Electrooxidation of trifloxystrobin at the boron-doped diamond electrode: electrochemical mechanism, quantitative determination and degradation studies

Joseany M. S. Almeida\textsuperscript{a}, Carlos A. T. Toloza\textsuperscript{a}, Rafael M. Dornellas\textsuperscript{b}, Andrea R. da Silva\textsuperscript{c} and Ricardo Q. Aucélio\textsuperscript{a}

\textsuperscript{a}Chemistry Department, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rio de Janeiro, Brazil; \textsuperscript{b}Chemistry Institute, Universidade Federal de Uberlandia, Uberlandia, Brazil; \textsuperscript{c}Centro Federal de Educação Tecnológica Celso Suckow da Fonseca (CEFET/RJ), Valença, Brazil

\textbf{ABSTRACT}

The boron-doped diamond (BDD) presents attractive electrochemical sensing characteristics that are useful in analytical applications based on voltammetry and amperometry. It has a wide potential window in aqueous solutions enabling the quantification of the fungicide trifloxystrobin, measured at +1744 mV (versus Ag/AgCl), by square-wave anodic voltammetry in a Britton–Robinson (BR) buffer (0.04 mol L\(^{-1}\); pH 4.00)/acetonitrile 70/30% v/v. The activation of the electrode was made using galvanostatic chronopotentiometry and cyclic voltammetry (CV). The linear analyte addition curve, \(I_p (\mu A) = (1.0 \times 10^{-1} \pm 4.8 \times 10^{-5}) C (mol L^{-1}) + (8.8 \times 10^{-2} \pm 1.1 \times 10^{-3})\); \(R^2 = 0.997\), was obtained using amplitude of 40 mV, frequency of 30 Hz, step potential of 20 mV. The instrumental limit of detection (LOD) was \(1.4 \times 10^{-7}\) mol L\(^{-1}\) (0.058 mg L\(^{-1}\)) and the dynamic linear range covered three decades (up to \(1 \times 10^{-5}\) mol L\(^{-1}\) or 4.1 mg L\(^{-1}\)). The samples were analysed with recoveries about 80% in orange juice samples and from 92.4% to 104.0% in water samples. A study to evaluate potential interferences was made in the presence of other fungicides. Diagnostic studies indicated that oxidation of trifloxystrobin in aqueous medium at the surface of the BDD is irreversible, involving two steps, each one with two electrons. The UV degradation of trifloxystrobin was evaluated using the proposed electrochemical method and the kinetics of degradation established with half-life of 1.07 min.

\textbf{ARTICLE HISTORY}

Received 11 March 2016
Accepted 9 July 2016

\textbf{KEYWORDS}

Trifloxystrobin; voltammetry; boron-doped diamond electrode; natural waters; orange juice; photodegradation

\section*{1. Introduction}

Pesticides play a key role in agriculture, but the proper control of their residues in food and in the environment is crucial to evaluate potential harm to the population \cite{1}. The application of fungicides in fruit trees and in vegetables is made by spraying the leaves so that the active molecule is gradually and steadily absorbed, allowing prolonged and uniform plant protection \cite{2}. Trifloxystrobin is a synthetic strobilurin class fungicide used...
in the production of important commodities such as soybean and orange. In function of the high quantities of pesticide employed in large plantations, often nearby water-bodies, the level of trifloxystrobin must be monitored. According to the Brazilian National Health Surveillance Agency, that follows criteria adopted by other international health agencies, the acceptable daily intake value for trifloxystrobin is 30 μg kg^{-1} body weight [3]. In addition, the Codex Alimentarius [4] establishes maximum residue limits for trifloxystrobin of 40 μg kg^{-1}, thus the level of this pesticide must be monitored to guarantee no harm for population nearby plantations and also to evaluate if the crop production meets adequate standards to be exported to countries with rigid sanitary regulations.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) were the main tools for the determination of strobilurins (including trifloxystrobin). GC with tandem mass spectrometry (MS/MS) was used to determine trifloxystrobin, along with other pesticides, in fruits and vegetables after liquid–liquid extraction (LLE), with recoveries from 89% to 106% [5]. The determination of strobilurin fungicides (trifloxystrobin included) in wheat, apples and grapes was made using GC with three different types of detection and previous extraction of analytes from samples using LLE and clean-up by gel permeation chromatography (GPC). The recoveries varied from 70% to 110% and LOD for trifloxystrobin in samples varied from 0.004 to 0.014 mg kg^{-1} [6]. Bo et al. used gas chromatography– mass spectrometry (GC-MS) to determine eight strobilurin fungicides in fruits, vegetables, beverages, cereals, nuts, meat, eggs and milk [7]. The extraction of the analytes was performed by GPC. The limit of quantification (LOQ) for trifloxystrobin was 0.005 mg kg^{-1} with recoveries from 69% to 120%. Seven strobilurins were determined in baby food samples by GC-MS using solid-phase microextraction (SPME) on a fibre polydimethylsiloxane-divinylbenzene [8]. The LOQ for trifloxystrobin was 0.024 μg kg^{-1} with 96% of recovery. Strobilurin fungicides were also determined, in grapes and wines, using a variant of this method by using a programmed vapourisation temperature of sampling, LLE and clean-up using solid-phase extraction (SPE) with stationary phase of a double-layer graphite/ethylenediamine-N-propyl [9]. The LOQ for trifloxystrobin was 0.002 and 0.003 mg kg^{-1} for white wine and red wine, respectively, with recoveries within 98–101%. GC-MS was also used for the determination of seven strobilurins (including trifloxystrobin) along with other fungicides in fruits and fruit juices. Pretreatment of the samples was performed with liquid–liquid microextraction (LLME) assisted by ultrasound. The LOD was 0.01 μg L^{-1} for trifloxystrobin with recoveries from 101% to 117% [10].

Four strobilurin fungicides were determined in grape and wine by HPLC with absorption photometric detection (HPLC UV) at 210 nm [11]. The analytes were extracted by LLE and the clean-up/pre-concentration performed by SPE on a silica cartridge. For trifloxystrobin, the LOD was 0.150 mg kg^{-1} in grapes (about 0.150 mg L^{-1} for wines). The recoveries were from 85% to 94%. The determination of seven strobilurins in fruits using HPLC UV was made after the pretreatment of samples by adsorptive extraction on a stirring bar modified with an adsorbent layer. The LOQ was 0.9 μg kg^{-1} for trifloxystrobin (recoveries from 89% to 98%) [12]. HPLC UV was also used to determine strobilurin fungicides in fruit juice samples after LLME procedure assisted by ultrasound. LOD of 4 μg L^{-1} was obtained for trifloxystrobin (recoveries in two fortification levels ranging from 88.5% to 97.5%) [13]. In 2015, Yang et al. [14], combined magnetic solid-
phase microextraction (MSPME) and dispersive LLME with a ionic liquid, to determine four pesticides including triﬂoxystrobin. The analytes were determined in water samples and were extracted from the sample to the adsorbent and then desorbed in acetonitrile prior to analysis. The LOD was 0.03 µg L⁻¹ for triﬂoxystrobin.

The efficient separation of species on a chromatographic column, the ability to determine a large number of pesticides of different classes in a single run and the consolidated maturity of GC and HPLC make these techniques obvious choices for analytical monitoring of pesticides. However, complex sample cleaning/extraction procedures are required and the detection is usually poor by photometric absorption (near 210 nm) in the case of strobilurins. In addition, both cost of instrumentation and often required maintenance, particularly for approaches in tandem, are the driving force for the development of simpler analytical methods to be used in the field and for screening of fruit and fruit extracts to be liberated for food processing and also to evaluate the effect of pesticide crop application in nearby waterbodies.

Literature reports the successful use of boron-doped diamond (BDD) for the voltammetric determination of pesticides such as picloram [15,16], atrazine [17], metamitron [18], linuron [19], mehiocarb [20], triclopyr [21] and fenfuram [22]. Currently, the literature does not report any electrochemical method for the determination of triﬂoxystrobin although recently reported amperometric and voltammetric methods have been addressed the determination of other strobilurin class fungicides [23–26]. The present work describes the triﬂoxystrobin redox behaviour on a BDD establishing a plausible mechanism. The voltammetric method, developed taking into advantage the electrochemical response, was used to determine triﬂoxystrobin in natural waters and in orange juice samples. The method was also used to monitor the UV-induced degradation of the fungicide to stabilise its kinetics parameters.

2. Experimental

2.1. Instrumentation

The electrochemical studies were made using a potentiostat/galvanostat (µ-AUTOLAB Type III, Metrohm, The Netherlands) interfaced to a personal computer and operating in the voltammetric analysis square-wave mode, cyclic voltammetric mode and in the galvanostatic chronopotentiometric mode. The working electrode was a 1.0 cm² polycrystalline BDD (p-doped; 1.0–1.5 micrometer thick; 6000–8000 mg kg⁻¹ boron doping) from Adamant Technologies, Switzerland. The Ag/AgCl (in saturated KCl) electrode was used as the reference and a platinum wire was used as the auxiliary electrode. The solutions were placed in a laboratory-made cylindrical cell made of Teflon (15 mL total volume). The auxiliary and the reference electrodes were immersed into solution from the top of the cell while the BDD, set on the top of a copper plate (to establish an electrical contact with the potentiostat) was placed at the bottom of the cell.

UV-vis absorption spectra were acquired on a Perkin Elmer Lambda 35 double-beam spectrophotometer using 1 cm quartz cuvettes. GC experiments were made on a model TRACE 1300 ISQ Single Quadrupole gas chromatographic system (Thermo Scientiﬁc, USA) with MS detector. A chromatographic open tube column DB-5MS (60 m × 0.25 mm and 0.25 µm from J&W Scientiﬁc, USA) was used. A pH meter (model mPA-210, MS Tecnopon,
Brazil) with a pH-combined glass electrode with an Ag/AgCl (in saturated KCl) reference electrode was used. A total carbon analyser, model TOC-V CNP, Shimadzu, was used to determine the organic, inorganic and the total carbon present in the water samples. The conductivity of the sample was measured with a model MA150, MS conductivimeter from Technopon. A lab-made photochemical reactor was used to expose trifloxystrobin solutions to the UV light [27,28]. The reactor consisted of six mercury lamps (germicide mercury lamp of 6 W) placed at the internal side of a cylindrical structure (reaction chamber) covered with aluminium foil in order to reflect radiation inside the reactor chamber. Only two of the lamps, in opposite sides, were kept on during the experiment. The quartz tubes were placed in a rotatory turret enabling them to receive the same radiance exposure from the lamps during a rotation cycle (five cycles per minute).

2.2. Reagents and samples

All solutions were prepared using ultrapure water (resistivity less than 18 MΩ cm) obtained from a water purifier Milli-Q Gradient System A10, Millipore (USA). Fluoxastrobin (99.0%), kresoxim-methyl (99.0%), trifloxystrobin (99.0%), picoxystrobin (99.0%), tebuconazole (99.0%) and ciproconazole (99.0%) were from Riedel-de-Haen (Seelze, Germany). Acetonitrile (ACN), boric acid, hydrochloric acid, sulphuric acid, phosphoric acid and sodium hydroxide were from Merck (Germany).

Water samples were obtained from the Rodrigo de Freitas Lagoon (Rio de Janeiro, Brazil), from the Rainha Creek (Rio de Janeiro, Brazil). In addition, tap water and commercial mineral water were also analysed. Fermented soy orange juice sample were bought in the local market.

2.3. Solutions, standard solutions and sample preparation

Pesticide stock solution (1.0 × 10^{-3} mol L^{-1} or 0.41 g L^{-1}) was prepared in ACN and, when required, diluted in BR buffer (0.040 mol L^{-1}; pH 4.0) to prepare standards of lower concentrations. The supporting electrolyte, BR buffer (0.040 mol L^{-1}), that consists of boric acid, phosphoric acid and acetic acid was prepared in water and the pH adjusted by adding appropriate amounts of sodium hydroxide 1 mol L^{-1}.

The samples of mineral water, tap water, water from the Rainha Creek and the water from the Rodrigo de Freitas Lagoon did not require any pretreatment prior to analysis. Volume of 5.00 mL of water samples were fortified with two analyte concentration levels (5 × 10^{-6} mol L^{-1} or 2.0 mg L^{-1} and 7 × 10^{-6} mol L^{-1} or 2.9 mg L^{-1}). After analyte fortification, 1.00 mL of each water samples was introduced into the electrochemical cell that contained 10.0 mL of supporting electrolyte (BR buffer 0.040 mol L^{-1}; pH 4.0). For orange juice, a fraction of 2.5 mL of sample was diluted in ultrapure water to 5.0 mL and fortified with trifloxystrobin. These samples were loaded into a C-18 SPE cartridge (3.0 mL and 500.0 mg of the solid phase, Varian, USA) and washed with 20.0 mL of ultrapure water. The cartridge had been previously treated with 2.0 mL of ACN, followed by 2.0 mL of water. The retained analyte was eluted with ACN (1.0 mL) and then diluted to 2.0 mL with ultrapure water. An aliquot of 1.00 mL of this eluted solution was added to the electrochemical cell containing 10.0 mL of supporting electrolyte. Quantifications were performed using the analyte addition procedure.
2.4. Characterisation of the water samples

Natural water samples were analysed in order to obtain the total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) values. For the Rainha Creek water, results were 8.68 mg L\(^{-1}\) of TOC, 9.68 mg L\(^{-1}\) of TC and 0.99 mg L\(^{-1}\) of IC. The Rodrigo de Freitas Lagoon water, which is a mixture of seawater and sweet water coming from several water streams, was analysed after a 1:1 dilution with ultrapure water. The results (after correcting for the dilution factor) indicated 9.50 mg L\(^{-1}\) of TOC, 10.51 mg L\(^{-1}\) of TC and 1.01 mg L\(^{-1}\) of IC. The pH of the Rainha Creek water (sweet water) and Rodrigo de Freitas Lagoon water (mixture of sweet water and salty water) samples were, respectively, 6.95 and 7.63 with their conductivities of 144.6 \(\mu\)S cm\(^{-1}\) and 12.94 mS cm\(^{-1}\).

2.5. Electrochemical measurements

Pretreatment of the BDD (activation procedure) was performed daily prior to analysis using galvanostatic chronopotentiometry. Initially, the BDD was immersed in H\(_2\)SO\(_4\) 0.10 mol L\(^{-1}\) and subjected to an anodic pretreatment by applying a current of +0.01 A, during 1000 s, then a cathodic pretreatment was made with −0.01 A, during 1000 s. In addition, a sequence of 10 voltammetric cycles (100 mV s\(^{-1}\)) was applied from −500 to +1500 mV, until signal stabilisation in H\(_2\)SO\(_4\) 0.10 mol L\(^{-1}\). Diagnostic studies of the redox process were made using cyclic voltammetry (CV) with a scan rate up to 400 mV s\(^{-1}\) and a step potential of 2 mV in a potential range from +1050 to +2300 mV using BR buffer (0.040 mol L\(^{-1}\)) as the supporting electrolyte, within the pH range from 2.0 to 12.0, adjusted by adding appropriate amounts of sodium hydroxide. Square-wave voltammetry (SWV) was also used to evaluate the redox process.

For the determination of trifloxystrobin by SWV, BR buffer (pH 4.0; 0.040 mol L\(^{-1}\)) was used as the supporting electrolyte. After 15 s of equilibration time, the scanning potential was made from +1400 to +2300 mV with signal measurement made at +1744 mV. A frequency of 30 Hz, step potential of 20 mV and pulse amplitude of 40 mV completed the electroanalytical conditions. Often, when a systematic loss of sensitivity was observed, the signal response from the BDD was restored through multiple pulse amperometry by applying pulses at +1200 mV pulses (every 0.03 s) and at +1800 mV pulses (every 0.3 s) during about 1000 s, using H\(_2\)SO\(_4\) 0.10 mol L\(^{-1}\) as the supporting electrolyte. Then, 30 sequential cyclic voltammetric scans (within the +1200 to +2200 mV range at a scan rate of 100 mV s\(^{-1}\)) were made until the stabilisation of the measured current. When the cleaning procedure was not able to restore the analyte signal, the electrode activation procedure was performed.

2.6. Study of trifloxystrobin degradation

The UV degradation study was conducted by placing 0.2 \(\mu\)mol of trifloxystrobin (200 \(\mu\)L of trifloxystrobin of the stock solution) in the quartz tubes. The tubes were taken to a water bath (set as 60°C) to gently evaporate the solvent. Then, the tubes were placed in the reactor to expose to the UV the analyte solid film deposited at the walls of the bottom of the tube. One of the tubes was withdrawn from the reactor every 5 min until the last one at 20 min. A volume of 2.0 mL of ACN was added to the tubes to redissolve the solid material adhered to
Then, a 100 µL aliquot of the solution was added to the electrochemical cell to be analysed using the developed voltammetric method. The results were represented as a percent value related to the concentration of the analyte in a solution not exposed to the UV (100%). The experiment was repeated three times in order to get an authentic replicate for each UV exposition time. The experiment with incidence of UV was also made by exposing 0.2 µmol of trifloxystrobin dissolved in 10.00 mL ACN/H$_2$O (40/60% v/v) solution. One of the tubes was withdrawn from the reactor every 5 min until the last one at 20 min. Then, a 100 µL aliquot of each solution was added to the electrochemical cell to be analysed using the developed voltammetric method.

2.7. GC-MS experiments

Analyses using GC-MS system were conducted using an injection volume of 1 µL with a temperature of 300°C, in the transfer line, and 230°C for the ion source. The ionisation was by electron impact (EI) with split injection mode. The analyses used a heating ramp (35–150°C at a rate of 2°C min$^{-1}$) followed by a 15°C min$^{-1}$ temperature ramp in the range from 150°C to 300°C.

3. Results and discussion

3.1. Preliminary studies

As a sensing material, the nanocrystalline BDD has been used for the construction of electrodes that allow the determination of chemical species that are oxidised or reduced in aqueous medium also at potentials above ±1000 mV, which is difficult when using other traditional materials for electrodes [29]. The qualitative assessment of the voltammetric response of trifloxystrobin using the BDD was carried out by CV and the characteristic voltammograms of the analyte in solution were observed over the entire pH range tested, presenting two oxidation peaks (through the anodic scan) with current maxima, respectively, at about +1740 mV ($E_{p1}$) and +2010 mV ($E_{p2}$). As the scan was reversed (cathodic scan), no reduction peaks were found as counterparts of the oxidation ones. In Figure 1(a), typical trifloxystrobin cyclic voltammograms (at pH 4.0) are shown in the potential range from +1200 to +2400 mV. The sequential voltammogram cycles (each cycle made within 2 s and with analyte mass transport to the electrode–solution interface minimised by no mechanical agitation of the solution) showed the continuous decreasing of the trifloxystrobin (at 8.0 × 10$^{-4}$ mol L$^{-1}$ or 0.33 g L$^{-1}$) oxidation peak intensities. By performing the sequential cyclic voltammetric scans for the first peak alone (cyclic scanning from +1200 to +1900 mV), the decreasing of peak intensity to blank level occurred at the 19th cycle (Figure 1(b)). Such behaviour indicates that the first oxidation peak does not depend upon the second oxidation process that occurs at a higher potential (which was avoided in the experiment). On the other hand, by isolating the second peak (cyclic scanning from 1800 to 2400 mV) its intensity is smaller than the one observed when scanning started from +1200 mV, showing a probable dependence of the second oxidation process upon the first oxidation process. The second oxidation peak intensity reaches the blank level already after five voltammetric cycles (Figure 1(c)). In addition, the variation of the scan velocity of the experiment did not affect the overall behaviour of the process concerning the second peak.
The CV study indicated the irreversibility of the mass transfer controlled diffusion process as there was a linear relationship ($R^2 = 0.989$) between the peak intensity ($I_p$), measured at +1740 mV and $\nu$, where $\nu$ was the potential scan velocity varied between 10 and 400 mV s$^{-1}$ (Figure 2(a)). For the second peak (measured at +2010 mV), such linear relationship also held ($R^2 = 0.990$) as seen in Figure 2(b). Voltammograms can be seen in Figure 2(c). The log($I_p$) versus log($\nu$) indicated adsorption-controlled processes as the slope of graphs were 0.988 for the first oxidation (Figure 3(a)) peak and 0.980 for the second oxidation (Figure 3(b)) peak. The linear increasing of $I_p$ as the frequency of the applied pulses ($f$) is increased (up to 70 Hz using SWV) confirmed the irreversibility of the first (Figure 3(c)) and second (Figure 3(d)) oxidation processes.

For irreversible systems, the SWV peak width at half height ($\Delta E_{p/2}$) is modelled by the equation $\Delta E_{p/2} = (65.5 + 0.5)/n\alpha$, where $n$ is the number of electrons involved in the process and $\alpha$ is the electronics transfer coefficient [30,31]. Considering systems with $\alpha$ value equal to 0.5, the experimental $\Delta E_{p/2}$ can be used to calculate the number of moles.
of electrons involved in the redox reaction. As $\Delta E_{p/2}$ was 80 mV, the value for $n$ is 1.6, suggesting that the oxidation process relative to the first peak involves two electrons per molecule. For the second reaction, a two electron ($n = 1.8$) process was also found.

Irreversible reactions are characterised by the linear relationship (with slope equal to $-2,3RT/\alpha nF$) between the maximum peak potential ($E_p$) and the log($f$) [30]. For trifloxystrobin (first oxidation peak), such a linear relationship held (see detail in Figure 3(c)). Through the inclination of the curve, the $\alpha n$ product (product of the electron transfer coefficient and the number of electrons involved in the electrode reaction) was close to 1, which would lead roughly to a value of $\alpha$ of 0.5 for two electrons involved in the reduction. Similar results are found for the second oxidation peak (see detail in Figure 3(d)).

The dependencies of the $E_p$ values in function of pH were studied for both peaks, in the range from pH 3.0 and 6.0. In both cases, neither a clear tendency nor significant variations for the $E_p$ values was found as expected since trifloxystrobins and possible its first oxidation product present no relevant acid/basic characteristics.

According to the literature [32,33] and based on the information collected for each of the oxidation processes, a mechanism for the oxidation can be proposed (Figure 4). The first step resembles the already proposed mechanism for the strobilurin kresoxim-methyl [23], that involves the loss of an electron which gives rise to a radical cation (structure II) followed...
by the abstraction of a proton leading to a radical. The loss of another electron generates a carbocation, which is stabilised by resonance due to electron delocalisation in the aromatic ring. Once the process occurs in aqueous medium, it is possible that one water molecule promotes the nucleophilic attack on the deficient carbon forming the species of structure V. Finally, a cleavage might occur with the formation of two neutral molecules, one of them an acetophenone-oxime (structure VI). Tallec and Tarvel demonstrated that acetophenone-oximes can undergo irreversible electrochemical oxidation by the loss of two electrons leading to the formation of ketones \cite{34} at potentials above +1800 mV and in the presence of 15% water. Therefore, it is believed that in step 2, the acetophenone-oxime (produced in the step 1) might be cleaved with the loss of two electrons and a proton (per molecule), thereby forming the aromatic ketone (structure XI). Thus, the proposed mechanisms justify the irreversible oxidation, promoted by the loss of two electrons for each of the oxidation processes.

3.3. Optimisation of experimental and instrumental conditions

Over the pH range tested (2.0–12.0), both oxidation peaks were more intense between pH 4.0 and 7.0. In this range, the relative intensities of each of the peaks, evaluated using
SWV, were statistically similar (analysis of variance at 95% confidence limit) no matter the pH used. In Figure 1(d), the areas of the voltammetric peak with maximum at +1744 mV are shown over the entire studied pH range. It was observed a clear decreasing of response in the extreme acid conditions and over the basic pH range. The supporting electrolyte at pH 4.0 was chosen to proceed with the development of the method because it produced more reproductive results (below 2% based on 10 sequential measurements on the same solution).

In order to achieve the best experimental conditions for the determination of trioxystrobin using SWV and BDD, a univariate optimisation was performed taking into account the critical parameters affecting the intensity and signal reproducibility. The studied parameters were: (i) pulse frequency \( f \) and (ii) pulse amplitude \( a \). The monitored analytical signal was the area of the peak with maximum at +1744 mV due to the fact that the first voltammetric peak presents better definition and higher signal, obtained at a less positive potential. The step potential was kept at 20 mV. The anodic scanning was applied without forcing any previous deposition of material onto the electrode surface as this strobilurin (or any reaction product) did not accumulate onto the surface of the BDD. All studies were performed in triplicate.

The increase of pulse amplitude, studied in the range from 10 mV to 100 mV, improved the measured signal (peak area) until the amplitude 80 mV. The selected pulse amplitude was 40 mV because of the sharpness (relationship between peak height and peak width). The peak area increased as the pulse frequency was varied from 10 to 70 Hz. The best compromise between peak area and peak width was obtained at 30 Hz. Besides, under such conditions, the scan velocity (the product of \( f \) and \( \Delta E \)) is not too fast, enabling the oxidation reaction to proceed efficiently. Thus, with these parameters chosen, the scan velocity was 600 mV s\(^{-1}\).

**Figure 4.** Proposed trioxystrobin electrochemical oxidation mechanism mediated by BDD.
3.4. Analytical figures of merit

The analytical figures of merit were obtained using the selected experimental conditions to determine trifloxystrobin (Table 1). A sequence of voltammograms of a solution containing increasing concentrations of analyte is shown in Figure 5(a). The analytical signal (current) was directly and linearly proportional to the concentration trifloxystrobin in the electrochemical cell (Figure 5(b)). The analytical curve equation (with the standard deviations of sensitivity and linear coefficient) was \( I_p (\mu A) = (1.0 \times 10^{-1} \pm 4.8 \times 10^{-6}) C (\text{mol L}^{-1}) + (8.8 \times 10^{-2} \pm 1.1 \times 10^{-3}) \); \((R^2 = 0.997)\) and dynamic linear range covered three decades \((10^{-7} - 10^{-5} \text{ mol L}^{-1})\). The measurements were performed in triplicate and the associated errors of sensitivity and linear coefficient in the curve were calculated as the standard deviation. For quantitative purposes, integrated peak area was used as the measured signal. The instrumental LOD was \(1.4 \times 10^{-7} \text{ mol L}^{-1} (0.058 \text{ mg L}^{-1})\) and the instrumental LOQ was \(4.6 \times 10^{-7} \text{ mol L}^{-1} (0.19 \text{ mg L}^{-1})\). The LOD was calculated using \(3s_b/m\) and the LOQ using \(10s_b/m\), where \(s_b\) is the standard deviation estimated by 10 consecutive measurements of the peak of the lower analyte concentration of the analytical addition curve and \(m\) is the slope of the analytical curve. In Figure 5(c), the signal produced by the analyte at the LOQ level is shown against the blank signal.

The instrumental precision was obtained as the variation coefficient of 10 consecutive measurements of the signal produced by the analyte standards. Four different concentrations: \(1 \times 10^{-6} (0.4 \text{ mg L}^{-1})\); \(5 \times 10^{-6} \text{ mol L}^{-1} (2.0 \text{ mg L}^{-1})\); \(1 \times 10^{-5} (4.1 \text{ mg L}^{-1})\) and \(5 \times 10^{-5} \text{ mol L}^{-1} (20 \text{ mg L}^{-1})\) were used. Each of the measurements was made after a solution agitation step to replenish the solution–electrode interface. The intermediate precision was determined by comparing the results obtained from the analysis (10 independent replicates) of a trifloxystrobin-fortified simulated potable water sample at two concentration levels in the electroanalytical cell: \(1 \times 10^{-6} \text{ mol L}^{-1} (0.4 \text{ mg L}^{-1})\) and \(1 \times 10^{-5} \text{ mol L}^{-1} (4.1 \text{ mg L}^{-1})\). Instrumental precision was about 3% at the \(10^{-6} \text{ mol L}^{-1}\) concentration level, decreasing to about 2% at the \(10^{-5} \text{ mol L}^{-1}\) concentration level. The intermediate precision values, estimated by the variation coefficient obtained in analyte-fortified mineral water, were about 10% (at \(1 \times 10^{-6} \text{ mol L}^{-1}\) and 7% (at \(1 \times 10^{-5} \text{ mol L}^{-1}\)).

Studies were conducted to evaluate the potential interference imposed by other fungicides (other strobilurins and triazoles) in the electroanalytical signal produced by trifloxystrobin. The interference was evaluated by the ratio between the signal from a solution containing only the analyte and the signal measured from a solution containing the mixture of the analyte together with other fungicide (strobilurin or triazole), in this case, the analyte concentration is the same in both solutions (\(1 \times 10^{-5} \text{ mol L}^{-1}\) or \(4.1 \text{ mg L}^{-1}\)).

Table 1. Experimental conditions selected for the square-wave voltammetric determination of trifloxystrobin using the BDD.

| Parameter                  | Value                                                                 |
|----------------------------|----------------------------------------------------------------------|
| Supporting electrolyte     | Britton–Robinson buffer (pH 4.0; 0.040 mol L\(^{-1}\))               |
| Amplitude (\(a\))          | 40 mV                                                                |
| Scan increment (\(\Delta E_s\)) | 20 mV                                                              |
| Frequency (\(f\))          | 30 Hz                                                                |
| Monitored signal           | Peak with maximum at +1734 mV                                        |
| Scan potential range       | of +1400 for +2300 mV                                                |
These $I_{\text{analyte}}/I_{\text{analyte} + \text{other fungicide}}$ values near unity did not indicate interference. Values greater than 1 indicate a decrease in the analyte signal due to the presence of another fungicide, while values below unity indicated increasing of the measured signal due to the contribution from the other fungicide. These tests were carried out with fixed concentration of analyte and two different analyte:other fungicide proportions (1:0; 1:1; 1:2 and, in some cases also 1:3). Measurements were performed in triplicate and the calculations were based on the integrated peak area with results shown in Table 2.

Picoxystrobin and kresoxim-methyl were found to interfere when they were twice the concentration of trifloxystrobin, increasing the measured signal due to the proximity of the oxidation peaks from these strobilurins with the ones of the analyte. Fluoxastrobin and tebuconazole interfere in the determination when present at the same concentration of the analyte. For tebuconazole, the interference seems to decrease the analyte signal. In this case, tebuconazole might have the preference in reacting at the electrode surface, slowing down the kinetics of oxidation of the analyte. The interference observed in the presence of cyproconazole has the effect of increasing the measured voltammetric signal to values higher than the expected one.
It is unusual the combined use of different fungicides of the strobilurin class in a specific crop, but the two evaluated triazoles (tebuconazole and cyproconazole) might be associated with trifloxystrobin in some commercial products. Thus, it is important that the analyst get previous information on what products have been used to treat a crop before performing analysis.

### 3.5. Application of method

The proposed method utilising SWV with the BDD was used to determine trifloxystrobin in water samples and in a commercial orange juice fermented with soy milk. The juice sample was chosen as trifloxystrobin is indicated to be used in both citrus and soybean crops. The signals from the original samples were measured before they were fortified with the analyte at $7.0 \times 10^{-6}$ mol L$^{-1}$ (2.9 mg L$^{-1}$) to be quantified by analyte addition method.

In Table 3, the recovery results (authentic triplicates performed in two different days) are shown. Percent recoveries in water samples varied from 92.4% to 104.0%, which showed the good accuracy of the method. For orange juice samples, recoveries were in the 80% range, probably affected by the loss of analyte during SPE sample cleaning procedure. Despite this loss, the method can indicate the contamination of these samples. The accuracy in the analysis of the juice sample might be improved if analyte standard used in the analyte addition curve is also passed though the SPE cartridge to compensate the loss of analyte in the treated sample solution.

![Table 2. Evaluation the interference imposed by other strobilurin and triazole fungicides in the trifloxystrobin (1 $\times$ 10$^{-5}$ mol L$^{-1}$ or 4.1 mg L$^{-1}$) electroanalytical signal$^a$ (n = 3).](image-url)

| Fungicide         | Proportion (trifloxystrobin/other fungicide) | $I_{(trif.)/I_{(trif. + other fungicide)}} \pm s_{ratio}^b$ |
|-------------------|---------------------------------------------|---------------------------------------------------------|
| Fluoxastrobin     | 1:0                                         | 1.00 ± 0.07                                             |
|                   | 1:1                                         | 1.22 ± 0.15                                             |
|                   | 1:2                                         | 2.27 ± 0.17                                             |
| Picoxystrobin     | 1:0                                         | 1.00 ± 0.13                                             |
|                   | 1:1                                         | 1.10 ± 0.14                                             |
|                   | 1:2                                         | 1.47 ± 0.16                                             |
|                   | 1:3                                         | 1.63 ± 0.15                                             |
| Kresoxim-methyl   | 1:0                                         | 1.00 ± 0.04                                             |
|                   | 1:1                                         | 1.15 ± 0.06                                             |
|                   | 1:2                                         | 1.26 ± 0.04                                             |
| Tebuconazole      | 1:0                                         | 1.00 ± 0.05                                             |
|                   | 1:1                                         | 1.98 ± 0.12                                             |
|                   | 1:2                                         | 2.87 ± 0.19                                             |
| Cyproconazole     | 1:0                                         | 1.00 ± 0.01                                             |
|                   | 1:1                                         | 0.84 ± 0.11                                             |
|                   | 1:2                                         | 0.53 ± 0.07                                             |
|                   | 1:4                                         | 0.47 ± 0.09                                             |
|                   | 1:3                                         | 0.53 ± 0.01                                             |
|                   | 1:4                                         | 0.47 ± 0.09                                             |

$^a$For instrumental conditions, see Table 1.
$^b$The standard deviation is the propagated one considering the ratio of signals and their standard deviations:

$$s_{ratio} = I_{(trif.)/I_{(trif. + other fungicide)}} \times [s_{I_{(trif.)}}/I_{(trif.)} + s_{I_{(trif. + other fungicide)}}/I_{(trif. + other fungicide)}]^{1/2}$$
3.6. *UV-induced trifloxystrobin degradation*

The photodegradation of trifloxystrobin upon exposure to UV was evaluated by monitoring the SWV peak current at +1744 mV. The results showed a rapid degradation of trifloxystrobin upon the exposition to the UV light as only 15% of the analyte remains intact after only 5 min of exposition (*Figure 6*(a)).

The degradation profile indicated a second-order kinetics as the linear relationship ($R^2 = 0.987$) was found between the inverse of the determined concentration of trifloxystrobin (1/[trif]) and the UV exposition time (*Figure 6*(b)). The rate constant ($k$) was $3.7 \times 10^{-4}$ L mol$^{-1}$ min$^{-1}$ and the time of half-life ($t_{1/2}$) of 1.07 min. This second-order model of degradation indicated that after 20 min only about 1.3% of trifloxystrobin remained in its

---

**Table 3. Recoveries for trifloxystrobin ($n = 3$ in two different days) in analyte-fortified samples ($7.0 \times 10^{-6}$ mol L$^{-1}$ or 2.90 mg L$^{-1}$).**

| Sample                     | Day | Recovered concentration | Percent recovery (%) |
|----------------------------|-----|-------------------------|----------------------|
|                            |     | mol L$^{-1}$ a           | mg L$^{-1}$ b        |
| Queen Creek water          | 1   | $(7.3 \pm 0.5) \times 10^{-6}$ | $104.0 \pm 6.3$     |
|                            | 2   | $(7.0 \pm 0.1) \times 10^{-6}$ | $100.3 \pm 0.7$     |
| Rodrigo de Freitas Lagoon water | 1   | $(6.8 \pm 0.4) \times 10^{-6}$ | $96.7 \pm 6.6$     |
|                            | 2   | $(6.5 \pm 0.6) \times 10^{-6}$ | $93.1 \pm 8.5$     |
| Mineral water              | 1   | $(6.5 \pm 0.1) \times 10^{-6}$ | $92.4 \pm 2.1$     |
|                            | 2   | $(6.8 \pm 0.8) \times 10^{-6}$ | $96.4 \pm 11.5$    |
| Fermented soy Orange juice | 1   | $(6.0 \pm 0.1) \times 10^{-6}$ | $85.9 \pm 0.6$     |
|                            | 2   | $(5.7 \pm 0.2) \times 10^{-6}$ | $81.9 \pm 2.9$     |

a The recovered values in mol L$^{-1}$ are associated with the standard deviations for $n = 3$.

b The recovered values in mg L$^{-1}$ are associated with their confidence intervals considering the standard deviation for $n = 3$ and a confidence limit of 95%.

---

*Figure 6.* UV exposure of trifloxystrobin: (a) Analyte percent degradation in function of time; (b) The relationship between the inverse of the concentration of trifloxystrobin and time ($R^2 = 0.987$). Instrumental parameters of the analyte determination indicated in Table 1.
original form. For comparison purposes, Dornellas et al. [24] investigated the UV photo-induced degradation of pyraclostrobin, irradiating the pyraclostrobin solution (in ACN). The authors also observed a second-order kinetics of degradation but in a much slower reaction, with \( t_{1/2} \) of 9.8 min. Similarly, performing the experiment irradiating trifloxystrobin in solution \( (1 \times 10^{-3} \text{ mol L}^{-1}) \) in water/ACN 60%/40% v/v). In contrast to what was observed previously by irradiating the solid film of the fungicide, the photodegradation in solution was faster to the point that no voltammetric signal of the original analyte could be measured after 5 min of exposure (first point of the experiment).

The solution containing the photodegraded trifloxystrobin was analysed by GC-MS. The chromatogram showed initially four peaks at retention times (RT) 14.68, 14.88, 15.66 and 15.86 min (circulated in Figure 7(a)). These peaks are characteristic of the four isomers (geometric ones) of the trifloxystrobin (EE, EZ, ZE, ZZ) as discussed by Banerjee et al. when

![Figure 7](image_url)

**Figure 7.** (a) GC chromatogram for the degraded solution trifloxystrobin. (b) Mass spectrum of one of the isomers of trifloxystrobin (RT = 14.64 min).
investigating the exposition of a trifloxystrobin solution to the light form a xenon source [35]. In the mass spectroscopic data, no matter the trifloxystrobin isomer, it was observed peaks at m/z 132, 145, 149 and 207 (Figure 7(b)), which are characteristics of the fragmentation mechanism of the original analyte [36]. Besides the four isomers, the chromatogram shows additional peaks one at RT of 34.00 min and another at RT 52.07 min (Figure 7(a)). The mass spectrum of the chemical species with RT 34.00 min present a peak at m/z 207 whose fragments indicates one of the possible photodegradation products of trifloxystrobin (Figure 8(a)). The structure of the other main possible photodegradation product of the original analyte can be predicted, as the mass spectrum of the peak at RT 52.07 min produce an intense fragment with m/z 173, compatible with the structure shown in the detail in Figure 8(b).
4. Conclusion

A simple and easy to use voltammetric method was developed for the quantification of trifl oxystrobin. The choice of BDD was crucial to enable a measurable signal at a high oxidation potential. The method was sensitive enabling a LOD of $1.4 \times 10^{-7}$ mol L$^{-1}$ (0.058 mg L$^{-1}$) enough for the screening for trifl oxystrobin in field applications. The procedure used to separate the analyte from orange juice sample can be also use to pre-concentrate the analyte in the solid-phase extraction (C18) cartridge improving at the least 10 times the detection of analyte in the sample. Precision and accuracy achieved indicated the reliability of the voltammetric method. The method presents analytical characteristics that are competitive with other electroanalytical methods (used for the detection of strobilurins or for the separation of strobilurins before the detection of their natural absorbance) found in the literature (see Table S1) [24, 26, 37–40]. The proposed method was used to establish the UV-degradation second-order kinetics for trifl oxystrobin. Oxidation on the BDD occurred in two steps and a mechanism was proposed based on diagnostic experiments and literature data. The irreversible oxidation process suggests that the BDD can also be used to degrade trifl oxystrobin in remediation procedures.

Acknowledgements

Aucelio thanks the Brazilian scientific agencies CNPq (474220/2013-3) and FAPERJ (E-26/201.406/2014 and E-26/110.740/2012) for research grants and scholarships. Almeida thanks CNPq for scholarship (141.071/2014-1), Dornellas thanks CNPq for scholarship (400.408/2015-5) and Toloza acknowledges his FAPERJ scholarship (E-26/200.435/2016).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico: [Grant Numbers 302888/2013-6; 141.071/2014-1; 400.408/2015-5]; Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ): [Grant Numbers E-26/110.740/2012; E-26/201.406/2014; E-26/200.435].

References

[1] A.L. Frank, R. McKnight, S.R. Kirkhorn and P. Gunderson, Annu. Rev. Publ. Health 25, 225 (2004). doi:10.1146/annurev.publhealth.25.101802.123007.
[2] H. Balba, J. Environ. Sci. Health B 42, 441 (2007). doi:10.1080/03601230701316465.
[3] BRASIL, Ministério da Saúde. Agência Nacional de Vigilância Sanitária – ANVISA. D.O.U. (8September 2009), p. 50.
[4] CODEX ALIMENTARIUS, Pesticide Residues in Food and Feed. Base. <http://www.codexalimentarius.net/pestres/data/pesticides/details.html?id=229>. <http://www.codexalimentarius.net/pestres/data/pesticides/details.html?id=199>. 
[32] O. Hammerich and H. Lund, *Organic Electrochemistry* (Marcel Dekker, New York, 2001), p. 471.
[33] U. Bussy, V.F. Ferchaud-Roucher, I. Tea, M. Krempf, V. Silvestre and M. Boujtit, Electrochim. Acta. 69, 351 (2012). doi:10.1016/j.electacta.2012.03.007.
[34] L. Bencharif, A. Tallec and R. Tardivel, Electrochim. Acta. 42, 3509 (1997). doi:10.1016/S0013-4686(97)00047-9.
[35] K. Banerjee, A.P. Ligon and M. Spiteller, Anal. Bioanal. Chem. 382, 1527 (2005). doi:10.1007/s00216-005-3336-8.
[36] K. Banerjee, A.P. Ligon and M. Spiteller, Anal. Bioanal. Chem. 388, 1831 (2007). doi:10.1007/s00216-007-1382-0.
[37] W.F. Pacheco, A. Doyle, D.R.A. Duarte, C.S. Ferraz, P.A.M. Farias and R.Q. Aucelio, Food Anal. Methods 3, 205 (2010). doi:10.1007/s12161-009-9109-9.
[38] R.M. Dornellas, R.A.A. Franchini and R.Q. Aucélio, Electrochim. Acta. 97, 202 (2013). doi:10.1016/j.electacta.2013.02.125.
[39] C.F. De Souza, A.L.M.C. Cunha and R.Q. Aucélio, Chromatographia 70, 1461 (2009). doi:10.1365/s10337-009-1318-2.
[40] K. Wang, G. Chen, X. Wu, J. Shi and D. Guo, J. Chromatogr. Sci. 52, 157 (2014). doi:10.1093/chromsci/bmt001.