6}$ mol L^{-1} MBC), representing a satisfactory relative error of the method if one considers the use of a CPE as the working electrode [30, 49].

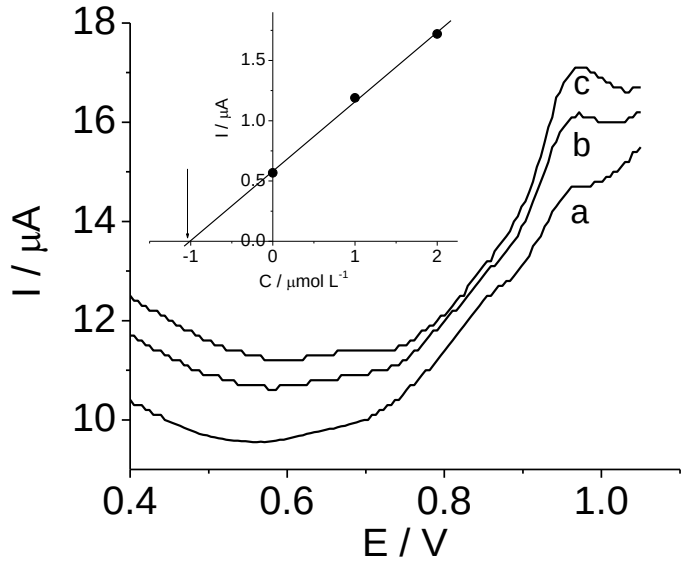

Figure 8. Determination of *Carbendazim* in spiked Labe river sample. Adsorptive stripping differential pulse voltammetric curves obtained for spiked river water sample (curve **a**) and successive standard additions of *Carbendazim* (curves **b** and **c**). The inset shows the corresponding analytical plot.

As confirmed, the method was selective over *Linuron* (a herbicide with related structure), as well as over some naturally frequent inorganic anions (namely: SO_4^{2-}, NO_3^-, Cl^- and Br^-); all species investigated being added at a 10- and 100-fold excess in concentration.

Finally, the applicability of newly proposed and developed procedure employing TCP-CPE and DPAdSV was tested on the determination of MBC in Elbe river, when using model spiked samples. A typical result of such an analysis is given in Fig. 8, illustrating the respective quantification method — the standard additions (in inset) — and showing good linearity (with r = 0.994), as well as a very tight recovery rate of 101.9 %.

4. CONCLUSIONS

In this article, the tricresyl-phosphate-based carbon paste electrode, TCP-CPE, has for the first time been employed for the effective pre-concentration of *Carbendazim* (MBC) fungicide, with the subsequent anodic detection in the DPAdSV mode. Voltammetric signals of MBC were investigated at tricresyl phosphate-, silicone oil- and mineral oil-based CPEs. The sensitivity of the respective voltammetric measurements has depended mainly on the composition of the carbon paste electrode material, with additional benefit by means of electrochemical pretreatment of the CPE surface. Also, the TCP-CPE as the electrode with the most hydrophilic pasting liquid exhibited the best analytical performance concerning the overall signal-to-noise ratio and the actual intensity.

The DPAdSV working regime combined with the TCP-CPE and the selection of Britton–Robinson buffer with pH 4.0 was found to be the optimal constellation for the determination of the substance of interest, when the analytical performance of the TCP-CPE could nearly doubly be enhanced via the addition of 2-hydroxypropyl-β-cyclodextrin as the modifier. As a result, the method developed has offered the linearity in the concentration range of $5 \cdot 10^{-7} – 1 \cdot 10^{-5}$ mol L^{-1} MBC (with r = 0.995) and the analyte could be detected down to $3 \cdot 10^{-7}$ mol L^{-1}. Finally, the voltammetric results were compared with the reference HPLC/DAD analysis, resulting in a satisfactory recovery of about 102% for a model sample of river water spiked with MBC at concentrations from $1 \cdot 10^{-6}$ to $3 \cdot 10^{-6}$ mol L^{-1}.

It can be stated that the procedure developed within this study can be recommended as convenient screening approach for initial / preliminary monitoring, as well as for quick but sufficiently accurate determination of MBC fungicide in real water samples. The method can also be characterized as simple (as not incorporating any complicated clean-up step), inexpensive, attractive from saving-time point of view, as well as obeying the present day's ecologically oriented demands [50].

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Development of new materials for application in environmentally friendly technologies for the cost-effective remediation of contaminated sites threatening cross-border regions) also. The contents of this document are the sole responsibility of the University of Novi Sad Faculty of Sciences and can under no circumstances be regarded as reflecting the position of the European Union and or the Managing Authority.

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