



Polished silver solid amalgam electrode and cationic surfactant as tool in electroanalytical determination of methomyl pesticide

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ABSTRACT

The polished silver solid amalgam electrode (p-AgSAE) combined with square wave voltammetry (SWV) was used in the development of an analytical procedure to Methomyl determination in natural water samples. The experimental and voltammetric parameters were optimized and the use of cationic surfactant cetyltrimethyl ammonium bromide promoted a considerable change in the kinetic and mechanism of the Methomyl reaction. Was observed the presence one-single reduction peak, related to a totally irreversible two-electrons transfer, followed by chemical reaction involving two protons. Analytical parameters (linearity range, analytical curve equations, correlation coefficient, detection and quantification limits, recovery efficiency, and relative standard deviation for intraday and interday experiments) were evaluated indicating that the proposed voltammetric procedure is suitable for determination of Methomyl. The application of the proposed procedure in natural water analysis indicated high robustness with only simple filtration, without pre-concentration steps, good stability and suitable sensitivity to determination of Methomyl in natural water samples.

1. Introduction

Carbamate pesticides represent one of the chemical classes that have attracted most interest in the development of analytical methods for the monitoring their residues and their metabolites in surface and groundwater samples, soil and food [1,2]. The contamination by carbamates promote a disrupt of thyroid functions by interaction of carbamates with thyroid hormone receptor or thyroid binding proteins [3,4]. Some carbamates pesticides present anti-estrogenic activity interfering in reproductivity capacity, and other some carbamates can participate in the development of some types of cancer [5,6].

Among them, the Methomyl {S-methyl-N-[(methylcarbamoyl)oxy] thioacetimidate}, called here as MT, is used in agricultural practices as insecticide and nematocide, and chemically belongs to the family of carbamic acids compounds. Furthermore, MT is a principal metabolite in degradation process of other carbamates, including Thiodicarb, Alanicarb and Aldicarb [7]. MT is an endocrine disrupt compound due to modify the estrogen production, interfering in reproductive capacity, for this, is highly toxic to animals and humans [8]. So, its quantification in drinking water has a high scientific importance.

According to Environmental Protection Agency (EPA) [9], the separation and measurement of the MT in natural water can be realized by high-performance liquid chromatography coupled to ultraviolet

detector (HPLC/UV-Vis), producing a limited sensitivity due considerable interference from complex samples and low wavelengths used in carbamates detection. For this, the use of HPLC coupled to mass spectrometry has (HPLC-MS) become a suitable tool in environmental analysis of MT and other carbamates pesticides [10,11]. Gas chromatography coupled to mass spectrometry (GC-MS) has been reported as highly sensitivity MT analysis in water samples promoting additional selectivity and sensitivity [12,13].

However, the use of chromatographic requires the solid phase extraction (SPE) to reach limits of detection sufficient low for trace analysis [14–16]. Nevertheless, SPE presents long time to analysis, are expensive due to consumption of organic solvents, and considerable volume of samples are necessary and mainly, only a small portion is injected onto the chromatographic column. For this, some non-chromatographic techniques, such as fluorescence spectroscopy [17] and electrochemical [18,19] techniques also have been used in MT determination.

The use of biosensor based on acetylcholinesterase enzyme has been reported to MT analysis, however, the sensitivity is usually lower than obtained with the traditional chromatographic techniques, due to the reversible behavior of inhibition reaction of the acetylcholinesterase to acetylcholine promoted by MT [20,21].

Zapp and co-workers [22] proposed the use of montmorillonite, a

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clay mineral, modified by platinum nanoparticles and ionic liquid, as a support to Lacasse immobilization. This biosensor was used in MT determination in carrot and tomato samples, but was not used in natural water samples. *Fernandes* and co-workers [23] shown a sol-gel derived carbon ceramic biosensor for the determination of MT in vegetable extracts samples. The substrate used was the esculetin, that resulted in a decrease in enzymatic activity when fresh vegetable samples containing MT were added to the reaction medium. In this research, natural water samples also were not analyzed.

In these works, the analytical results were dandified by Lacasse immobilization method and by pH of the samples, by ionic strength and by content of the samples employed. It's occurred due to biocatalytic processes are complex and some components in the samples also may inhibit the enzymatic activity. So, *Costa* and co-workers [24] employed a boron doped diamond electrode to determination of MT in river water, tap water, and commercial formulations. However, the oxidation potential of MT presented value greater than 1.5 V, promoting interference of other redox processes in complex samples.

According to our knowledge, until this moment, no work showing the use of others solid electrodes has been reported to electroanalytical determination of the MT. For this, the goal of this work was the development of reliable, fast, inexpensive and environmental friendly analytical procedure to MT determination in natural water using p-AgSAE allied to square wave voltammetry (SWV), an electroanalytical technique extremely sensitive in the pesticides detection [25,26].

2. Experimental

2.1. Reagents and equipments

In this work, all chemicals employed presented high analytical purity and the solutions employed were prepared with water purified by a Gehaka model OS20 LX system ($18.2 \text{ M}\Omega \text{ cm}^{-1}$ at 25°C). MT (99.50%) has obtained from Ouro Fino Agricultural Company, from Minas Gerais, Brazil, and used without purifications steps. The MT stock solutions were prepared by dissolving a suitable quantity of it in pure acetonitrile to obtain concentrations of $1.00 \times 10^{-3} \text{ mol L}^{-1}$ and $1.00 \times 10^{-4} \text{ mol L}^{-1}$. To prevent degradation process, these solutions were then stored in a dark flask and kept in a refrigerator.

H_3PO_4 , H_3BO_3 and CH_3COOH , in a concentration of the 0.04 mol L^{-1} plus 0.1 mol L^{-1} NaClO_4 were used to prepare the Britton-Robinson (BR) buffer, which had the desired pH values adjusted by addition of the amounts of 1.0 mol L^{-1} NaOH solution. The surfactant cetyltrimethyl ammonium bromide (CTAB) was used in the concentration from $1.00 \times 10^{-5} \text{ mol L}^{-1}$ to $1.00 \times 10^{-3} \text{ mol L}^{-1}$, which corresponds to critical micellar concentration (c.m.c.) [27].

A potentiostat ($\mu\text{Autolab}$, from Metrohm-Eco Chemie) coupled to NOVA software version 2.1.2 was used in voltammetric experiments. The pH values of the solutions were measured using a pH meter (mPA 210, from Tecnopon) equipped with a 3.00 mol L^{-1} Ag/AgCl/KCl-glass combined electrode.

2.2. Electrochemical cell and working electrode

An electrochemical cell containing 10 mL of the BR buffer as supporting electrolyte, was used. A platinum wire and an Ag/AgCl 3.0 mol L^{-1} electrode were used as auxiliary and as reference electrode, respectively, and a home-made p-AgSAE was used as working electrode.

The p-AgSAE was constructed using a natural amalgamation process, according description presented in a previous works [28,29]. For this, 0.35 g analytical grade liquid mercury and 0.15 g of fine silver powder (particle size: 5–9 μm , purity: 99.90%, Sigma-Aldrich) were vigorously blended. The paste resulting was compacted inside a Pyrex® glass capillary tube with an inner diameter of about 0.30 mm. After 24 h, an electric contact was inserted by use a copper wire and silver adhesive. The resulting disks were polished with an emery paper (up to

2000 grit sandpaper, 3 M).

Subsequently, p-AgSAE was inserted in electrochemical cell containing 0.20 mol L^{-1} KCl and the potential of the -2.20 V was applied for 600 s under a stream of purified N_2 (White Martins). This procedure, called as electrochemical activation, allowed a considerable reduction in the resistance to charge transfer and a consequent increase in the analytical responses, and ever is necessary before each day of experiments or after every delay of longer than one hour without use of the electrode [30]. Besides, between each voltammetric scan is applied -2.2 V for 30 s to removal of the adsorbed product reaction from the p-AgSAE [31,32].

2.3. Voltammetric experiments

Preliminary experiments were performed using 10 mL of the BR buffer in pH values from 2.0 to 12.0, plus $3.00 \times 10^{-5} \text{ mol L}^{-1}$ of MT standard solutions in the electrochemical cell. Prior to each one, the solution was deaerated by bubbling in N_2 for 10 min. SWV experiments were realized using scan potential from 0.00 V to -1.50 V at $26 \pm 1^\circ\text{C}$, with the pulse potential frequency (f) of 100 s^{-1} , amplitude of the pulse (a) of 50 mV and the height of the potential step (ΔE_s) of 2 mV. These experiments were done with and without the use of CTAB surfactant to observe the effects of its addition in concentrations below, near and above its c.m.c [27].

The voltammetric parameters related do SWV (f , a and ΔE_s) were individually evaluated in relation to the maximum value of peak current (sensitivity) and the minimum half-peak width (selectivity) [25,26]. Besides, these parameters were used to evaluate the redox reaction mechanism of MT at p-AgSAE.

In this work, the experimental and voltammetric parameters were individually evaluated by analysis of resultant peak currents (I_p), the peak potentials (E_p) and the variation in the half-peak width ($\Delta E_{p/2}$), responsible by sensitivity and selectivity suitable in the voltammetric procedures.

After the experimental and voltammetric optimization, the analytical curves were constructed by the standard addition method. The detection and quantification limits (LOD and LOQ, respectively) were calculated according to guidelines recommended by IUPAC [33], using of the standard deviation of the mean current measured at the reduction potential of MT for 10 voltammograms of the blank solution in purified electrolyte (S_b) together with the slope of the straight line of the analytical curves (s).

The recovery experiments were done to attest the efficiency of the methodology. For this, a known amount of MT was added to the supporting electrolyte followed by the standard additions from the MT stock solution and plotting the resulting analytical curves. The recovery efficiencies (%R) were calculated considering the value of the perceptual of the relationships between $[\text{MT}]_{\text{added}}$ and $[\text{MT}]_{\text{recovered}}$, which refer to the concentration of MT intentionally added in the electrochemical cell, and the concentration obtained by extrapolating the analytical curves, respectively [34]. All measurements were performed in triplicate.

The precision of the proposed procedure was evaluated based on the reproducibility of experiments carried out using five different standard solutions of MT on five different days (interday). The accuracy was evaluated based on repeatability experiments that included ten replicated determinations in the same solution of MT (intraday). The relative standard deviations (RSD) were calculated for the reproducibility and repeatability measures, using the relationships between the standard deviation and the mean of the obtained peak current values [34].

2.4. Application of methodology

The proposed methodology was applied in natural water samples analysis. Three samples were used to evaluate the interfering effects

from complex samples, which were collected in the Paranaíba River, a local river located at Patos de Minas city, Minas Gerais state, Brazil. The samples used were:

- Sample 1: collected in a local before the city limits (-18.6554° ; -46.5128°) where the river is relatively free of the urban or industrial pollution and is located near agricultural areas that produce corn, soy, bean and coffee;
- Sample 2: collected along the stream length (-18.6024° ; -46.5405°), located around the urban area where the degree of pollution is high;
- Sample 3: collected after the river crossing the city (-18.4983° ; -46.5603°), thus containing a high degree of organic matter including agricultural, domestic and industrial residues, received throughout the city.

These samples were filtrated in a $0.5\ \mu\text{m}$ paper filter to remove all particulate. After this, these samples were used to prepare the BR buffer solution in pH 9.0, by dissolution of the BR buffer components. The resultant solution was maintained in a refrigerator even its use. So, the evaluation of the robustness of the proposed procedure was realized using 100% of the samples in preparation of the supporting electrolyte, without any clean up or pre-treatment steps, exception of the preliminary filtration to remove particulate in the samples.

10 mL of each sample was artificially contaminated with $3.00 \times 10^{-7}\ \text{mol L}^{-1}$ of MT and the SWV experiments were performed. The peak current values obtained were inserted in calibration curve equation, and the resultant concentrations were calculated and used to evaluate the effects of interferences present in these samples in the SWV responses from differences between the MT calculated values and MT added concentration in the electrochemical cell.

Recovery experiments were performed to attest the efficiency in the proposed procedure in natural water samples. For this, 10 mL of BR buffer prepared with each sample, was artificially contaminated by $3.00 \times 10^{-7}\ \text{mol L}^{-1}$ of MT and the standard addition method was employed to performed recovery curves. All recovery calculations were realized similar to described early to pure BR buffer.

3. Results and discussion

3.1. Experimental parameters

Preliminarily, the electrochemical reduction of MT on the p-AgSAE was investigated in BR buffer with pH values adjusted from 2.0 to 12.0, and the SWV responses indicated that the increase in pH provoked an increase in I_p values until pH 4.0, after this value, the I_p had considerable decrease. As the maximum value of I_p was observed at BR buffer adjusted for pH 4.0, which is lower than the pK_a reported in the literature (pK_a 13.27) [35], is possible to affirm that, in this case, the MT reduction occurs without participation of protonation equilibrium preceding the electrons transfer reaction. Additionally, in pH above 4.0, the responses were accompanied by an expressive change in the voltammetric profile, characterized by a higher variation in $\Delta E_{p/2}$.

Besides, can be observed that in pH bellow 9.0 the E_p do not however seem to be affected by pH of medium, E_p remains practically constant, confirming the absence of any protonation step in the electron transfer reaction. However, in pH above 9.0 the E_p shifted to less negative values with increasing pH, which indicated that a transfer of protons was coupled to the reduction process [36]. The relationships between E_p versus pH, presents two linear portions which can be represented by equations:

$$E_p = -0.963 - 0.007\text{pH}(\text{First inclination}) \quad (1)$$

$$E_p = -1.520 + 0.058\text{pH}(\text{Second inclination}) \quad (2)$$

where values of potential are given in volt. The intersection between

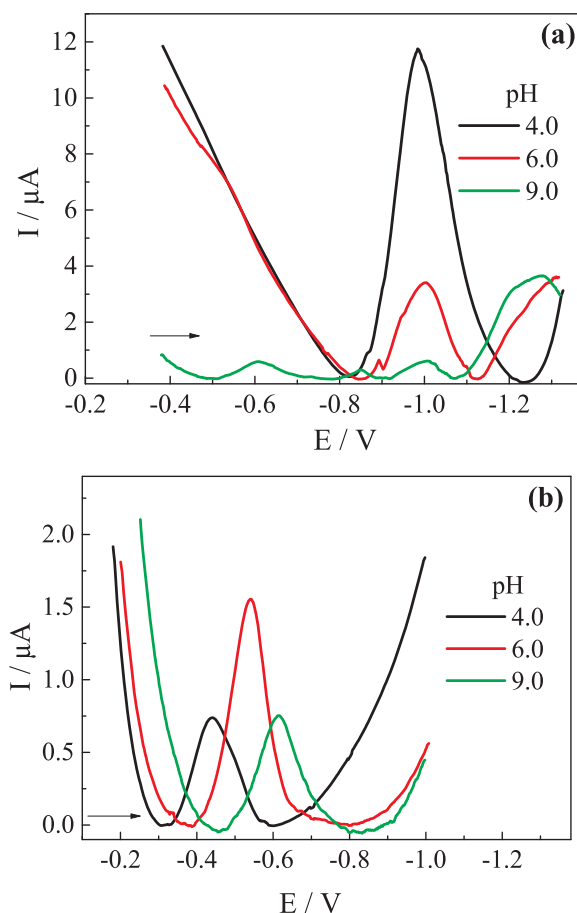


Fig. 1. Square wave voltammograms for reduction of the $3.00 \times 10^{-5}\ \text{mol L}^{-1}$ of MT at p-AgSAE using $0.04\ \text{mol L}^{-1}$ of BR buffer pH 4.0, 6.0 and 9.0, with $\alpha = 50\ \text{mV}$, $\Delta E_s = 2\ \text{mV}$ and $f = 100\ \text{s}^{-1}$. (a) Without CTAB addition. (b) With $1.00 \times 10^{-5}\ \text{mol L}^{-1}$ of CTAB addition.

the inclinations occurred in pH values lower than the reported pK_a value of MT. Besides, the slope in second inclination is around $0.0592\ \text{V}$, typical values expected to redox reactions involving one electron and one proton transfer [36].

Further experiments were successively performed indicating a considerable I_p decrease, probably related to a strong products adsorption of the redox process on the electrode surface. According to a previous work, the p-AgSAE is previously polarized applying $-2.20\ \text{V}$ and the use of CTAB, a non-electroactive surfactant with positive charge, can produce a thin layer at p-AgSAE surface promoting the stabilization of the voltammetric signal [28]. So, $1.00 \times 10^{-5}\ \text{mol L}^{-1}$ CTAB, below c.m.c ($\sim 9.2 \times 10^{-4}\ \text{mol L}^{-1}$) [27], was added in electrochemical cell and SWV experiments were performed in BR buffer with pH 4.0, 6.0 and 9.0.

The comparison between the voltammetric responses without (Fig. 1a) and with (Fig. 1b) CTAB addition indicated that the voltammetric behavior of MT was considerably altered in CTAB medium, and the intensities in these alterations were related to the pH of BR buffer, confirming that considerable effects occurred on the mechanisms and kinetics of electron transfer occurred. In addition, the use of CTAB promoted a considerable dislodgment in E_p to less negative values, which is related to a lower energy to redox process and more favorable redox potential, despite the decrease in I_p values.

New experiments were performed using BR buffer in pH values from 2.0 to 12.0 with $1.00 \times 10^{-4}\ \text{mol L}^{-1}$ of CTAB and $3.00 \times 10^{-5}\ \text{mol L}^{-1}$ of MT, presented in Fig. 2a, where can be observed a significant increase in I_p values when pH was increased, with a maximum I_p in pH 9.0, above this value the I_p decreased. Besides, the E_p shifts

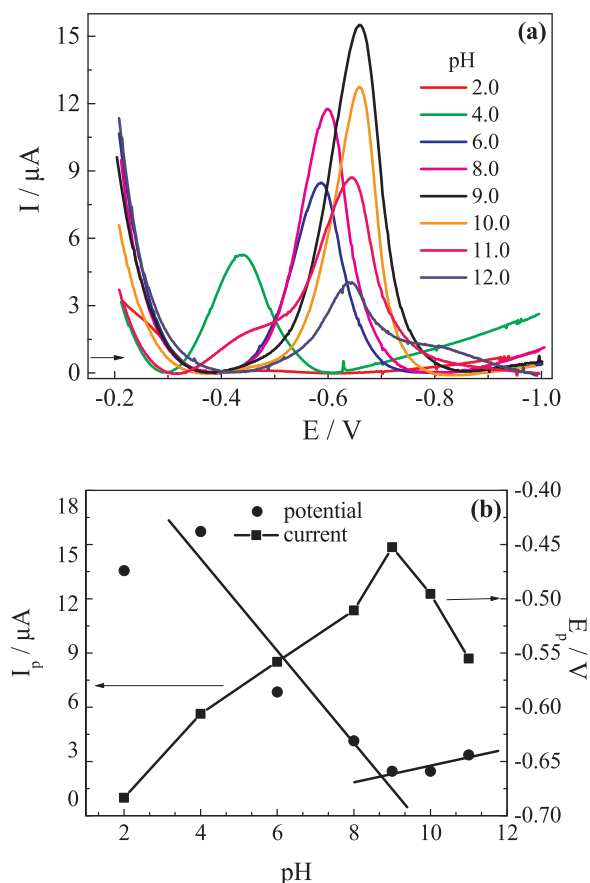


Fig. 2. (a) Square wave voltammograms for reduction of the 3.00×10^{-5} mol L⁻¹ of MT at p-AgSAE in 0.04 mol L⁻¹ of BR buffer pH 9.0 and 1.00×10^{-4} mol L⁻¹ of CTAB, with $\alpha = 50$ mV, $\Delta E_s = 2$ mV and $f = 100$ s⁻¹. (b) Relationships between peak current and peak potential versus pH obtained from square wave voltammograms presented in (a).

towards more negative values as the pH increases until pH 9.0, characteristic of the reactions involving protons during electrons transfer reaction. After pH 9.0, the E_p was independent of pH values, indicating that the MT reduction occurs only by electrons transfer, without

protons involving. These behaviors confirmed the change in redox mechanisms after the CTAB addition.

The relationship between E_p and pH values, Fig. 2b, shown a linear dependence, represented by two straight lines described by following equations:

$$E_p = -0.291 - 0.0336\text{pH (First inclination)} \quad (3)$$

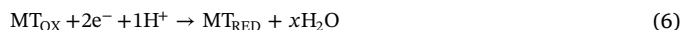
$$E_p = -0.729 + 0.007\text{pH (Second inclination)} \quad (4)$$

where values of potential are given in volts.

The almost vertical line observed in pH values below 9.0 (Eq. (3)) indicated that occurs a redox equilibrium involving both protons and electrons. Additionally, comparison between the theoretical value predict to Nernst Equation, Eq. (5), the first inclination of the 0.0336 V by pH unit can be related to a redox reaction involving one protons and two electrons, in determining step of the redox mechanism, as initially proposed in Eq. (6).

$$E_p = E^0 - 0.0592 \left(\frac{H^+}{n} \right) \text{pH} \quad (5)$$

where H^+ is number of the protons involved in the reaction and the n is electrons number [37].



Eq. (4) is represented by a horizontal line, with inclination only 0.007 V by pH unit, probably related to the electrons transfers without involvement of protons. Besides the values of pH where occurs the interception of two straight lines represent equilibrium between the MT protonated and MT non-protonated and is lower than the value of pK_a reported in literature [35]. On the base of these results, the pH 9.0 was chosen for further analysis.

By analysis of MT structure, MT present 1 group donor of protons and four group acceptors of protons. This way is probable that in $\text{pH} < pK_a$ occurs the liberation of one proton (Eq. (7)), a chemical step, and in $\text{pH} > pK_a$ occurs the reception of protons (Eq. (8)), both followed by electrons transfer [38].



The effects of CTAB addition was evaluated using concentrations from 1.00×10^{-5} to 5.00×10^{-4} mol L⁻¹ of CTBA, and I_p and E_p values obtained were shown in Fig. 3. The differences observed in I_p

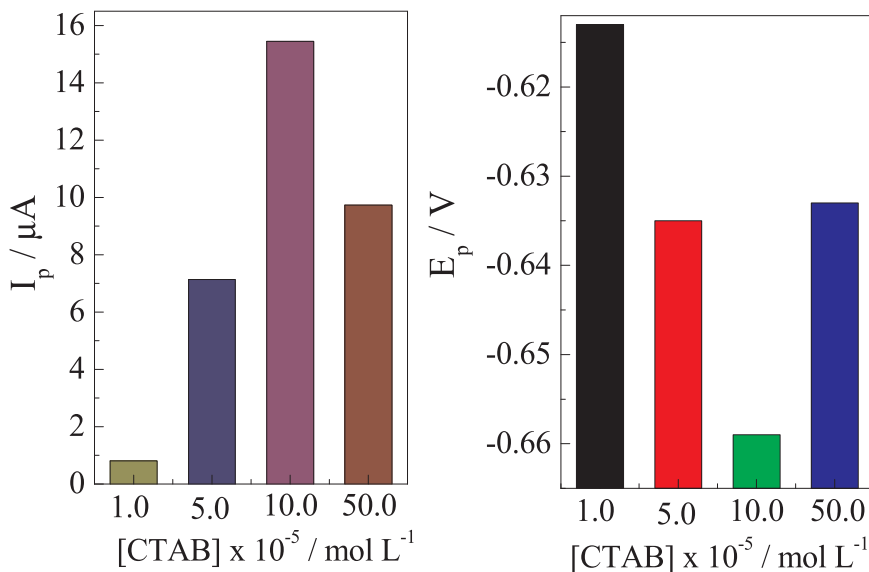


Fig. 3. Signal of peak current (a) and peak potential (b) from SWV experiments performed using 3.00×10^{-5} mol L⁻¹ of MT at p-AgSAE in 0.04 mol L⁻¹ of BR buffer pH 9.0, with $\alpha = 50$ mV, $\Delta E_s = 2$ mV and $f = 100$ s⁻¹ and different CTAB concentrations.

were related to dependence of the amount CTAB adsorbed onto the p-AgSAE. Probably, the CTAB was adsorbed due to the electrostatic attraction between the charged head group of the surfactant and the opposite charge of the p-AgSAE previously polarized. In CTAB concentrations below c.m.c. there is no interaction between the CTAB molecules either solution on solid surface.

Additionally, the CTAB concentrations near the c.m.c. can form full coverage aggregates in electrode surface, increasing the electrons transfers resistance and reducing the analytical sensitivity [27]. So, in this work, the increase in CTAB concentration initially promotes the CTAB adsorption and a little interaction of between each CTAB molecule. The increase in CTAB concentration, adsorption also increases, and aggregates begin to form in p-AgSAE surface, which decrease the I_p e increase E_p values. So, $1.00 \times 10^{-4} \text{ mol L}^{-1}$ of CTBA was chosen to subsequently experiments.

CTAB effects can be demonstrated by *Graphical Abstract*. In absence of CTAB, the p-AgSAE presents a negative charge from polarization at -2.2 V , and in $\text{pH} < \text{pK}_a$ the MT deprotonated presented similar charge of the p-AgSAE, occurring an electrostatic repulsion and producing higher E_p values. However, after the CTAB addition, in low concentration, the first stage of the adsorption from solution is thought to result from neutralization of the surface of the p-AgSAE minimizing the electrostatic repulsion between MT and p-AgSAE and for this, decreasing the E_p to reactions.

3.2. Voltammetric parameters and electrochemical behavior

Using BR buffer ($\text{pH } 9.0$), $1.00 \times 10^{-4} \text{ mol L}^{-1}$ of CTAB solution and $5.00 \times 10^{-7} \text{ mol L}^{-1}$ of MT in electrochemical cell, SWV experiments were conducted for the determination of voltammetric parameters f , a and ΔE_s that promote the acquisition of best analytical signal in terms of I_p , E_p and $\Delta E_{1/2}$. So, the ΔE_s , was varied from 2 to 10 mV and SWV responses shown that the increase in ΔE_s promoted an increase in the I_p signal without linear dependence between I_p and ΔE_s , which, according to SWV theory [25,26], is typical of the voltammetric responses in reactions involving reactants and/or products adsorbed on the electrode surface. Additionally, this I_p increase was accompanied by a widening of the peak diminishing the selectivity of the analysis, so 2 mV was used in subsequent experiments.

The a values was considered from 5 to 50 mV and SWV responses demonstrated that an increase in a promoted a linear increase in the I_p values, without considerable shift in E_p or in the $\Delta E_{1/2}$, typical behavior of totally irreversible redox process. Also, it was concluded that the best value of a which MT can be detected is 50 mV.

The f value was changed from 10 to 250 s^{-1} , which is in the near the maximum values permitted by equipment used in this work. The variations in the f promoted proportional increases of the I_p and a fast displacement of the E_p to more negative values. Thus, the relationships between I_p and f values were directly proportional to f , indicating a redox process involving reagent adsorption and/or product adsorption as the rate determining step [25]. Besides, it was possible to observe that the I_p increased linearly with f values until 100 s^{-1} without signal distortions. Thus, in further experiments the value employed to assure the maximum sensitivity was 100 s^{-1} .

The analysis of forward (I_f), backward (I_b) and resultant (I_r) components of currents in SWV were used to obtain mechanistic information of electrochemical process. These components of current were evaluated in different f because their values change the time scale and the apparent reversibility electrode kinetic. So, by SWV responses, can be observed a single sharp I_r and the presence of I_f and I_b current components, with the I_f presenting values higher than obtained for I_b component. The precise shape and location of components currents is function of f values employed.

As occur a decrease in the ratio I_b/I_f and decreased in the separation between forward and backward peaks by the increase in f values, is possible to consider that the kinetic of MT reduction is very slow.

Besides, the considerable asymmetry between I_f and I_b was an indicative that occurs an irreversible or quasi-reversible redox process. As the theoretical models of SWV [25] were used to confirm the nature of redox process, which a quasi-reversible electron transfer, the ratio of the I_p and the corresponding f plotted as the function of the f present a parabolically profile, and occurs a maximum called as “quasi-reversible maximum”, thus, this relationship was evaluated to MT. However, the responses obtained shown never maximum present, confirming that reaction is irreversible, under voltammetric conditions employed. This irreversible nature of MT redox process also was confirmed by cyclic voltammetry experiments, which are characterized by absence of anodic voltammetric peak in opposite scan potential, data not shown.

To MT reaction on the p-AgSAE, the I_f component corresponds to the reduction of reactant and the I_b component corresponds to the oxidation of product. The oxidation process was restricted by a deep diffusion layer of the product that is developed during the scan from 0.0 to -1.2 V , and the backward responses correspond to a reduction reaction. So, the electrochemical behavior of MT corresponds to a totally irreversible reduction and the backward component, in this potential range, is also the reduction current, from a slow diffusion process and a slow electron transfer [25].

The MT reaction also was evaluated using the analysis of the relationship between E_p and f values, where was observed that the E_p values shift towards more negative values by increase in f , typical of totally irreversible redox process. Additionally, the E_p values are varying linearly with the logarithmic value of the f according to:

$$\frac{\Delta E_p}{\Delta \log(f)} = \frac{-2.3RT}{\alpha nF} \quad (9)$$

where R is the gas constant; T is the temperature; α is the electron transfer coefficient; n is the number of electrons; and F is the Faraday constant. From Eq. (9), a plot of ΔE_p vs $\Delta \log(f)$ gives a straight-line curve, with a slope of the 106.1, which correspond to αn values. Considering $\alpha = 0.5$, and substituting the known values of R , F , and T , the room temperature, into the right-hand side of Eq. (9), and the known value of the slope, n was determined to be equal to 1, in the determining step of the reduction process, called as slow step.

Based on the above-mentioned results, in conjunction with information in literature reports [28,39] allied to structure of MT molecule, we believe that probably, in the experimental and voltammetric conditions employed in this work, the electro-reduction of imide group is the most favorable that carbonyl group reduction. So, the possible mechanism for the reduction of MT involves a slow one-electron reduction of imide group producing a radical anion. The heteroatom N contributes to the stability of the electrochemically generated radical, which immediately abstract protons from the solution, as demonstrated in Fig. 4. So, the reaction occurred in two irreversible steps, one electrochemical involving two-electron transfer, followed by a chemical step involving two-protons.

3.3. Analytical curves

Using the optimized parameters described early, analytical curves was constructed for the range of concentration values from $1.00 \times 10^{-7} \text{ mol L}^{-1}$ to $1.00 \times 10^{-6} \text{ mol L}^{-1}$. For this, aliquots from the MT stock solutions were consecutively added into the electrochemical cell containing 10 mL of the BR buffer and CTAB, and SWV experiments were performed.

The SWV results showed a proportional increase in I_p with the increase of the concentration of MT, without displacement in E_p , confirming that not occur adsorption of the products reaction. In this step, three analytical curves were constructed, and the results were used in the calculus of analytical parameters, which represent a medium of the all obtained analytical curves.

The analytical parameters, or merit figures, were calculated to

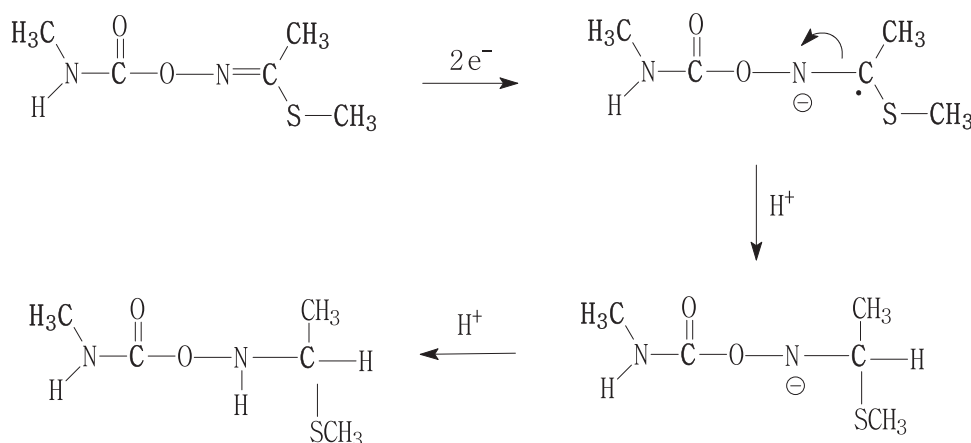


Fig. 4. Mechanism proposed to MT reduction process at p-AgSAE.

Table 1

Analytical parameters for the determination of MT in BR buffer on p-AgSAE, where r: correlation coefficient; S_b : standard deviations of the arithmetic mean of ten blank solutions; s: slope of the analytical curves; LOD: detection limits and LOQ: quantification limits.

Parameter	This work	Laccase biosensor [23]	DDB [25]
Concentration range (mol L^{-1})	1.00×10^{-7} to 1.00×10^{-6}	9.80×10^{-7} to 9.00×10^{-6}	6.60×10^{-6} to 4.10×10^{-4}
r	0.9958	0.9993	0.9934
s ($\text{A} / \text{mol L}^{-1}$)	16.3	NR	0.20
S_b (A)	1.68×10^{-7}	NR	NR
LOD (mol L^{-1})	3.09×10^{-8} ($5.01 \mu\text{g L}^{-1}$)	2.35×10^{-7} ($38.1 \mu\text{g L}^{-1}$)	1.90×10^{-5} (3.10 mg L^{-1})
LOQ (mol L^{-1})	1.03×10^{-7} ($16.7 \mu\text{g L}^{-1}$)	7.80×10^{-7} ($126 \mu\text{g L}^{-1}$)	6.30×10^{-5} (10.2 mg L^{-1})
Repeatability (%)	1.49	2.10	2.50
Reproducibility (%)	5.62	4.40	3.40
Recovery (%)	100.2 (electrolyte)	105.9 (tomato)	79.2 (tap water)

NR = no reported.

validate the proposed methodology to analysis of MT. The concentration range, which demonstrate the concentrations values where the I_p increase proportionally by concentration added and correlation coefficient (r), which determines the degree of linearity between the concentration of MT and analytical responses, were initially evaluated. Besides, the standard deviation of the arithmetic means of ten blank solutions for SWV experiments (S_b), the slope of the analytical curve (s), the detection limit (LOD), and the quantification limit (LOQ), repeatability, reproducibility and recovery values in supporting electrolyte were calculated and are presented in Table 1, which will be discussed below.

The relationships between I_p and MT concentrations added can be represented by:

$$I_p(\text{A}) = 8.01 \times 10^{-7} (\mp 3.20 \times 10^{-7}) + 16.28 (\mp 0.60) [\text{MT}] (\text{mol L}^{-1}) \quad (10)$$

which presented a correlation coefficient of 0.9958. The linearity information was evaluated by analysis of the correlation coefficient (r), presented a value of the 0.9958, which is considered evidence of acceptable fit of the data to the regression line ($r > 0.99$) [34].

The sensitivity of the proposed procedure was evaluated by analysis of the LOD and LOQ values, which were calculated as described in the Section 2. So, ten replicate blanks were analyzed to calculate the mean value of the I_p related to the E_p value to reduce the MT and the standard deviation of its values. The LOD and LOQ were individually calculated and the obtained values were $3.09 \times 10^{-8} \text{ mol L}^{-1}$ ($5.01 \mu\text{g L}^{-1}$, ppb) and $1.03 \times 10^{-7} \text{ mol L}^{-1}$ ($16.7 \mu\text{g L}^{-1}$, ppb), respectively.

These LOD and LOQ values are lower than those obtained by other works that employed Laccase enzyme inhibition responses [22,23] and boron doped diamond electrode [24]. Besides, the sensitivity presented in this work was very close to those obtained by use of traditional chromatographic techniques allied to solid phase extraction [9–16]. This way, the proposed procedure using p-AgSAE allied to CTAB

addition and SWV as analytical technique is an adequate, fast, and sensitivity methodology and can be used to analysis of ultra-trace of MT in complex samples.

All results in this work shown that p-AgSAE presents the great advantages of minimizing adsorptive problems related to the use of solid surfaces and the inactivation of immobilized enzymes related to the biosensor uses. Additionally, the LOD and LOQ values calculated in the present work are satisfactory as they are lower than the maximum values allowed by CONAMA (the National Counsel for the Environment – Brazil) for wastewaters, i.e., $10 \mu\text{g L}^{-1}$ (10.0 ppb) [40].

The precision was evaluated from data of the reproducibility and repeatability using multiple SWV experiments with $5.00 \times 10^{-7} \text{ mol L}^{-1}$ MT and previously optimized parameter. The reproducibility calculus included five different measurements in different solutions at various days (*interday precision*) and the RSD was 5.60%. The repeatability experiments included ten subsequent experiments performed in the same solution and at the same time (*intraday precision*) and the RSD calculate was 2.20%. Both RSD were calculated as described in the Section 2 and obtained values are indicative of the good precision of the proposed methodology.

Recovery curve was used to evaluate the accuracy of proposed procedure and were constructed by spiking a known concentration of the MT ($3.00 \times 10^{-7} \text{ mol L}^{-1}$, $[\text{MT}]_{\text{added}}$), either supporting electrolyte and evaluating the voltammetric responses by the standard addition method [41]. The recovery percentages were used to evaluate and quantify the MT that was added. In this way, the recovery concentration ($[\text{MT}]_{\text{recovered}}$) was identified graphically, with the abscissa axis denoting the concentration of Methomyl in the electrochemical cell. Extrapolating the curve along this axis yields the spiked concentration, allowing for the calculation of the recovery values (%R), as shown in Eq. (12):

Table 2

Analytical parameters obtained in natural water samples for MT detection using p-AgSAE combined with SWV. The same parameter evaluated in supporting electrolyte. All data were evaluated using medium values from three analytical and recovery curves.

Sample	From Calibration Curves [MT] calculated (mol L ⁻¹)	From Recovery Curves		
		[MT] recovered (mol L ⁻¹)	Recovery(%)	[MT] calculated (mol L ⁻¹)
1	8.90×10^{-7}	3.70×10^{-7}	123.3	3.08×10^{-7}
2	2.73×10^{-6}	3.03×10^{-7}	101.0	3.01×10^{-7}
3	1.32×10^{-6}	3.84×10^{-7}	126.7	1.00×10^{-7}

$$\%R = \frac{[MT]_{\text{recovered}}}{[MT]_{\text{added}}} \times 100 \quad (12)$$

Recovery curve also was evaluated in triplicate, using three different solutions of BR buffer. The average concentration recovered was 3.01×10^{-7} mol L⁻¹ and the %R calculated was 100.2%. These results shown that the proposed procedure to be in a suitable range for analytical applications, which are acceptable values from 70% to 130% for recovery percentages [41], indicating that the present methodology can be successfully applied to the analytical detection of MT in complex samples, such as, natural waters.

3.4. Analytical applications

This proposed procedure was applied in natural water samples collected in three different points in a river located at Patos de Minas, Minas Gerais state, as described in the Section 2. The collecting points were selected based on the different levels of organic matter, including industrial and domestic pollution. The water samples were used, as received, with only one filtration step having been previously carried out to prepare 0.04 mol L⁻¹ BR buffer, which was the pH adjusted to 9.0.

Preliminarily each sample was contaminated by 3.00×10^{-7} mol L⁻¹ of MT and SWV experiments were performed. In each sample, the I_p obtained was added in Eq. (10) and resultant MT concentration calculated, called were as $[MT]_{\text{found}}$. The difference observed between MT concentrations measured and MT concentration intentionally spiked, could be used to evaluate the level of contamination of the samples by MT residues.

However, as the samples were employed without pretreatment or purification steps, these responses also can be related to other organic and inorganic compound presents in these samples, which could be reduced at around the E_p of MT, and this way, to interfere in analytical responses. To confirm this suspicion, recovery curves were constructed in these samples, similar to effectuated in electrolyte support. The recovery values calculated are shown in Table 2.

The quantity of MT in each natural water sample ($[MT]_{\text{found}}$) was calculated from difference between the spiked intentionally (3.00×10^{-7} mol L⁻¹) and the values obtained from the use of calibration and recovery curves. The dates showed in Table 2, indicate that the $[MT]_{\text{found}}$ from calibration curves presented values higher than obtained by recovery curves, either the components of the natural water contributed to the analytical responses. The use of recovery curves promotes the interference elimination, by use of addition standard method, which, the only effects due to MT addition is measured. Thus, the $[MT]_{\text{found}}$ from recovery curves, probably, are related to a real contamination of the natural water by MT which is employed in agricultural practices around the local of the collect of the samples.

So, by analysis of recovery values calculated, it is possible to confirm that the proposed procedure has therefore proven to be applicable for use in complex samples, such as natural water. The values $[MT]_{\text{found}}$

calculated indicated that the proposed procedure presented a high robustness, with suitable recovery in 100% of the interference, since these samples no were previously clean up or pre-concentrates.

4. Conclusions

The square wave voltammetric responses of MT pesticide on the p-AgSAE showed the presence of one well-defined voltammetric peak towards the negative sweep direction, which the E_p values were strongly dependent of pH of the medium. The use of CTAB promoted a considerable change in the kinetic and mechanism of the redox reaction, for this the CTAB concentration was previously optimized.

The best voltammetric responses were obtained in 0.04 mol L⁻¹ BR buffer pH 9.0 solution on the p-AgSAE, with 1.00×10^{-4} mol L⁻¹ of CTAB, $f = 100 \text{ s}^{-1}$, $\alpha = 50 \text{ mV}$, $\Delta E_s = 2 \text{ mV}$. Analysis of a purified laboratory electrolyte using the p-AgSAE allowed for a very low detection limit of $5.008 \mu\text{g L}^{-1}$ associated with a high level of repeatability and reproducibility. The analytical sensitivity is comparable to results previously published using high performance liquid chromatographic coupled to ultraviolet/visible detector, with a previously solid phase extraction step, and lowers than electroanalytical data obtained using biosensor.

After the optimization of the experimental and voltammetric parameters was observed that the redox process is related to an irreversible redox reaction, controlled by adsorption, involving two-electron transfer of imide group, producing a radical anion, which immediately abstract protons from the solution.

The application of the proposed procedure in MT analysis in natural water samples, using the calibration and recovery curves, to determine MT concentration led to very coherent results, indicating that the proposed electroanalytical procedure is an important tool to detect MT residues in small concentrations, without pre-treatment of the samples and/or pre-concentration steps. This method provides information about the redox mechanism, which is of considerable importance since it allows for a better understanding of the MT behavior in the environment and of the possible interferences in the application of this procedure in others complex samples.

Finally, this work demonstrated that the p-AgSAE can be considered an environmentally friendly tool and a very interesting alternative for mechanistic studies and the analytical determination of MT and could be applied to other carbamates analysis in natural waters.

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