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A simple and sensitive detection of diquat herbicide using a dental amalgam electrode
A comparison using the chromatographic technique

Luisa Célia Melo[a], Giancarlo Richard Salazar-Banda[b], Sergio Antonio Spinola Machado[b], Pedro de Lima-Neto[a], Djenaine De Souza[c,*], Adriana Nunes Correia[a]

[a] *Universidade Federal do Ceará, Centro de Ciências, Departamento de Química Analítica e Físico Química, Bloco 940 Campus do Pici, 60455-970 Fortaleza, CE, Brazil*
[b] *Instituto de Química de São Carlos, Universidade de São Paulo, Cx. Postal 780, 13560-970 São Carlos, SP, Brazil*
[c] *Universidade Federal de São Carlos, Centro de Ciências Exatas e de Tecnologia, Departamento de Química - Rod, Washington Luiz, Km 235 Monjolinho, Cx. Postal 676, 13565-905 São Carlos, SP, Brazil*

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ABSTRACT

This paper describes the use of a dental amalgam electrode (DAE) to evaluate the electrochemical behaviour and to develop an electroanalytical procedure for determination of diquat herbicide in natural water and potato samples. The work was based on the square wave voltammetry responses of diquat, which presented two well-defined and reversible reduction peaks, at −0.56 V (peak 1) and −1.00 V (peak 2). The experimental and voltammetric parameters were optimised, and the analytical curves were constructed and compared to similar curves performed by high performance liquid chromatography coupled to ultraviolet–visible spectrophotometric detector (HPLC/UV–vis). The responses were directly proportional to diquat concentration in a large interval of concentration, and the calculated detection limits were very similar, around 10 $\mu g\,L^{-1}$ (10 ppb) for voltammetric and chromatographic experiments. These values were lower than the maximum residue limit established for natural water by the Brazilian Environmental Agency. The recovery percentages in pure electrolyte, natural water and potato samples showed values from 70% to 130%, demonstrating that the voltammetric methodology proposed is suitable for determining any contamination by diquat in different samples, minimising the toxic residues due to the use of liquid mercury or the adsorptive process relative to use of other solid surfaces.

1. Introduction

Since the development of polarography by Heyrovsky, around 1920, the use of mercury electrodes has become a suitable tool for the analytical determination and electrochemical behaviour study of the organic and inorganic compounds in different samples [1]. However, the use of liquid mercury working electrodes presents some drawbacks, such as the difficulty of field analysis, or its employment in industries and flowing systems, the use in analysis of compounds in negative potentials, and principally the generation of toxic wastes [2].

For this, the investigation of new materials for the construction of working electrodes that avoid the use of liquid mercury represents an area where the majority of work has been published in the field of electroanalytical research [3].

Different solid surface electrodes, either bare or modified, with different organic and inorganic compounds, have been designed to evaluate the analytical responses and to study the redox mechanisms of different compounds [4–7]. Nevertheless, in this type of electrode, the renovation of the electrodic surfaces can be complicated, due to the memory effects associated with the strong adsorption process of the organic compounds, which, in general, results in a decrease in the reproducibility of the voltammetric responses.

On the other hand, the working electrode constructed by the modification of the electrodic surface by enzymes is thought to improve the sensitivity in analytical determinations, and, in most cases, it presents high costs and difficulties involving the maintenance of its enzymatic activity, which can promote a loss in the analytical responses.

In the last two decades, there has been increasing interest in the construction of solid electrodes using metallic amalgam. These working electrodes are prepared by mixing a fine metal powder (Ag, Cu, Au, Bi, Sn, or Zn) together with liquid mercury [8–15].

Besides, preliminary works published by Mikkelsen and co-workers have demonstrated that dental amalgam is an excellent alternative to common working electrodes for the analytical determination of heavy metals in different samples [16–22].

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[*] Corresponding author. Fax: +55 16 3356 8350.
E-mail address: djenaine@gmail.com (D. De Souza).

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In dental amalgam, silver and mercury are the main constituents and are bound as a stable Ag_2Hg_3 alloy, without free liquid mercury and its construction procedure is very simple and well established in dental clinical practice [23].

These electrodes show a high overpotential to the hydrogen evolution reaction [3,8–24] and besides it can be employed in flowing systems and electrochemical detection in high performance liquid chromatography or capillary electrophoresis for the detection of electrochemically reducible organic and inorganic compounds with trace level sensitivity. The electrodic surface of dental amalgam electrodes allows easy renewal by mechanical polishing or electrochemical regeneration, and presents high mechanic stability, homogeneous surface, and long durability.

So, the main objective of this work was to evaluate the applicability of the dental amalgam electrode (DAE) in the development of an analytical procedure for the quantification of diquat pesticide (1,1′-ethylene-2,2′-bipyridilium dibromide), a compound largely employed as herbicide, which is extremely hazardous to human health and environment [25,26].

Among the electroanalytical techniques presently available for use with amalgam electrodes, and various electrodic surfaces, square wave voltammetry (SWV) [27] has proved to be extremely sensitive for the detection of pesticides [28–30]. The use of SWV allows valuable information concerning the characteristics of the charge transfer processes involved in a given reaction to be obtained. For this, SWV was employed in this work.

2. Experimental

2.1. Equipment and reagents

All the voltammetric measurements were recorded using a potentiostat (Autolab PGSTAT 30, Metrohm-Eco Chemie) controlled by a personal computer, using a GPES version 4.9 software (General Purpose Electrochemical System, Metrohm-Eco Chemie). A conventional cell with a three-electrode system, consisting of a Ag/AgCl/sat.KCl electrode as the reference electrode, platinum wire as the auxiliary electrode, and a DAE as the working electrode, was used in all experiments.

The DAE was constructed from amalgams produced using a similar technique used in dental practice, using the same mass percentages of silver and liquid mercury (Ag/Hg; m/m), since these metals are the main components in dental amalgams [31,32].

A Micronal B474 pH meter equipped with a Ag/AgCl/sat.KCl-glass combined electrode was used to adjust the pH values. All the solutions were prepared with water purified by a Milli-Q system (Millipore Corp.).

A high performance liquid chromatographic (HPLC) system from Shimadzu, model SCL 10 AVP, equipped with an UV–vis spectrophotometric detector (HPLC/UV–vis) model SPD 10 AVP, in conjunction with a STR ODS-II PEEKl LC-18 column (250 × 4.60 mm, with 5 μm particle size) from Shinwa Chemical Industries was used in chromatographic experiments.

The standard diquat used was purchased from Riedel-de Haën with a purity of 99.40%. Standard solutions of diquat were prepared using 1.00×10^{-2} mol L^{-1} and 1.00×10^{-4} mol L^{-1} of herbicide dissolved in water. Commercial diquat solution, with a content of 21.70% and a density of 1.27 g mL^{-1}, was provided by Syngenta, Brazil. This solution was used to prepare a 1.00×10^{-4} mol L^{-1} diquat solution to compare the effects of the components of the commercial formulation in analytical responses on DAE.

All reagents used in this work were of analytical grade, prepared using purified water (18 MΩ cm) in a Millipore Milli-Q system.

2.2. Electrochemical working procedure

Before each series of experiments, or after a pause longer than 1 h, the electrode was potentiostatically activated as described previously [8–24]. All experimental and voltammetric parameters were then evaluated considering the best electrochemical response measured in terms of the highest analytical signal and improved reproducibility.

Analytical curves were obtained in pure electrolyte using the standard addition method. The standard deviation of the mean current (S_b) measured at the reduction potentials (peak 1 and peak 2) of diquat for 10 voltammograms of the blank solution in pure electrolytes together with the slope of the straight line of the analytical curves (s) were used in the determination of the quantification and detection limits (*QL* and *DL*, respectively), according to the guidelines recommended by IUPAC [33,34].

The recovery experiments were done in order to attest the methodology's efficiency. All measurements were performed in triplicate. The recovery efficiencies (%*R*) were calculated considering the ratio between the value of the concentration obtained by extrapolating the analytical curves of the corresponding spiked samples and the concentration previously added [35].

The precision and accuracy of methodology were tested with different standard solutions of diquat and the relative standard deviations (RSD) were calculated, considering the standard deviation of the mean current values obtained and the mean peak current values.

2.3. HPLC conditions

The chromatographic determination of diquat herbicide was performed after a system optimisation that started by using the chromatographic conditions reported by Chichila and Walters [36,37]. Thus, diquat was analysed using isocratic conditions on an HPLC/UV–vis, as described earlier. The mobile phase consisted of 600 mL of a 0.14 mol L^{-1} NaCl solution previously adjusted to a pH of 2.2 with 1.00 mol L^{-1} HCl and 400 mL of acetonitrile, at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL, and the working wavelength for quantitative analysis was 310 nm.

These experiments were performed in order to compare the results obtained with SWV on DAE. Additionally, in HPLC/UV–vis analysis, the *QL* and the *DL* were determined, considering that the standard deviation (S_B) is the average of the y-intercepts of the six curves constructed at low concentration of the diquat and the slope of the fit (s) of the analytical curves [33,34].

2.4. Application of methodology

To evaluate the applicability of the proposed methodology, interfering effects were studied using a water sample collected from the Jacaré River, a local river in the city of Brotas, São Paulo state, Brazil. The sampling point, located away from the city, is relatively free from urban or industrial pollution and is located near agricultural areas producing sugarcane cultures. In Brazil, the use of diquat herbicide is authorised by the government for use in sugarcane and citrus cultures [38].

The sample was used as collected, and 1.0 mL of this sample was added into a cell containing 9.0 mL of a supporting electrolyte. A similar procedure was applied to construct the analytical and the recovery curves in a natural water sample, where the sample was artificially contaminated with 9.90×10^{-7} mol L^{-1} of diquat herbicide solution.

In order to determine the presence of diquat herbicide in potato samples, additions of 1.00×10^{-5} mol L^{-1} of diquat stock solution were inserted into Erlenmeyer flasks and added to 10.0 g of potato sample and 20.0 mL of a solution of 0.01 mol L^{-1} $Na_2B_4O_7$. The flasks were sealed and shaken at 25 °C with agitation for 30 min. After this, the samples were transferred to 50.0 mL centrifuge tubes and centrifuged for 5 min at 1500 rpm. From the supernatant, 1.0 mL aliquots were transferred into electrochemical cells containing 9.0 mL of the supporting electrolyte and analysed according to the procedure developed for the pure electrolyte.

3. Results and discussion

According to previous work [23], the AgSAE presents a surface with globular structures, formed by different individual globules, which remained globular structure with solid and compact surfaces even after the polishing, which enables easy renewal of the electrode surface via mechanical polishing. Additionally, the quantities of silver and mercury in the DAE were 43% and 57%, respectively, and that there was no oxide in the composition of the electrode, and the surface was homogeneous as to the distribution of the metals. For this, the DAE was used here.

3.1. Preliminary experiments

Cyclic voltammetry was performed to determine the electroanalytical responses of diquat herbicide on the DAE, and the voltammetric responses showed the presence of four well-defined voltammetric peaks, two of which were observed during the direct scan, the first at −0.62 V (peak 1) and the second at −1.04 V (peak 2), and two of which were observed in the reverse scan, with peak potential values at −0.90 V (peak 3) and −0.45 V (peak 4).

The square wave voltammetric responses of diquat herbicide on the DAE showed the presence of two well-defined voltammetric peaks towards the negative sweep direction, the first one at −0.56 V (peak 1) and the second at approximately −1.00 V (peak 2) *vs.* Ag/AgCl/sat.KCl. This response is similar to that seen in previous works published using other electrodic surfaces and other bipyridilium compounds [6,39–43].

3.2. Experimental and voltammetric optimisation

The effects of the medium in the voltammetric responses for diquat on DAE were evaluated in 0.1 mol L^{-1} Britton–Robinson buffer solution (pH 6.0), 0.1 mol L^{-1} Na_2SO_4, 0.1 mol L^{-1} $NaClO_4$ and 0.05 mol L^{-1} $Na_2B_4O_7$, using SWV experiments with 1.00×10^{-5} mol L^{-1} of diquat solution, f = 100 s^{-1}, a = 50 mV and ΔE_s = 2 mV. The responses measured in terms of the highest analytical signal and by better reproducibility in the responses, were obtained in 0.05 mol L^{-1} $Na_2B_4O_7$.

Besides, some ionic strength, characterised by different $Na_2B_4O_7$ concentrations, were evaluated from 0.01 to 0.05 mol L^{-1} $Na_2B_4O_7$ solutions, and the results indicated that the increase in the ionic strength promoted a decrease in peak current values for peak 1. For peak 2, the peak current values stay practically constant.

Considering the Debye–Hückel theory [44], the influence of the electrolyte composition and the ionic strength were related to secondary kinetic electrolyte effects due to electrostatic interactions between the ions present in solution and solvation effects based on the radius of the anion involved. So, the 0.05 mol L^{-1} $Na_2B_4O_7$ solution was defined for this work.

SWV experiment studies were carried out for the determination of voltammetric parameters that afforded the best analytical signal in terms of peak current (I_p) and peak potential (E_p). Also, the frequency of the pulse potential (f), amplitude of the pulse (a) and scan increment (ΔE_s) were evaluated.

The f in SWV experiments was evaluated in the interval from 10 to 400 s^{-1}. The results showed that an increase in the f is accompanied by an increase in I_p, for both peaks 1 and 2, up to 200 s^{-1}. Above this value, the I_p values stay practically constant. In addition, a linear relationship between the E_p and the square root of the f was observed up to 100 s^{-1}.

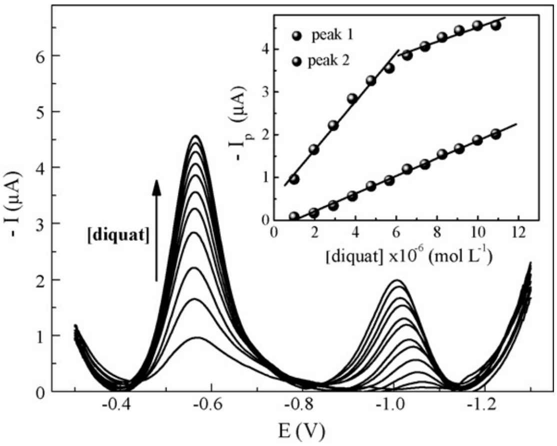

Fig. 1. Square wave voltammograms for diquat herbicide in 0.05 mol L^{-1} $Na_2B_4O_7$ solution on the DAE, with f = 100 s^{-1}, a = 50 mV, ΔE_s = 2 mV, and concentrations in the interval from 4.96×10^{-7} to 4.76×10^{-6} mol L^{-1} of diquat. The insert presents the media of the currents obtained for three analytical curves, for both voltammetric peaks.

According to the theoretical model developed for SWV [27], this behaviour may indicate a diffusion controlled electrode process, similar to that observed by cyclic voltammetric experiments. Moreover, the E_p showed a linear shift when the f was increased. This effect thus provides a good criterion for the diagnosis of a totally reversible electrochemical process [27,28].

The influence of a on I_p intensities was also considered for values of a from 5 to 50 mV. The results demonstrated that the increase in a values promoted an increase in I_p for two voltammetric peaks, but the increase only presented linear relationships until 30 mV. Above this value, the increase in I_p was not linear. Additionally, the E_p showed no variation as a function of a.

For ΔE_s, the increase in this value will also increase the signal and the sensitivity of the technique. However, for large values of ΔE_s, a widening of the peaks may occur, thus diminishing the resolution of the analysis. For this reason, ΔE_s was evaluated for the diquat reduction process on the DAE. The results show that, for peak 1, the increase in ΔE_s practically does not affect the responses. However, for peak 2, the increase in ΔE_s promotes a decrease in I_p values, which indicates that the electron transfer is complicated due to the adsorption process.

So, all parameters discussed earlier, indicated that the voltammetric parameters that can be employed for the analytical determination of diquat on the DAE are: ΔE_s = 2 mV, a = 30 mV and f = 100 s^{-1}.

3.3. Analytical curves

The analytical curves were constructed in supporting electrolyte media, considering the peaks 1 and 2, and the above mentioned parameters for a range of concentrations from 9.90×10^{-7} to 1.09×10^{-5} mol L^{-1}. Aliquots from the stock diquat solution were consecutively added to the electrochemical cell and voltammetric responses were recorded according previously described in Section 2.

The analytical curves obtained showed a linear increase in the responses as a function of the increase in the analytical concentration of diquat for two voltammetric peaks. The voltammograms obtained and the linear relationships between the peak currents and concentrations added are presented in Fig. 1 for both peaks.

By the analysis of Fig. 1, it is possible to observe a shift in peak potential values for peak 2 when the diquat concentration

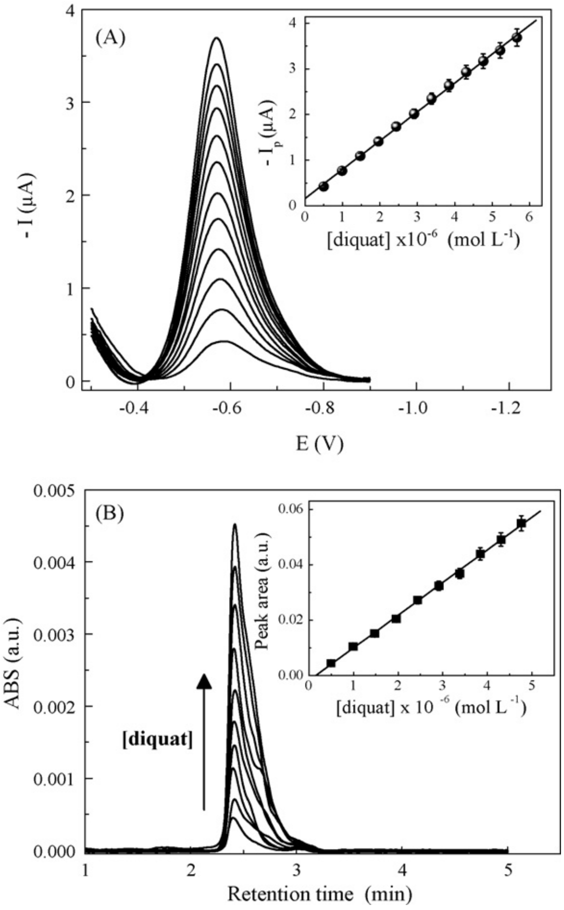

Fig. 2. (A) Square wave voltammograms for diquat herbicide in 0.05 mol L^{-1} $Na_2B_4O_7$ solution on the DAE with scan potentials from −0.30 to −0.90 V (only peak 1), with f = 100 s^{-1}, a = 50 mV, ΔE_s = 2 mV, and concentrations from 4.96×10^{-7} to 5.66×10^{-6} mol L^{-1} of diquat and its analytical curve resultant. (B) Chromatograms of diquat solutions with different concentrations from 4.95×10^{-7} to 4.76×10^{-6} mol L^{-1} on a STR ODS-II PEEKl LC-18 column (250 × 4.6 mm, 5 μm). The mobile phase was 0.14 mol L^{-1} NaCl solution (pH 2.2)/acetonitrile (60:40, v/v), at a flow rate of 1.0 mL min^{-1}. Injection volume was 20 μL, and detection was performed at 310 nm. Inset is the analytical curve resultant.

Table 1
Analytical parameters obtained from linear regression curves for diquat determination using SWV on the DAE and HPLV/UV–vis experiments.

Parameters	DAE	HPLC/UV–vis
Correlation coefficient	0.9993	0.9993
Standard deviation	6.10×10^{-3} μA	1.07×10^{-10} a.u.
Slope of calibration curve	0.631 μA mol^{-1} L^{-1}	0.012 a.u. mol^{-1} L^{-1}
Detection limit (mol L^{-1})	2.90×10^{-8} (10 μg L^{-1})	2.73×10^{-9} (10 μg L^{-1})
Quantification limit (mol L^{-1})	9.67×10^{-8} (33 μg L^{-1})	9.09×10^{-8} (33 μg L^{-1})
Recovery (%)	99.78 ± 1.90	94.23 ± 4.08
Repeatability (%)	1.93 (n = 10)	–
Reproducibility (%)	1.71 (n = 5)	–

is increased. This behaviour indicates that a strong adsorption process occurs in the electrodic surface. Additionally, the insert shows that a change in the analytical curve slope for peak 1 can be associated to changes in the electrodic mechanism or in the adsorption isotherm.

Some experiments were performed consecutively using different procedures to renew the electrodic surface, such as washing with pure water, ethanol, or acetonitrile and also polishing with ultrafine sandpaper. However, the results obtained indicate that using only simple solution agitation completely renews the surface and results in good reproducibility between experiments, indicating that the adsorption process of the reactant and product is not very strong. Thus, before each experiment, the solution was vigorously stirred for 5 min with gaseous nitrogen to remove all adsorbed products in the electrodic surface.

New analytical curves were constructed for a range of concentrations from 4.95×10^{-7} to 4.76×10^{-6} mol L^{-1}, considering only peak 1, for which the potential scans were performed from −0.30 to −0.90 V. The voltammograms obtained and the resultant analytical curve is shown in Fig. 2A.

Analytical curves were also constructed by a chromatographic technique (HPLC/UV–vis) in order to compare the results obtained with SWV on the DAE. The chromatographic determinations of diquat were performed using the experimental parameters presented in Section 2, which are similar to the chromatographic conditions already reported by Chichila and Walters [36,37].

This chromatography procedure is very simple, considering that for majority of the conventional procedures, diquat is usually pre-concentrated from water before the chromatographic determination, which is performed using surfactants as mobile phase [45].

The analytical curve in pure Milli-Q was then obtained by the use of the standard addition method, considering the same range of concentration used earlier for the SWV experiments. The chromatograms obtained are presented in Fig. 2B, which shows diquat signals at a retention time of 2.4 min. Additionally, a linear dependence of the peak area can be observed with the increase in the diquat concentration. The analytical parameters or figures of merit were evaluated for the proposed procedure as well as HPLC/UV–vis experiments, as described in Section 2.

The correlation coefficient (r), which determines the degree of linearity between the concentration of diquat and analytical responses, the standard deviation of the arithmetic mean of ten blank solutions for SWV experiments or the average of the y-intercepts of the six curves constructed in a low concentration of diquat for HPLC/UV–vis experiments (S_b), the slope of the analytical curve (s), the detection limit (DL), and the quantification limit (QL) are shown in Table 1 for SWV experiments on the DAE and for HPLC/UV–vis experiments.

The values of the DL and QL for diquat on the DAE fall very close to those determined by HPLC/UV–vis. Besides, the voltammetric results obtained on DAE presented sensibly similar to the methodologies which employed mercury electrode [40,41], gold microelectrode [6,39], chemically modified electrodes [46,47] and diamond electrodes [42].

Furthermore, the DL and QL found by the methodology using the DAE as the working electrode are lower than the maximum value allowed by CONAMA (National Council for the Environment, Brazil) for wastewaters (i.e., 100 μg L^{-1}) [48] and lower than the maximum value recommended by the Environmental Protection Agency of the United States (EPA) in natural water samples (i.e., 20.00 μg L^{-1}) [49].

In this sense, it can be observed that the use of DAE provides a new alternative for the electrochemical determination of diquat, substituting for traditional mercury electrodes that present the inconvenience of being highly toxic, other solid electrodes, which present a reproducibility affected by adsorptive processes in the electrodic surfaces, modified electrodes that can present high costs, or even chromatographic procedures that require preparation steps and pre-concentration of the samples.

Recovery experiments were also performed using the SWV on the DAE and HPLC/UV–vis experiments in order to evaluate and compare the efficiency of the proposed methodology. The recovery curves were constructed in triplicate and the results obtained are shown in Table 1 for SWV on the DAE and for HPLC/UV–vis experiments.

For the voltammetric experiments, the pure electrolyte was composed of 0.01 mol L^{-1} $Na_2B_4O_7$ solution and was spiked with 9.90×10^{-7} mol L^{-1}of diquat herbicide, followed by the addition of the standard solutions to observe and quantify the amounts of herbicide added. For the HPLC/UV–vis experiments, the recovery efficiencies were calculated using the relationship between the observed experimental concentration value and the expected concentration value.

Table 1 shows the methodologies using SWV on the DAE. The HPLC/UV–vis experiments presented recovery efficiencies of 99.78 ± 1.9% and 94.23 ± 4.08%, respectively. Based on the International Conference on Harmonization (ICH), these values are very satisfactory for analytical procedures, for which recovery efficiencies from 70% to 130% are accepted as an indication of the suitable analytical procedures [50].

The precision and accuracy of the use of the DAE in the analytical determination of diquat herbicide were also evaluated. The precision was evaluated from reproducibility experiments in which five different measurements, performed on different days, were effectuated to a standard solution containing 9.90×10^{-7} mol L^{-1} diquat using the procedure shown earlier. The calculated *RSD* was 1.71%.

The accuracy of the measurements was also evaluated from 10 repetitive determinations (repeatability) of the same standard solution containing 9.90×10^{-7} mol L^{-1} of diquat. The *RSD* obtained was 1.93%.

From the above results, it can thus be concluded that good repeatability and reproducibility in the reduction process for diquat herbicide on the DAE indicate that the DAE is an excellent material for use as a substitute for other electrodic surfaces and even for chromatographic procedures.

3.4. *Effects of interference from commercial formulations*

In order to compare the effects of the components present in commercial formulations of diquat herbicide on the analytical responses obtained on the DAE using SWV, analytical curves using the stock solutions of commercial diquat were constructed. For this, a commercial diquat solution with a 21.70% content and a density of 1.27 g mL^{-1}, provided by Syngenta, Brazil, was used to prepare a 1.0×10^{-4} mol L^{-1} diquat solution.

As diquat is very toxic, colourless, and odourless, in commercial formulation, the producer added a colour to prevent fortuitous poisoning cases. A commercial formulation was used to evaluate the effects of the colour in the analytical responses for diquat using DAE, and like this, study the robustness of the proposed procedure.

Analytical curves were constructed for a range of concentrations from 4.98×10^{-7} to 1.96×10^{-6} mol L^{-1} using a procedure similar to that previously described. The resultant curve is shown in Fig. 3, where it can be observed that the peak current is also directly proportional to the concentration of herbicide solution, with similar peak current values for responses obtained from standard or commercial solutions, and a slight increase in the slope for the standard solution.

Meanwhile, for greater concentration intervals, the slopes are similar for both solutions, indicating that the components of the commercial solutions do not interfere in the electrochemical responses of diquat on the DAE. The procedure described in this work can be employed with success to control the quality of the commercial formulation of diquat, indicating the real concentration of herbicide with good precision and accuracy. Besides, the responses obtained shown to be insensitive to variation in composition of the standard solution, indicating this way, a suitable robustness for the proposed procedure.

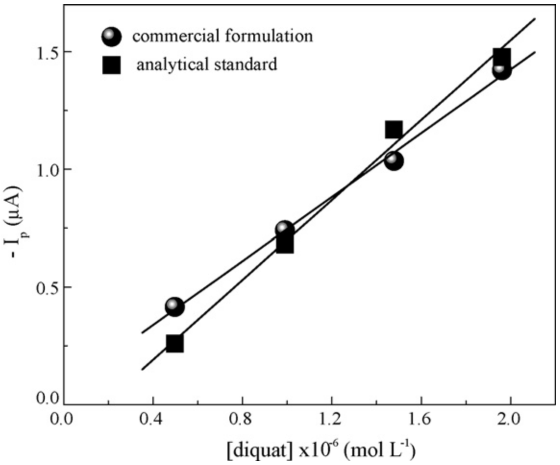

Fig. 3. Analytical curves obtained for peak 1 of the diquat reduction on the DAE in a medium of 0.05 mol L^{-1} $Na_2B_4O_7$ solution with scan potentials from −0.30 to −0.90 V with f = 100 s^{-1}, a = 50 mV, ΔE_s = 2 mV, and concentrations from 4.96×10^{-7} to 5.66×10^{-6} mol L^{-1} using a stock solution of diquat prepared with standard solution and commercial formulation.

3.5. *Applications in natural water and potato samples*

The natural water and potato samples were employed as described in Section 2 and used for the construction of analytical and recovery curves.

The potato sample was employed due to the fact that in Brazil, this herbicide is widely used in such applications and its use has been regulated by government agencies [48]. Those experiments were carried out with the objective of observing any possible interfering effects on the detection of diquat herbicide using the DAE as the working electrode.

Analytical curves were constructed for a range of concentrations from 4.98×10^{-7} to 3.38×10^{-6} mol L^{-1} in pure electrolyte, natural water and potato samples, and the responses obtained showed that the peak potential did not change significantly, indicating that diquat is not prone to interference. Between each experiment, the solution was vigorously stirred for a period of 5 min with gaseous nitrogen to remove all adsorbed products in the electrodic surface, similar to that effectuated earlier for the pure electrolyte.

The linearity between the peak currents and the added concentration of diquat obtained for pure electrolyte, natural water and potato samples, respectively, are described by the equations below:

$$I_p\ (\mu A) = 0.096\,(\mu A) + 0.673C\,(\mu A\,mol^{-1}\,L^{-1}) \quad (1)$$

$$I_p\,(\mu A) = -0.039\,(\mu A) + 0.869C\,(\mu A\,mol^{-1}\,L^{-1}) \quad (2)$$

$$I_p\,(\mu A) = 0.201\,(\mu A) + 0.563C\,(\mu A\,mol^{-1}\,L^{-1}) \quad (3)$$

A comparison between the slopes of the analytical curves in natural water and pure electrolyte showed some influence from the organic and inorganic components in the natural water samples, which contributed to an increase in the responses, characterised by an increase in the slope of the analytical curve. The sample of natural water employed in this work was collected from a river located in a rural area, free of domestic and industrial discharge and characterised by a large organic matter quantity, such as humic and fulvic acids, which are charged species that can be adsorbed in electrodic surfaces, promoting an increase in the responses.

For the potato samples, the equations above showed a decrease in the slope of the respective analytical curve compared to the pure electrolyte. This is the expected behaviour since those responses

Table 2
Results obtained from recovery curves of diquat in pure electrolyte, natural water and potato samples, considering peak 1 on the DAE and SWV.

Sample	$[\text{diquat}]_{\text{added}}$ (mol L^{-1})	$[\text{diquat}]_{\text{found}}$ (mol L^{-1})	Recovery (%)
Pure electrolyte	9.90×10^{-7}	9.88×10^{-7}	99.78 ± 1.9
Natural water	9.90×10^{-7}	1.15×10^{-6}	115.7 ± 2.7
Potato	1.00×10^{-6}	8.60×10^{-7}	86.10 ± 3.4

reflect interference of the starch, due to the presence of these compounds in high concentration in the samples. This interference does not affect the analytical sensitivity, and the proposed procedure can be employed with success in the evaluation of recovery curves.

Additionally, recovery experiments employing the samples prepared according to the methods described in Section 2 were conducted. The samples were artificially contaminated by the addition of a specific concentration of diquat herbicide. The recovery curves were constructed by the standard addition method, and the recovery percentage was obtained by the graphical method, in which the abscissa axis refers to the concentration of the diquat in the electrochemical cell. When the curve obtained is extrapolated in this axis, the sample concentration is obtained, and the recovery values can be calculated. All curves were constructed in triplicate. The data related to the recovery curves are shown in Table 2.

Table 2 shows that, for natural water samples, an increase in the calculated values occurs compared to the observed values in pure electrolyte. This behaviour is in close agreement with that observed in the construction of the analytical curves, where an increase in the slopes occurred, indicating an increase in the responses when natural water samples were used. Either the components of the natural water contributed to the analytical responses of the diquat herbicide, or the sample was contaminated with diquat, although there was no change in the peak potential value. To confirm this suspicion, experiments involving spectroscopic techniques, such as liquid chromatography coupled to a mass detector, could be performed in future studies in order to characterise the components.

For the present, it is possible to confirm that the recovery values calculated for pure electrolyte, natural water and potato samples were in a suitable range for analytical applications (from 70% to 130%) [50], indicating that the proposed procedure has therefore proven to be applicable for use in complex samples, such as natural water and potato.

4. Conclusions

The experimental and voltammetric parameter for electroanalytical determination of diquat on DAE were evaluated and the best responses were obtained in 0.05 mol L^{-1} $Na_2B_4O_7$ solution on the DAE, with $f = 100\ s^{-1}$, $a = 50$ mV, $\Delta E_s = 2$ mV. Analysis of a purified laboratory electrolyte using the proposed methodology allowed for a very low detection limit of 10 $\mu g\ L^{-1}$ associated with a high level of repeatability and reproducibility. The analytical sensibility is comparable to similar results previously published using mercury electrodes and solid surfaces, such as boron-doped diamond and gold electrodes.

The comparison between the analytical data obtained for the determination of diquat herbicide using SWV on the DAE and HPLC/UV–vis led to very coherent results, indicating that the proposed electroanalytical procedure is an important tool to detect diquat residues in small concentrations. This method provides information about the redox mechanism, which is of considerable importance since it allows for a better understanding of the herbicide behaviour in the environment and of the possible interferences in the application of this procedure in complex samples.

Finally, this work demonstrated that the DAE can be considered an environmentally friendly tool and a very interesting alternative for mechanistic studies and the analytical determination of diquat. In addition to presenting good sensibility and analytical stability, this technique also presents the great advantages of eliminating mercury waste and minimising adsorptive problems related to the use of other solid surfaces.

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References

[1] J. Wang, Electroanalytical Chemistry, third ed., Marcel Dekker, New York, 2006.
[2] S.E. Manahan, Environmental Chemistry, eighth ed., CRC Press, United States of America, 2000.
[3] B. Yosypchuck, L. Novotný, Crit. Rev. Anal. Chem. 32 (2002) 141.
[4] D. De Souza, R.A. de Toledo, A. Galli, G.R. Salazar-Banda, M.R.C. Silva, G.S. Garbellini, L.H. Mazo, L.A. Avaca, S.A.S. Machado, Anal. Bioanal. Chem. 387 (2007) 2245.
[5] F.W.P. Ribeiro, A.S. Cardoso, R.R. Portela, J.E.S. Lima, S.A.S. Machado, P. Lima-Neto, D. De Souza, A.N. Correia, Electroanalysis 20 (2008) 2031.
[6] D. De Souza, S.A.S. Machado, Electroanalysis 18 (2006) 862.
[7] S.L.C. Ferreira, P.A.P. Pereira, J.A. Nobrega, O. Fatibello-Filho, M.A. Feres, B.F. Reis, R.E. Bruns, F.R. Aquino-Neto, Anal. Lett. 41 (2008) 1494.
[8] B. Yosypchuk, L. Novotný, Electroanalysis 14 (2002) 1733.
[9] D. De Souza, R.A. de Toledo, H.B. Suffredini, L.H. Mazo, L.A. Avaca, S.A.S. Machado, Electroanalysis 18 (2006) 605.
[10] B. Yosypchuk, L. Novotný, Talanta 56 (2002) 971.
[11] M. Fojta, F. Jelin, L. Havran, E. Palecek, Curr. Anal. Chem. 4 (2008) 250.
[12] R. Piech, B. Bas, W.W. Kubiak, J. Electroanal. Chem. 621 (2008) 43.
[13] S. Hason, V. Vetterl, M. Fojta, Electrochem. Acta 53 (2008) 2818.
[14] J. Barek, J. Fischer, T. Navratil, K. Peckova, B. Yosypchuk, Sensors 6 (2006) 445.
[15] D. De Souza, R.A. Toledo, L.H. Mazo, S.A.S. Machado, Electroanalysis 17 (2005) 2090.
[16] Ø. Mikkelsen, K.H. Schrøroder, Anal. Chim. Acta 458 (2002) 249.
[17] Ø. Mikkelsen, K.H. Schrøroder, Anal. Lett. 33 (2000) 3253.
[18] Ø. Mikkelsen, K.H. Schrøroder, Electroanalysis 16 (2004) 386.
[19] Ø. Mikkelsen, K.H. Schrøroder, http://www.rsc.org/Publishing/Journals/AN/article.asp?doi=b008473pAnalyst 125 (2000) 2163.
[20] Ø. Mikkelsen, C. Nordhei, S.M. Skogvold, K.H. Schrøroder, Anal. Lett. 37 (2004) 2925.
[21] Ø. Mikkelsen, S.M. Skogvold, K.H. Schroder, M.I. Gjerde, T.A. Aarhaug, Anal. Bional. Chem. 337 (2003) 322.
[22] Ø. Mikkelsen, K. Strasunskiene, S. Skogvold, K.H. Schroder, C.C. Johnsen, M. Rydningen, P. Jonsson, A. Jonsson, Electroanalysis 19 (2007) 2085.
[23] R. Fradna, Anal. Lett. 37 (2004) 3255.
[24] B. Yosypchuk, I. Šestáková, Electroanalysis 20 (2008) 426.
[25] S.D. Murphy, Casarett and Doull's Toxicology, Macmillan, New York, 1986.
[26] E. Halfon, S. Galassi, R. Brüggemann, A. Provini, Chemosphere 33 (1996) 1543.
[27] V. Mirceski, S. Komorsky-Lovric, M. Lovric, in: F. Sholz (Ed.), Square Wave Voltammetry – Theory and Applications, Springer, Berlin, 2007.
[28] D. De Souza, L. Codognoto, A.R. Malagutti, R.A. de Toledo, V.A. Pedrosa, R.T.S. Oliveira, L.H. Mazo, L.A. Avaca, S.A.S. Machado, Quim. Nova 27 (2004) 790.
[29] S.Y. Ly, Microchim. Acta 163 (2008) 283.
[30] J.C. Chen, J.L. Shih, C.H. Liu, M.Y. Kuo, J.M. Zen, Anal. Chem. 76 (2006) 3752.
[31] N. Horosawa, H. Nakajima, J.L. Ferracane, S. Takahashi, T. Okabe, Dent. Mater. 12 (1996) 154.
[32] S. Nomoto, N. Suzuki, Y. Ohmori, T. Yamaguchi, T. Inoue, Nihon Univ. Dent. J. 60 (1986) 404.
[33] J. Mocak, A.M. Bond, S. Mitchel, G. Scollary, Pure Appl. Chem. 69 (1997) 297.
[34] Analytical Methods Committee, Analyst 112 (1987) 199.
[35] D.A. Skoog, D.M. West, F.J. Holler, Fundamentals of Analytical Chemistry, fifth ed., Saunders College, Philadelphia, 1996.
[36] T.M. Chichila, S.M. Walters, J. Assoc. Off. Anal. Chem. 74 (1991) 961.
[37] T.M. Chichila, S.M. Walters, J. Assoc. Off. Anal. Chem 76 (1993) 1323.
[38] http://www.anvisa.gov.br/toxicologia/monografias/index.htm, access in January 2009.
[39] D. De Souza, S.A.S. Machado, Anal. Chim. Acta 546 (2005) 85.
[40] A. Walcarius, L. Lamberts, J. Electroanal. Chem. 406 (1996) 59.
[41] R.M. Elofson, R.L. Edsberg, Can. J. Chem. 35 (1957) 646.
[42] L. Xiao, G.G. Wildgoose, R.G. Compton, New J. Chem. 32 (2008) 1628.
[43] C.L. Bird, A.T. Kuhn, Chem. Soc. Rev. 10 (1981) 49.
[44] A.O.M. Bockris, A.K.N. Reddy, Modern Electrochemistry 1: Ionics, second ed., Kluwer Academic Publisher, New York, 1998.
[45] J.L. Tadeo, S. Sánchez-Brunete, R.A. Pérez, M.D. Fernández, J. Chromatogr. A 882 (2000) 175.

[46] I.C. Lopes, D. De Souza, S.A.S. Machado, A.A. Tanaka, Anal. Bioanal. Chem. 308 (2007) 1907.
[47] P.M.S. Monk, C. Turner, S.P. Akhtar, Electrochim. Acta 44 (1999) 4817.
[48] http://www.mma.gov.br/port/conama/res/res86/res2086.html, access in January 2009.
[49] http://www.epa.gov/ogwdw/contaminants/dw_contamfs/diquat.html, access in January 2009.
[50] International Conference on Harmonization (ICH), Topic Q2B, Validation of Analytical Procedures: Methodology, Proceedings of the Commission of European Communities, Geneva, Switzerland, November 1996.