===== **ARTICLES** =====

Use of Multiple Square Wave Voltammetry for the Detection of Diquat Herbicide in Environmental Water, Foods and River Sediments[1]

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Abstract—This paper reports the use of multiple square wave voltammetry (**MSWV**) in the development of a simple, sensitive, fast and low-cost electroanalytical procedure for the diquat herbicide detection in different samples employing a custom-made gold microelectrode (**Au-ME**). The experimental and voltammetric parameters were optimized, and the use of eight pulses superimposed in each step in the MSWV yielded the detection limits two orders of magnitude greater than those obtained by SWV or previously published chromatographic procedures. Also, the Au-ME allied to MSWV was used to determine diquat in beetroot, onion, peach, environmental water and river sediments. All samples were spiked, and the obtained recovery percentage values were satisfactory for the analytical procedure, showing that Au-ME and MSWV is a suitable tool for diquat residues detection in complex samples.

Diquat (1,1'-ethylene-2,2'-bipyridylium dibromide) is one of the most widely employed agricultural pesticides. Belonging to the bipyridilium class, diquat is used as a defoliating herbicide. However, it exhibits mutagenic and lethal effects on human health [1] and as a result has been banned in some countries. Yet, it is still heavily used in more than 130 others principally due to its rapid biological inactivation upon contact with soil, very fast action and rain-fastness within minutes of application. Diquat is non-selective, which means it kills a wide range of annual grasses and broad-leaved weeds and the tops of established perennial weeds.

Due to the widespread use and poisonous characteristics of diquat, a great effort has been made in seeking sensitive methodologies for quantification of its residues. Procedures based on spectrophotometry [2, 3], capillary zone electrophoresis [4], gas chromatography [5], high performance liquid chromatography [6], immunological methods [7, 8], potentiometric [9, 10] and other electroanalytical methods [11−14] have been reported to date. Determination of diquat residues is complicated by their ionic nature, where cationic species exist in solution that are prone to strong adsorption onto organic and inorganic compounds present in environmental water, foods and soil. Thus, sample preparation steps are necessary before analytical quantification, e.g., liquid-solid extraction and pre-concentration of herbicide residues in a column packed with a cation-exchange material [15]. Application of the Environmental Protection Agency (EPA)-validated method, HPLC coupled to UV-Vis spectrophotometry (HPLC−UV-Vis), requires reactants that are costly, toxic and require skilled analysts [15].

Thus, the main objective of this work was the development of a simple, sensitive, fast and low-cost electroanalytical procedure for diquat residues detection in environmental water, foods, and river sediment samples employing a custom-made gold microelectrode. A secondary objective of this work was to do a comparative study between the responses obtained by the use of traditional square wave voltammetry (**SWV**) and by multiple square wave voltammetry as electroanalytical tools.

In general, the use of microelectrodes (**ME**) allows the improvement in the quality of experimental results because its use increases the resolution and current density and decreases the effect of resistance on resolution [16, 17]. For this, the ME can be used in the resistive solutions without added supporting electro-

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lytes, a property that is very important in methods involving low conductivity solutions such as environmental water or food samples. These effects make ME advantageous in many areas of electroanalytical chemistry, mainly in the direct determination of antibiotics, metals, dissolved oxygen and antioxidants in environmental and food samples [18].

EXPERIMENTAL

Reagents. The diquat herbicide standard was obtained from Aldrich (98.0 and 99.2% purity, respectively). A 1.000×10^{-3} M stock solution of diquat was prepared in Milli-Q water, which were stored at $-4°C$ and used in all work. For the electroanalytical measurements, several supporting electrolytes (Britton–Robinson buffer, sulfate, phosphate, perchlorate) were tested and the best results were obtained with a 0.1 M Na_2SO_4 electrolyte (adjusted by adding the appropriate amount of 0.1 M H_2SO_4). Water was purified by means of a Milli-Q system from Millipore Corporation and used to prepare all the solutions.

Instrumentation. A Metrohm model 682 pH-meter equipped with a 3 M Ag/AgCl/KCl-glass combined electrode was used to adjust pH values. Electrochemical measurements were all carried out using a PGZ 402 Voltalab potentiostat from Radiometer Analytical coupled to a Voltamaster 5.06. A two-electrode configuration was used with the reference electrode (Ag/AgCl in 3.0 M KCl) acting also as counter electrode, while the working electrode was a custom-made electrode constructed with gold micro wire.

Microelectrode construction and characterization. The microelectrode was constructed with 25 μm diameter gold wire (Goodfellow). The micro wire was inserted into a piece of glass tubing of approximately 0.5 mm internal diameter, which was later insulated with epoxy resin. After the epoxy resin dried, the ME was polished using a mechanical polisher with waterproof paper of different sizes (Norton Industries, Brazil). After polishing, the ME was cleaned with distilled water to obtain a disk surface.

The voltammetric characterization was carried out by studying the electrochemical response of potassium hexacyanoferrate(III) in acidic medium, since its behavior is well known and the voltammograms exhibit sigmoid profiles, characteristic of ME [17].

Before each series of experiments, the ME was polished with an ultra fine glass paper and ~200 cycles of potential from 0 to -1.3 V vs. Ag/AgCl were performed employing cyclic voltammetry at 0.5 V/s in the supporting electrolyte. This procedure was carried out to obtain good reproducibility in the voltammetric responses and was repeated at each sample change in application of the steps of the proposed procedure.

Analytical technique. MSWV was used as an analytical technique in the development of the electroanalytical procedure employing ME. In MSWV, the manner of potential application is similar to that of traditional SWV [19, 20] with the difference that, for each potential step, more than a single pair of pulse potentials of opposite signs or pulses with asymmetric profiles (where the forward pulse is different from the reverse pulse) can be applied [21, 22].

However, the optimization of the analytical procedure for MSWV was carried out following a systematic study of experimental parameters that affect the SWV responses, such as: the pulse potential frequency (f) related to total pulses duration, the pulse height (a) and the height of the potential step (ΔE_s) or scan increment. Additionally, the number of superimposed pulses in each step (N) was also evaluated. All parameters were optimized in relation to the maximum value of peak current and the maximum selectivity (half-peak width).

The MSWV method is complementary to SWV but is more sensitive (the detection limits are 10^2 to 10^3 times lower). For that reason, it has been used for the determination of trace amounts even in complex samples [23, 24].

Working procedure. All measurements were carried out under ambient conditions. A standard solution of herbicide and supporting electrolyte was transferred into the electrochemical cell and the optimizations of the experimental and voltammetric parameters were carried out. The pH of the medium, f, a, ΔE_s and N were properly optimized since their values exert a strong influence on the sensitivity of the voltammetric analysis.

In all experiments, the cell was placed in a Faraday case in order to minimize background noise. Analytical curves were obtained in pure electrolyte using the standard addition method, in which known portions of the standard solution of diquat were added in the electrochemical cell and the voltammetric responses evaluated for each addition. The standard deviation (s_b) as the average of the y-intercepts of the three curves constructed at low concentration of diquat together with the slope of the analytical curves (S) were used in the determination of the quantification and detection limits (**LOQ** and **LOD**, respectively), according to IUPAC guidelines [25].

The recovery experiments were carried out by adding a known amount of diquat to the supporting electrolyte, called artificial spike, followed by standard additions from the diquat stock solutions and plotting the resulting analytical curves. 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 recovery curves of the corresponding spiked samples and the previously added concentration [26].

The precision and accuracy of the method were tested with different standard solutions of diquat, and the relative standard deviations (**RSD**s) of the mean

current values obtained and the mean peak current values were calculated.

Application of the proposed procedure to environmental water, foods and river sediments samples. Before each experiment with the different samples, the Au-ME was polished with ultra fine glass paper to remove interfering substances adsorbed on its surface. To test the applicability of the proposed procedure, the effect of interference in analytical responses for diquat was evaluated using environmental water samples without chemical separation stages or pre-concentration. The environmental water samples were collected from river Mogi-Guaçu (state of São Paulo, Brazil). The samples were collected from different points of the river, which presented different levels of pollution characterized by chemical and biologic oxygen demand (**COD** and **BOD**). The supporting electrolyte was prepared by dissolving Na_2SO_4 in environmental water, and the measurements were performed without pre-treatment of the solutions.

The proposed method was employed with samples of foods for which diquat herbicide is used in their cultivation, according to the recommendations of the National Agency for Sanitary Vigilance of Brazil (ANVISA). The electroanalytical responses of diquat were evaluated in beetroot, peach and onion samples. All samples were obtained in shops of São Carlos city (São Paulo state, Brazil) and were employed without any pre-treatment or separation steps except adjustment of the pH values to 6.0 with appropriate additions of 0.1 M NaOH solution.

The samples of beetroot, peach and onion were ground in a domestic blender and their pH values adjusted. For these samples, two variants of the proposed procedure in analytical determination of diquat were evaluated. In the first one, called direct analysis, 20 g of each sample were transferred to an electrochemical cell and spiked with an appropriate concentration of diquat stock solution. Next, the mixture was mechanically stirred and electrochemical measurements were done under the experimental conditions described above by applying the standard addition method.

In the second procedure, called prepared samples, 10 g of each sample with 10 mL of the supporting electrolyte were spiked with appropriated concentration of diquat stock solution and kept under agitation for 60 min. After this, the samples were centrifuged and an aliquot of 1 mL of the supernatant was inserted in an electrochemical cell containing 9 mL of the supporting electrolyte. After this, the procedure employed for recovery curves in pure electrolyte was performed.

For application of the procedure to river sediments, the samples were collected following the procedure defined by CRC [27] from two different points of Mogi-Guaçu river, located in the city of Pirassununga (sediment 1) and Mogi-Guaçu city (sediment 2), respectively. T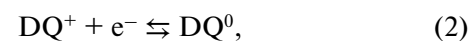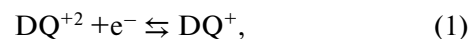he sampling points presented different physical and chemical characteristics defined by different values of organic matter, silt, clay and sand percentages.

In order to determine the herbicide in these samples, additions of 7.50×10^{-6} M of diquat stock solution were added to Erlenmeyer flasks each charged with 2.0 g of a river sediment sample and 20 mL of a solution of 0.1 M Na_2SO_4. The flasks were sealed and shaken at 25°C for 24 h. After 24 h of agitation, the samples were transferred to 50 mL centrifuge tubes and centrifuged for 20 min at 15000 rpm. From the supernatant, 10 mL aliquots were transferred into electrochemical cells and analyzed according to the procedure developed for pure electrolyte.

In all samples, the recovery curves were performed in triplicate, and the results shown in this work are the average of the values calculated.

RESULTS AND DISCUSSION

Electrochemical behavior and voltammetric optimization. Preliminary experiments using SWV were performed to determine the electroanalytical responses of diquat with the Au-ME and to evaluate the presence of forward and backward current components that can be applied to determine the reversibility of the electrochemical redox process. For this purpose, SWV experiments were performed for 1.00×10^{-4} M diquat in 0.10 M Na_2SO_4 pH 6.0, with $\Delta E_s = 2$ mV, $a = 50$ mV and $f = 100$ s^{-1}. The responses obtained showed that diquat gave two voltammetric peaks towards the negative sweep direction, the first one around -0.7 V and the second at approximately -1.0 V vs. Ag/AgCl, which according to previous works [28, 29] correspond to:

$$DQ^{+2} + e^- \leftrightarrows DQ^+, \qquad (1)$$

$$DQ^+ + e^- \leftrightarrows DQ^0, \qquad (2)$$

$$MV^0 + MV^{2+} \leftrightarrows (MV)_2^{2+}, \qquad (3)$$

where DQ represents the diquat molecule. In equation (1), a redox process in peak 1 is shown, associated with the redox couple, in equation (2) a quasi-reversible adsorption-controlled process generating the neutral molecule, and in equation (3) the subsequent formation of a dimer.

Based on these results, it can be inferred that the electrochemical process related to these peaks presented a completely reversible behavior, indicated by the presence of forward and backward currents with similar values of peak current, a behavior similar to that published in [11—14].

The effect of pH on the electrochemical behavior of diquat on Au-ME was investigated in media consisting of 0.1 M Na_2SO_4 with pH values adjusted from 2.0 to 6.0 by addition of 0.1 M H_2SO_4. The analysis of responses indicated no evidence of a shift in the peak

potential (E_p) values with pH, confirming the absence of any intervention of protons in the electrochemical reduction process. However, the peak current showed an increase with pH up to a value of 6.0, after which it decreased. Consequently, the results observed suggested that a pH of 6.0 represents an optimum value for the determination of diquat. At this point, the peak current is highest for both peak reductions, corresponding to the best values for the electrochemical reduction of the herbicide. Hence, this pH was chosen for subsequent measurements.

In practice, the MSWV is an expansion of the traditional SWV with the implementation one more pair of pulse potentials in each step of the staircase. All parameters of the SWV exert a marked effect on the voltammetric response and it can be initially optimized. Also, the effects of the pulse potential frequency (f) related to total pulses duration, the pulse height (a) and the height of the potential step (ΔE_s) or scan increment on the peak current and peak potential of diquat on Au-ME were studied.

For variation in the f values, the responses obtained demonstrated that for either peak, the intensities of the peak current (I_p) increased for values of f up to even 1000 s^{-1} without any loss of quality in the electrochemical signal. This feature is due to the employment of Au-ME that enabled rapid diffusion of species to or from the electrode surface [16–18].

In spite of the high frequency value used to promote an increase in analytical sensitivity, the maximum acceptable value to be used in an MSWV program is 250 s^{-1}, which corresponds to 0.004 s of duration for each step and was used in all subsequent experiments.

The a values were optimized to obtain the maximum response values. The results obtained for both voltammetric peaks showed that the values of I_p showed a practically linear variation with the pulse amplitude for values of a from 5 to 50 mV, while in this range E_p shows no variation as a function of a. Thus, a value of $a = 50$ mV was chosen for analytical applications.

Another important parameter evaluated was the effective rate of variation of potential, characterized by the product of the duration of all pulses and the scan increment. Therefore, as expected, an increase in the latter parameter will also increase the signal and the sensitivity of the technique. For large values of ΔE_s, a widening of the peaks may occur thus diminishing the resolution of the analysis. For this reason, a value of $\Delta E_s = 2$ mV was adopted for diquat determinations.

After optimization of the experimental and voltammetric parameters using the SWV, the number of pulse potentials in each step (N) was also studied to evaluate the effect of increasing N on the voltammetric signal and consequently an improvement in analytical sensitivity. For this, N was varied from two to eight, which

is the range provided by the instrument and software, and the results obtained showed that an increase in N produces a better analytical signal, as shown in Fig. 1. It can also be observed that the relation between I_p and N is linear for both peaks, demonstrating the great improvement in signal expected when multiple pulses were used. Based on these observations, analytical curves of the MSWV were constructed using eight potential pulses in each step; all pulses at equal durations and amplitudes.

Analytical curves by MSWV and SWV. A comparative study of the techniques. Using the optimized parameters above, analytical curves were obtained for diquat in an electrolyte prepared with pure water. For this, aliquots from the stock herbicide solutions were consecutively added to the electrochemical cell. The MSWV and SWV responses were recorded with $f = 250$ s^{-1}, $a = 50$ mV, $\Delta E_s = 2$ mV and $N = 8$. The analytical curves were constructed for a range of concentrations from 1.15×10^{-6} to 1.24×10^{-5} M and the voltammetric responses obtained by use of MSWV are showed in Fig. 2, where the insert represents the linearity between the peak current and concentration of diquat.

The linear relationships between the peak current and concentration of diquat in pure electrolyte, considering the use of SWV (peak 1 and peak 2) and MSWV (peak 1 and peak 2), respectively, are described by the equations below:

$$I_p \text{ (A)} = 1.609 \times 10^{-8} \text{ (A)} + 0.016c \text{ (A/M)}, \quad (4)$$

$$I_p \text{ (A)} = 1.511 \times 10^{-8} \text{ (A)} + 0.017c \text{ (A/M)}, \quad (5)$$

$$I_p \text{ (A)} = 8.921 \times 10^{-8} \text{ (A)} + 0.033c \text{ (A/M)}, \quad (6)$$

$$I_p \text{ (A)} = 8.883 \times 10^{-8} \text{ (A)} + 0.045c \text{ (A/M)}. \quad (7)$$

Besides, analytical curves were constructed by MSWV in the concentration range from 7.69×10^{-8} to 2.09×10^{-6} M, since the MSWV experiments were more sensitive than traditional SWV. In all analytical curves constructed, the analytical responses presented a similar profile, where a linear increase can be observed in the responses with the variation in the analytical concentration according to equations shown below, considering peaks 1 and 2, respectively. It is possible to observe that the slope of the analytical curves presented similar inclination to observed in high concentration interval:

$$I_p \text{ (A)} = -1.411 \times 10^{-9} \text{ (A)} + 0.024c \text{ (A/M)}, \quad (8)$$

$$I_p \text{ (A)} = -2.951 \times 10^{-10} \text{ (A)} + 0.047c \text{ (A/M)}. \quad (9)$$

By analysis of equations (1) to (4), it can be observed that the analytical curves resulting from MSWV represent a 2-fold increase in the peak intensity for peak 1 and 3.4-fold for peak 2, compared to SWV. The importance of such an increase cannot be underestimated since it indicates a suitable improvement in analytical sensitivity, thus concluding that a
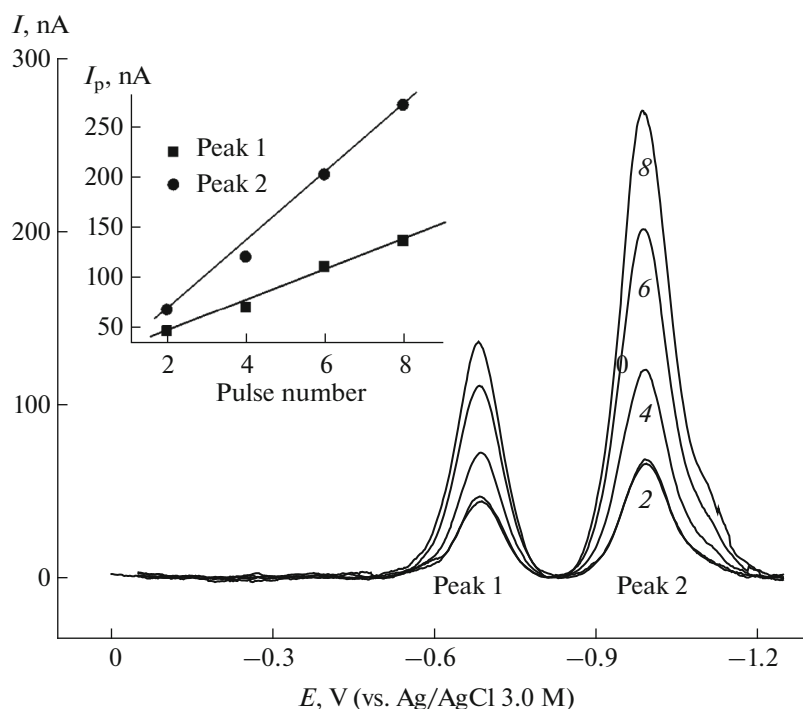
Fig. 1. Multiple square wave voltammograms for 5.0×10^{-5} M of diquat in Na_2SO_4 0.1 M, with $\Delta E_s = 2$ mV, $a = 50$ mV and $f = 250$ s^{-1} using different numbers of pulses in the same potential step. Insert: relationships between peak current and number of pulses.

higher sensitivity is obtained for MSWV compared to SWV. Besides, in low concentration range [equations (8) and (9)], the slope of the analytical curve presented a similar value that observed by curves constructed in greater concentration range. So, the proposed method can be used with success in a wide concentration range without loss in sensitivity.

The values of LOD and LOQ for both peaks were determined using SWV and MSWV, as defined under "Experimental." So, the correlation coefficient (r), which determines the degree of linearity of the relation between the concentration of herbicide and peak current, the average of the y-intercepts of the three ana-

lytical curves (s_b), the slope of the analytical curves (S), LOD and LOQ are shown in Table 1.

From the results presented in the table above, it can be observed that the smaller detection limit and the better sensitivity were obtained for the MSWV when eight potential pulses were applied within the same potential step. The values of LOD and LOQ obtained for the support electrolyte prepared from Milli-Q water using the SWV are very close to those published in literature using other electroanalytical methods [11−14].

Table 1. Parameters of analytical curves obtained for the determination of diquat in pure electrolyte on Au-ME

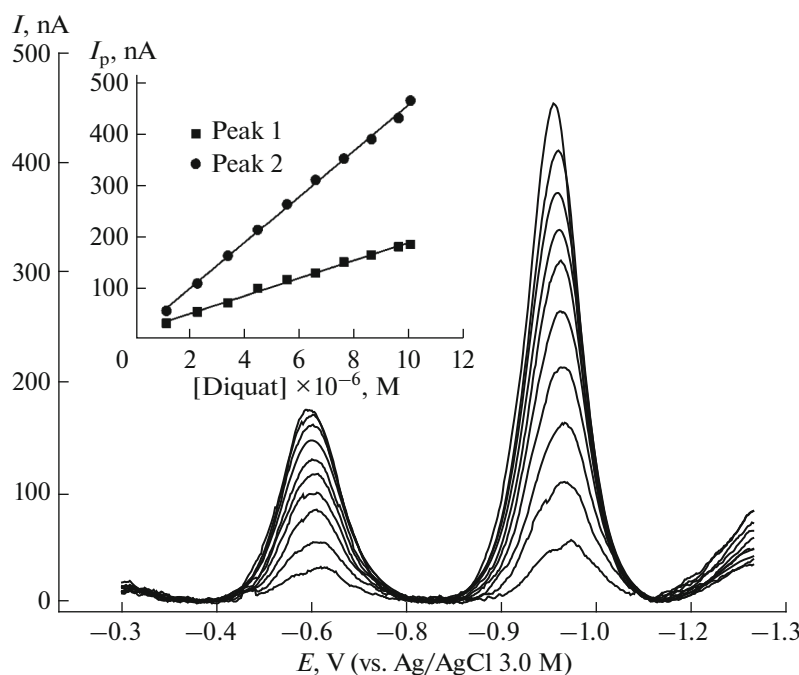| Parameter	SWV		MSWV	
	peak 1	peak 2	peak 1	peak 2
r	0.9952	0.9961	0.9980	0.9975
s_b (A)	3.18×10^{-10}	3.75×10^{-10}	1.02×10^{-10}	1.16×10^{-10}
S (A/M)	1.61×10^{-2}	1.71×10^{-2}	3.30×10^{-2}	4.51×10^{-2}
LOD, M	6×10^{-8} (20 ppb)	6×10^{-8} (23 ppb)	9×10^{-9} (3 ppb)	8×10^{-9} (3 ppb)
LOQ, M	1.3×10^{-7} (68 ppb)	2.1×10^{-7} (75 ppb)	3.1×10^{-8} (11 ppb)	2.5×10^{-8} (8.8 ppb)
Recovery, %	99.1	95.5	103.5	108.2
Repeatability, %	1.45	1.88	1.75	1.95
Reproducibility, %	1.76	2.05	1.85	2.20

Fig. 2. Multiple square voltammograms for diquat in 0.10 M Na_2SO_4 on Au-ME with $a = 50$ mV, $\Delta E_s = 2$ mV, $f = 250$ s^{-1}, $N = 8$ and concentrations between 1.50×10^{-6} and 1.24×10^{-5} M. Insert: analytical curves obtained considering the responses for peaks 1 and 2.

However, for peak 1, the LOD results changed from 5.93×10^{-8} M (20.4 µg/L) to 9.27×10^{-9} M (3.19 µg/L) when the MSWV was employed. For peak 2, the change were from 6.58×10^{-8} M (22.6 µg/L) to 7.72×10^{-9} M (2.65 µg/L) by use of the 8 potential pulses in the same steps of the potential. These changes correspond to a decrease in the LOD values around an order of magnitude of about two times less than those obtained when SWV was used.

In addition to the observations above, a comparative analysis of data obtained using liquid chromatography-electron spray ionization mass spectrometry and HPLC coupled to a spectrophotometric UV-Vis detector [2–10, 30] or electroanalytical procedures employing other electrode surfaces [11–14] showed that the values of LOD and LOQ obtained for MSWV still represent an order of magnitude of about two times less than those previously published.

This confirms the fact that the MSWV is very sensitive, making it possible to analyze ultra-traces even in more complex samples. Such results are very appropriate for determining traces of diquat in environmental water, where the recommended maximum residue stipulated by the European Community is 10 µg/L (10 ppb) [31].

To evaluate the efficiency of the proposed procedure, recovery experiments were carried out in pure electrolyte using the SWV and MSWV. The standard addition method was used and the percentage recov-ered obtained by a graphical method in which the abscissa refers to the concentration of the standard added to the electrolyte. When the curve obtained is extrapolated to this axis the percentage recovered is obtained.

The supporting electrolyte was artificially contaminated by addition of the 5.00×10^{-6} M of diquat, and the additions of the diquat stock solutions were inserted in the electrochemical cell and the responses were evaluated by SWV and MSWV. The recovery values calculated indicated that independent of the method used, the percentages recovered are satisfactory for analytical purposes (above 103% for peak 1, and above 108% for peak 2), which makes the application of the method quite practical for complex samples.

Similar experiments were realized using the supporting electrolyte contaminated by 2.500×10^{-7} M and MSWV. The recovery values calculated also presented values around 101% for both voltammetric peaks, indicating that the MSWV is efficient, the same in low concentration interval.

The precision of both techniques was evaluated using reproducibility experiments. For this, six different measurements of a standard solution containing 5.00×10^{-6} M of herbicide were evaluated, and the RSDs were calculated from current values measured for each voltammetric peak. Using the SWV and MSWV, a low value of reproducibility of 2% was

obtained for both peaks. Besides, the reproducibility of the MSWV also was evaluated in solutions containing 1.00×10^{-7} M, and the RSD values calculated presented values of the 2.15 and 3.05% for peak 1 and peak 2, respectively, indicating this way, that the use of MSWV in low concentration interval also presents a suitable reproducibility.

The accuracy of the measurements was evaluated from ten repetitive determinations (repeatability) of a standard solution containing 5.000×10^{-6} M of herbicide, and the RSD values obtained from peak currents in SWV and MSWV experiments were as low as 2%, for both voltammetric peaks. The reproducibility of the MSWV experiments in the concentration of the 1.000×10^{-7} M also determined and the RSD values calculated was 2.10 and 2.95% for peak 1 and peak 2, respectively.

The results presented in this work show that the analytical responses from the use of MSWV present a suitable improvement in the analytical sensitivity. Additionally, the responses obtained employing the commercial software for pulse potential application indicated that the development of an instrumentation setup and software which applies more than eight potential pulses in the same step would permit the acquisition of more sensitive results, and consequently, the electroanalytical determination of the organic and inorganic contaminants present in ultra-trace level in different samples with good sensibility, precision and accuracy.

Application of the proposed procedure to environmental water, foods and river sediments. Usually, the determination of diquat herbicide in natural water and food samples is performed using liquid chromatography–tandem mass spectrometry [32, 33], micellar electrokinetic chromatography [34] or liquid chromatography with UV-Vis detection [35]. When complex samples (such as food and natural water samples) were analyzed by chromatography methods, intense matrix effects occurred in analytical responses. Consequently, it is necessary to use preconcentration steps such as solid phase extraction. The chromatographic mobile phase is constituted by surfactants and organic solvents, which can be associated to instability in the chemical system and also, in the instrumental system. So, the use of procedures involving a direct analysis, without pre-treatment or clean-up steps, offers an excellent alternative to the use of chromatographic techniques.

For this, the procedure using MSWV was applied for the determination of diquat in environmental water, foods and river sediments samples. In practice, the samples used did not contain residues of the herbicide; they were employed to evaluate the influence of the composition of the samples on the analytical responses. The recovery curves were constructed in triplicate, using the same procedure effected for pure electrolyte.

In all experiments, cleaning of the Au-ME was necessary between each experiment to remove the components of the samples from the electrode surface. For this, the working electrode was washed with water and polished using an ultrafine sand paper. After the addition of herbicide, the samples were homogenized and the MSWV responses were evaluated employing the proposed procedure.

For environmental water samples, the supporting electrolyte prepared using these samples was spiked with 5.00×10^{-7} M of diquat by adding the standard solution, and the recovery experiments were carried out by the standard addition method. The values of the concentration recovered, in all environmental water samples, presented values proportional to the BOD and COD of each sample (sample 1: BOD = 5.50 mg/L and COD = 16.80 mg/L; sample 2: BOD = 7.05 mg/L and COD = 24.80 mg/L). However, the recovery values obtained were in the interval adequate for analytical procedures and indicated the suitability of the proposed method for environmental water samples (from 70 to 130%) [25]. In Table 2, the recovery values obtained from peak 1 are shown.

The proposed procedure was also employed for the determination of diquat in beetroot, peach and onion samples, spiked with diquat. The samples were prepared as described under "Experimental." In all the samples, the interferences due to adsorption processes were minimized by cleaning the electrodic surface between each experiment.

Compared to the experiments realized in the supporting electrolyte, the reduction potentials for diquat considering the two voltammetric peaks did not change significantly. The I_p value results demonstrated that the I_p for peak 1 is less inclined to interference by adsorption from components of the samples. However, peak 2 overlays the background current possibly related to other sample components which interfere with the electrochemical process that occurs with an intermediary adsorbed.

These responses were confirmed by experiments in which the same procedure was carried out in the samples before and after the addition of the herbicide, and are in agreement with previous works which showed the mechanism established for bipyridinium herbicides [11−14]. Certainly, this behavior is due to matrix interference effects on the electrode surface. This effect changes according to the composition of the samples employed, being more or less intense as a function of the adsorbed species and their concentrations. Thus, for all samples the recovery calculus was realized only for peak 1.

The proposed procedure was applied to beetroot, peach and onion samples contaminated with diquat. However, adsorption of diquat is higher than of paraquat. Therefore, in the direct analysis of real samples, where the responses were evaluated directly in the samples, the recovery efficiencies were lower than

Table 2. Recovery values obtained for diquat in different samples, according to the procedure employing Au-ME and MSWV ([diquat]$_{added}$ corresponds to the concentration diquat added in the samples and diluted in electrochemical cell, and [Diquat]$_{recovered}$ corresponds to the concentration obtained by recovery curve)

Sample	[Diquat]$_{added}$, M (g/g)	[Diquat]$_{recovered}$, M (g/g)	Recovery, %
Electrolyte 1	5.00×10^{-6}	5.18×10^{-6}	103 ± 2
Electrolyte 2	2.50×10^{-7}	2.53×10^{-7}	101 ± 3
Environmental sample water 1	5.00×10^{-7}	4.83×10^{-7}	97 ± 3
Environmental sample water 2	5.00×10^{-7}	4.39×10^{-7}	88 ± 3
Beetroot (direct analysis)	5.00×10^{-7} (9.09×10^{-9})	2.43×10^{-7} (4.40×10^{-9})	48 ± 7
Peach (direct analysis)	5.00×10^{-7} (9.09×10^{-9})	2.56×10^{-7} (4.64×10^{-9})	51 ± 6
Onion (direct analysis)	5.00×10^{-7} (9.09×10^{-9})	3.11×10^{-7} (5.63×10^{-9})	62 ± 5
Beetroot (diluting the sample)	2.50×10^{-7} (4.53×10^{-9})	1.94×10^{-7} (3.51×10^{-9})	78 ± 4
Peach (diluting the sample)	2.50×10^{-7} (4.53×10^{-9})	2.13×10^{-7} (3.85×10^{-9})	85 ± 4
Onion (diluting the sample)	2.50×10^{-7} (4.53×10^{-9})	2.04×10^{-7} (3.69×10^{-9})	82 ± 5
Sediment 1	7.5×10^{-6} (1.23×10^{-9})	5.83×10^{-6} (9.59×10^{-8})	78 ± 4
Sediment 2	7.5×10^{-6} (1.23×10^{-9})	5.77×10^{-6} (9.50×10^{-8})	77 ± 3

70%, which is lower than suitable values for analytical purposes (from 70 to 130%) [25]. These results are probably due to the adsorption of components of the samples on the electrode surface, which interferes in the electrochemical reduction of the adsorbed intermediaries.

In order to minimize the interferences of the adsorbed components on the electrodic surface, the samples were prepared by a simple dilution procedure and spikes, as described under "Experimental." With this method, the recovery percentages gave very satisfactory values, indicating that the proposed procedure can be employed successfully in complex samples. Table 2 shows the recovery percentages and RSD values obtained for diquat in food samples employing two experimental procedures described for use with the samples.

The proposed procedure was also applied to determine the diquat in river sediment samples spiked by standard additions of pesticide stock solution to the samples, ranging from 0.50 to 1.50×10^{-6} M, allowing the determination of the recovery percentages obtained by extrapolating the analytical curve, as was done for the food samples. In these samples the Au-ME was only washed with water between each experi-

ment. For these samples, again, only peak 1 was considered to calculate the recovery percentages, and the values obtained were between 75 and 80%, indicating that the procedure is very convenient for the determination of diquat residues in river sediments.

CONCLUSIONS

The results presented in this work demonstrated that the use of the custom-made microelectrodes allied to a very sensitive MSWV technique is suitable for easy application to a variety of environmental matrices such as foods and complex samples similar in nature without necessity in pre-treatment or chemical preparation stages. Beside the above-mentioned observation and conclusions, the use of the MEs makes direct analysis of the samples sensitive, precise and accurate. Even with a limited number of pulses in the same potential step, the MSWV represents an excellent choice for the determination of ultra-traces of diquat in all the samples employed, showing that the proposed procedure can be used for qualitative or quantitative determination of diquat residues in complex samples.

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