2012

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ANALYTICAL METHODS, CAMBRIDGE, v. 4, n. 8, supl. 2, Part 3, pp. 2348-2354, DEC, 2012
http://www.producao.usp.br/handle/BDPI/41955

Analytical Methods

Cite this: *Anal. Methods*, 2012, **4**, 2348

www.rsc.org/methods

PAPER

Evaluation of the detection and quantification limits in electroanalysis using two popular methods: application in the case study of paraquat determination

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Received 31st January 2012, Accepted 5th May 2012
DOI: 10.1039/c2ay25111f

In this work, the reduction reaction of paraquat herbicide was used to obtain analytical signals using electrochemical techniques of differential pulse voltammetry, square wave voltammetry and multiple square wave voltammetry. Analytes were prepared with laboratory purified water and natural water samples (from Mogi-Guaçu River, SP). The electrochemical techniques were applied to 1.0 mol L^{-1} Na_2SO_4 solutions, at pH 5.5, and containing different concentrations of paraquat, in the range of 1 to 10 μmol L^{-1}, using a gold ultramicroelectrode. 5 replicate experiments were conducted and in each the mean value for peak currents obtained −0.70 V *vs.* Ag/AgCl yielded excellent linear relationships with pesticide concentrations. The slope values for the calibration plots (method sensitivity) were 4.06×10^{-3}, 1.07×10^{-2} and 2.95×10^{-2} A mol^{-1} L for purified water by differential pulse voltammetry, square wave voltammetry and multiple square wave voltammetry, respectively. For river water samples, the slope values were 2.60×10^{-3}, 1.06×10^{-2} and 3.35×10^{-2} A mol^{-1} L, respectively, showing a small interference from the natural matrix components in paraquat determinations. The detection limits for paraquat determinations were calculated by two distinct methodologies, *i.e.*, as proposed by IUPAC and a statistical method. The values obtained with multiple square waves voltammetry were 0.002 and 0.12 μmol L^{-1}, respectively, for pure water electrolytes. The detection limit from IUPAC recommendations, when inserted in the calibration curve equation, an analytical signal (oxidation current) is smaller than the one experimentally observed for the blank solution under the same experimental conditions. This is inconsistent with the definition of detection limit, thus the IUPAC methodology requires further discussion. The same conclusion can be drawn by the analyses of detection limits obtained with the other techniques studied.

A. Introduction

The growing rate of discarded pesticides and drugs is associated with environmental contamination and subsequent risks to human health. Considering that most of these substances are synthetic, tiny amounts can result in effects not yet fully understood to health and reproduction in humans and livestock. Thus, detection and quantification of trace and ultratrace complex organic molecules is a constant challenge in analytical chemistry. Pico to nano moles per litre amounts of *e.g.* pesticides, hormones, drugs, should be determined with precision and reliability. Modern analytical techniques provide the possibility to run such determinations, frequently *in situ*, and in many cases in real time allowing remediation actions to be taken.

In order to present reliable results on the demanded concentration range, analytical techniques should feature, as one of the main characteristics, a low *detection limit*, together with reproducibility and robustness. This concept has been defined in different ways in the literature, but is defined as *an analyte concentration that yields a signal in the measurement instrument (y) which is significantly different from that obtained for the blank*.[1] Every discussion about methodologies employed to calculate the detection limit of a given analytical technique emerges from the definition of "significantly different". In this way, the International Union of Pure and Applied Chemistry (IUPAC) proposes that a good evaluation is given through considering a linear calibration curve, normal data distribution, and constant variance in every measurement[2], *i.e.*:

$$\mathrm{LOD} = 3S_B/S \quad (1)$$

where S_B is the standard deviation of 10 measurements taken from the signal obtained from the blank (a solution identical to that analysed but without the analyte) and S the slope of the calibration curve (sensitivity of the analytical method). The number 3 comes from the required 90% level of confidence in the difference between the observed signal and the blank response. Such methodology is popular when applied to analytical techniques, *e.g.* electrochemical and spectrophotometry, mainly due

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to the simplicity of the statistical procedure. Consequently, it is found that a very large number of papers in the literature employ such a methodology.[3–7] In Brazil, the Government regulatory agencies adopted a similar concept of detection limit. In this way, the *Instituto Nacional de Metrologia, Normalização e Qualidade Industrial* published in March 2003 a guidance only document, where this concept is defined, in a similar fashion to the IUPAC definition.[8] On the other hand, the *Agência Nacional de Vigilância Sanitária (ANVISA)* postulated that the detection limit should be calculated from:

$$\mathrm{LOD} = 3A/S \quad (2)$$

where A is the standard deviation of the intercept of the linear calibration curve of, at least, three independent curves. Of course, eqn (2) is similar to eqn (1), only with a different methodology to calculate S_B. However, a conceptual criticism of such an approach, although it is widely applied being established by usage methodology, concerns the use of standard deviation of the blank signal as the determining parameter in the calculation of detection limit. As observed in eqn (1), the smaller the value of S_B the smaller the detection limit of the methodology. But S_B is defined as:[1]

$$S_B = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{(n-1)}} \quad (3)$$

where x_i is the analytical signal value and n is the number of repetitions done. As can be observed if n increases, S_B diminishes and, thus the detection limit also gets lower. Consequently the n value should be carefully standardized, which does not seems to be the case. Several papers that use eqn (1) report values of n between 3 and 20. In this way, the reliability of detection limit determination is bonded to the measurement of the smallest analytical signal (the blank one), with an unavoidable loss of precision.

Another far from logical consequence in the utilization of such methodology is concerned with the fact that, as the standard deviation of the blank solution is normally very low, the numerical values of detection limit obtained are so small that they are not detectable in the calibration curve.[9–11] In this way, the physical meaning of such quantitites is not straightforward.

Paraquat is used worldwide as a non-selective herbicide and is sold under commercial names of Gramoxone, Weedol or Panthclear.[12] Its molecular structure is presented in Fig. 1. Paraquat has been determined by several electrochemical techniques[13–19] due to its reversible electrochemical response, its good solubility in water and the importance of trace determination of this pesticide in foodstuff, since it is extremely hazardous to human health. The considerable number of publications available in literature makes this pesticide an excellent molecule model for discussion of detection limit methods in different matrices.

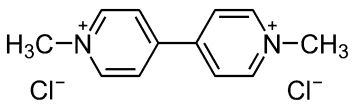

Fig. 1 Molecular structure of the paraquat herbicide.

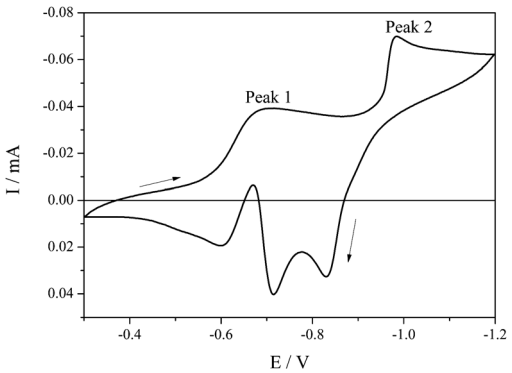

Fig. 2 Stationary state cyclic voltammogram on UME-Au in a 4.2 × 10^{-3} mol L^{-1} paraquat + 0.1 mol L^{-1} Na_2SO_4 at 0.1 V s^{-1}.

This work aims to determine the detection limits of the herbicide paraquat in laboratory matrices and in water samples from the Mogi-Guaçu River, Brazil, using three different electroanalytical techniques: differential pulse voltammetry, square wave voltammetry and multiple square wave voltammetry. The results are compared to those obtained by IUPAC methodology using a far less popular statistical methodology.

B. Experimental

Reagents and equipment

All electrochemical experiments were performed at room temperature, using a 25 mL volume electrochemical cell made of borosilicate glass with a polytetrafluorethylene cover. In this cover there were special holes to accommodate the entrance and exit of N_2 gas (White Martins, SS, bubbled in the solution 15 minutes prior to the experiment with the flow kept over the surface during the measurements) and the reference (Ag/AgCl, 3.0 mol L^{-1} KCl), auxiliar (Pt foil with 2 cm^2 geometric area) and working electrodes (a home-made Au ultramicroelectrode – UME-Au – obtained by embedding a Goodfellow 99.95% Au wire with 25 μm diameter in epoxy resin). The electrochemical experiments of square wave voltammetry (SWV), multiple square wave voltammetry (MSWV) and differential pulse voltammetry (DPV) were performed in a model PGZ 402 Voltalab potentiostat/galvanostat controlled by Voltamaster 4.05 software from Radiometer Analytical. During the voltammetric experiments the electrochemical cell was kept in a Faraday cage to minimize background noise.

A 1.0 × 10^{-3} mol L^{-1} stock solution of paraquat (Aldrich, 98%) was prepared with ultrapurified Milli-Q (Millipore Inc.) water. For the electroactivity of paraquat on the UME-Au measurements, we used a support electrolyte composed of 1.0 mol L^{-1} Na_2SO_4 solution, with pH adjusted to 5.5 with NaOH or H_2SO_4 1.0 mol L^{-1}. All reagents were supplied by Merck PA and used without any further purification.

Experimental procedure

The experimental setup, support electrolyte, hydrogenionic concentration, voltammetric parameters as pulse amplitude (a), scan increment (ΔE_s) and frequency (f) related to the SWV, were

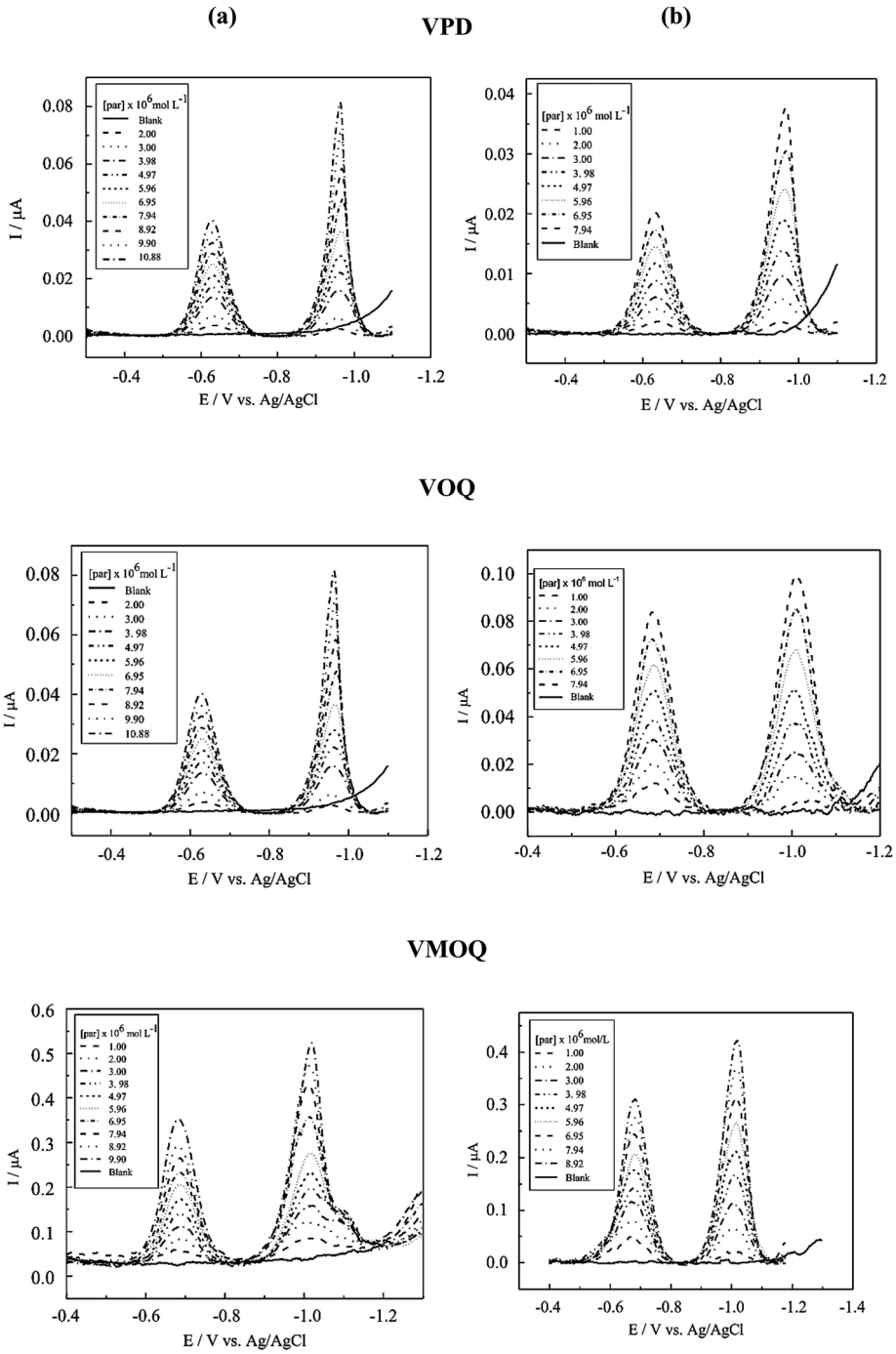

Fig. 3 Voltammograms for various paraquat concentrations in 0.1 mol L^{-1} Na_2SO_4 on UME-Au using $a = 50$ mV, $\Delta E_s = 2$ mV, $f = 250$ s^{-1}, $N = 8$ e $v = 20$ mV s^{-1}. In (a) the results were obtained in electrolyte prepared with purified water and in (b) with water from the Mogi-Guaçu River.

already optimized for these measurements by our research group.[12,20] The MSWV parameters were the same as SWV except for a multiple pulse (N) programming overlayed on a single potential step, allowing variations from 2 to 8 pulses, on the same step. DPV parameters were also evaluated.

Analytical curves were obtained by the consecutive addition of standard solution aliquots to the support electrolyte prepared either with ultrapurified or natural water, collected from the Mogi-Guaçu River in São Carlos, São Paulo, Brazil, to investigate the matrix effect of such natural sample. Under these experimental conditions detection and quantification limits were determined for the three electrochemical techniques: DPV, SWV and MSWV.

Statistical treatment of analytical data

In this work, the statistical method described by Miller and Miller[1] is used to evaluate experimental error, confidence limits, and to calculate the detection and quantification limits. Firstly, the product-moment correlation coefficient (r) is calculated:

Table 1 Figures of merit for paraquat quantification in electrolytes prepared with Milli-Q water and river water

	Purified water			Natural water		
Parameters	DPV	SWV	MSWV	DPV	SWV	MSWV
Repeatability (relative standard deviation %)	1.98	2.03	1.99	1.99	2.01	1.96
Reproducibility (relative standard deviation %)	2.34	2.20	2.25	2.54	2.58	2.76
r	0.9986	0.9994	0.9998	0.9961	0.9986	0.9996
Sensitivity (b) (A mol^{-1})	$4.06 \times 10^{-3} \pm 1.47 \times 10^{-4}$	$1.07 \times 10^{-2} \pm 3.54 \times 10^{-4}$	$2.95 \times 10^{-2} \pm 5.19 \times 10^{-4}$	$3.60 \times 10^{-3} \pm 1.65 \times 10^{-4}$	$1.06 \times 10^{-2} \pm 4.96 \times 10^{-4}$	$3.35 \times 10^{-2} \pm 5.90 \times 10^{-4}$
Intercept (a) (μA)	$3.42 \times 10^{-3} \pm 9.80 \times 10^{-4}$	$-5.19 \times 10^{-3} \pm 1.70 \times 10^{-4}$	$-2.58 \times 10^{-2} \pm 2.67 \times 10^{-3}$	$1.02 \times 10^{-3} \pm 7.79 \times 10^{-4}$	$1.04 \times 10^{-3} \pm 2.36 \times 10^{-4}$	$-1.01 \times 10^{-2} \pm 3.33 \times 10^{-3}$

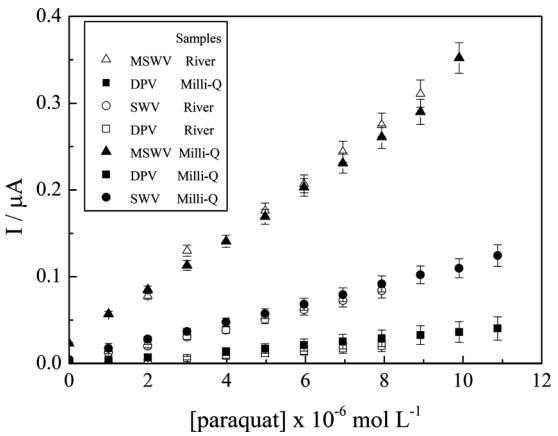

Fig. 4 Analytical curves for paraquat on UME-Au in electrolytes prepared with pure and natural waters using DPV, SWV and MSWV.

$$r = \frac{\sum_i \{(x_i - \bar{x})(y_i - \bar{y})\}}{\left\{\left[\sum_i (x_i - \bar{x})^2\right]\left[\sum_i (y_i - \bar{y})^2\right]\right\}^{1/2}} \quad (4)$$

where ($\bar{x}$, $\bar{y}$) is the centroid of the experimental data, *i.e.*, the arithmetic mean value of all x (standard concentrations) or y (equipment signal) values. A value of $r = +1$ describes a perfect correlation, *i.e.*, all experimental data is exactly linear. Secondly, the slope (b) and intercept (a) of the straight line is calculated:

$$b = \frac{\sum_i [(x_i - \bar{x})(y_i - \bar{y})]}{\sum_i (x_i - \bar{x})^2} \quad (5)$$

$$a = \bar{y} - b\bar{x} \quad (6)$$

The random errors in slope and intercept were also evaluated. In order to do so, the statistic parameter ($S_{x/y}$) was obtained, which estimates random errors in the y-direction:

$$S_{y/x} = \left\{\frac{\sum_i (y_i - \hat{y}_i)^2}{n - 2}\right\}^{1/2} \quad (7)$$

The $\hat{y}_i$ symbol represents the points on the calculated regression line (eqn (6)) corresponding to the individual x-values. This $\hat{y}_i$ parameter can be used to calculate the standard deviation of both slope and intercept:

$$s_b = \frac{S_{y/x}}{\left\{\sum_i (x_i - \bar{x})^2\right\}^{1/2}} \quad (8)$$

and

$$s_a = S_{y/x}\left\{\frac{\sum_i x_i^2}{n\sum_i (x_i - \bar{x})^2}\right\}^{1/2} \quad (9)$$

Table 2 Spreadsheet for the calculation of statistical parameters for paraquat quantification in pure water by MSWV

x (μmol L^{-1})	y (μA)	$x - \bar{x}$	$y - \bar{y}$	$(x - \bar{x})(y - \bar{y})$	$(x - \bar{x})^2$	$(y - \bar{y})^2$	$\hat{y}$	$(y - \hat{y})^2$	x^2
0.000	−0.0270	−4.3058	0.12567	−0.54109	18.53972	0.01579	−0.02579	0.00000146	0.0000
0.999	−0.0570	−3.3068	0.09567	−0.31635	10.93478	0.00915	−0.05523	0.00000314	0.9980
1.996	−0.0850	−2.3098	0.06767	−0.15629	5.33507	0.00458	−0.08461	0.00000016	3.9840
2.991	−0.1130	−1.3148	0.03967	−0.05215	1.72864	0.00157	−0.11392	0.00000086	8.9461
3.984	−0.1410	−0.3218	0.01167	−0.00375	0.10354	0.00014	−0.14319	0.00000477	15.8723
4.975	−0.1690	0.6692	−0.01633	−0.01093	0.44786	0.00027	−0.17239	0.00001147	24.7506
6.951	−0.2310	2.6452	−0.07833	−0.20721	6.99720	0.00614	−0.23061	0.00000015	48.3164
7.937	−0.2610	3.6312	−0.10833	−0.39338	13.18577	0.01174	−0.25967	0.00000178	62.9960
8.919	−0.2900	4.6132	−0.13733	−0.63355	21.28182	0.01886	−0.28860	0.00000196	79.5486
38.752	**−1.3740**	**0.0000**	**0.00000**	**−2.31471**	**78.55441**	**0.06823**	**−1.37400**	**0.00002574**	**245.4119**
Mean									
4.306	−0.15267								
b	a								
−0.02947	−0.02579								
$S_{y/x}$	S_b	S_{az}	Lodi (μA)	LOD (μmol L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})			
0.00192	0.00022	0.00113	−0.02918	0.11503	21.4235	71.4117			

Table 3 Limits of detection calculated by the two methodologies employed

		IUPAC			Miller and Miller		
		DPV	SWV	MSWV	DPV	SWV	MSWV
Purified water	LOD (μmol L^{-1})	0.16	1.95×10^{-2}	1.98×10^{-3}	0.30 ± 0.001	0.20 ± 0.0017	0.11 ± 0.0027
	LOD (ppb)	29.43	3.63	0.37	55.35 ± 0.18	37.50 ± 0.32	21.42 ± 0.51
	LOQ (ppb)	98.17	12.11	1.24	184.49 ± 0.18	125.00 ± 0.32	71.41 ± 0.51
Natural water	LOD (μmol L^{-1})	0.27	2.76×10^{-2}	2.96×10^{-3}	0.38 ± 0.0008	0.28 ± 0.0024	0.13 ± 0.0034
	LOD (ppb)	50.93	5.14	0.55	70.52 ± 0.15	52.76 ± 0.19	23.59 ± 0.63
	LOQ (ppb)	169.77	17.13	1.84	235.06 ± 0.15	175.86 ± 0.19	78.62 ± 0.63

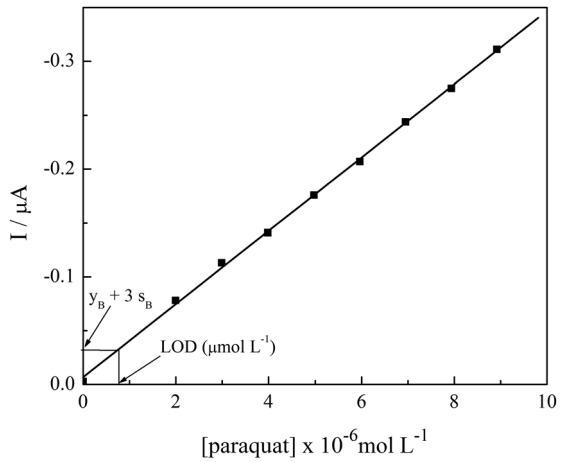

Fig. 5 Analytical curve for paraquat on UME-Au for MSWV experiments in purified water.

The standard deviation values above allow the calculation of confidence limits for the slope ($b \pm t_{(n-2)}s_b$) and the intercept ($a \pm t_{(n-2)}s_a$), where t is the Student parameter.

After obtaining the analytical curve, by statistic parameters, the detection and quantification limits were calculated for each electroanalytical methodology employed. These limits values were calculated in two different ways, *i.e.*, following IUPAC recommendations, using eqn (1) with $n = 10$ and using the statistical method proposed[1] as the analyte concentration gives a current signal defined by:

$$Y_{LOD} = y_B + 3S_B \quad (10)$$

where y_B is the analytical signal of the blank and S_B its standard deviation. The limit of quantification is defined in an analogous way as:

$$Y_{LOQ} = y_B + 10S_B \quad (11)$$

It is important to notice that, with this methodology, it is not necessary to perform several repetitions of the blank measurement since the S_B parameter is obtained directly from the analytical curve, as shown above. Thus, the standard deviation of the blank is not affected further by the different number of blank signal determinations. The intercept value (a) from the linear regression curve can be utilized as a precise estimative of the y_B value, which standard deviation is given by the behavior of the entire curve and not from just one point under the worst possible conditions *i.e.* smallest signal obtained for the blank. In this way its error is significantly reduced.

C. Results and discussion

The SWV responses for a 4.25×10^{-3} mol L^{-1} paraquat solution with the UME-Au in 0.10 mol L^{-1} de Na_2SO_4, pH = 5.5, with $f = 250\ s^{-1}$, $a = 50$ mV and $\Delta E_s = 2$ mV, showed two reduction peaks around −0.70 and −1.00 V, with voltammetric profiles similar to those previously published.[12,20,21] These results are presented in Fig. 2 and the two peaks are associated with two different reduction processes[21], the second reduction process is followed by chemical dimerization.

$$PQ^{2+} + e^- \leftrightharpoons PQ^{+} \text{ (peak 1 at } -0.70 \text{ V)} \quad (12)$$

and

$$PQ^{+} + e^- \leftrightharpoons PQ \text{ (peak 2 at } -1.00 \text{ V)} \quad (13)$$

The introduction of MSWV expanded the application range of electroanalysis toward lower concentration values in the ultra-trace analysis domain. MSWV consists of applying several potential pulses (N) to each potential step in the electrode programme. These pulses activate the electrode surface to yield a more intense analytical signal.[22–25] Due to equipment limitations in this work, N was chosen as 8 pulses for each step. Moreover, as peak 1 is associated with an electrochemical process that does not involve adsorption of reagents or products on the electrode surface, N was selected to furnish the analytical signal in the following determinations.

In DPV experiments, the voltammetric parameters were optimized and the highest analytical signal was obtained using $a = 50$ mV, $\Delta E_s = 2$ mV and scan rate (v) = 20 mV s^{-1}.

Analytical curves for DPV, SWV and MSWV and statistical treatment

Using the experimental setup described in the Experimental section, analytical curves were obtained for paraquat in two different supporting electrolytes, *i.e.*, 1.0 mol L^{-1} Na_2SO_4 + 1.0×10^{-3} mol L^{-1} paraquat solutions prepared either with Milli-Q or Mogi-Guaçu's river water. Aliquots of stock solutions were consecutively added to the electrochemical cell. The different voltammetric responses obtained for the concentration range of pesticide between 1.0 and 11.0 μmol L^{-1} are displayed in Fig. 3. It

is observed that all peak currents showed a direct relationship with pesticide concentration.

Voltammetric experiments, with different pesticide concentrations in both water samples, were repeated 5 times each. The reproducibilities (*i.e.*, the relative standard deviations for experiments performed on different days, with different solutions) and repeatability (experiments performed on the same day, in the same electrochemical cell with the same samples) are presented in Table 1, which also includes figures of merit of the electroanalytical experiments. The mean value of peak current in each measurement for each experimental technique are plotted against the paraquat concentration and the respective analytical curves are given in Fig. 4. The slopes of the analytical curves are associated with the sensitivity of the analytical methodology, *i.e.*, the maximum analytical signal possible to obtain for each concentration of analyte. These different sensitivity values are included in Table 1.

The values presented in Table 1 indicate that MSWV presents a much higher sensitivity than the other two techniques (3 times that of SWV and 10 times that of DPV) as expected. This confirms the efficiency of multipulses application to activate the electrode surface. Additionally, it can be observed that the slopes of the calibration curves are quite similar for purified and river waters, in all three analytical techniques. Such experimental observations are tested by running *t*-tests[1] for $n = 5$ and $P = 0.05$ between each pair of slope values (for purified and river water samples). In all cases, no significant difference was observed. This indicates that the matrix effect is very small in river water and organic (humic and fulvic acids, contaminants) and inorganic (cations and anions) components of samples do not interfere significantly in the determination of paraquat.

In order to perform a statistical treatment in the set of data acquired in the experiments, initially the product-moment correlation coefficient was calculated as described in eqn (4) for all analytical curves obtained. The resulting values are incorporated into Table 1 and, together with the visual analysis of the straight lines presented in Fig. 4, they confirm the linear relationship between analytical signals and paraquat concentrations.[1]

In the next step, the linear regression equations are calculated for all straight lines using eqn (5) and (6). With these equations and the other parameters included in a Microsoft Inc. Excel spreadsheet, the other parameters as slopes (b), intercepts (a), standard deviations of the slopes (S_b), intercepts (S_a), points in the analytical curves ($S_{y/x}$) and the respective confidence intervals were calculated. These data are displayed in Table 1. The spreadsheet used for MSWV in electrolyte prepared with purified water is presented in Table 2.

The detection and quantification limits for all experimental methods employed, both in electrolyte prepared in the laboratory and in natural water, were calculated by two different methods: one recommended by IUPAC (using eqn (1)) and the statistical method of Miller and Miller (eqn (10) and Excel spreadsheet). In the former, the blank standard deviation was obtained from 10 different determinations. In the latter, the blank standard deviation was obtained as the error of the intercept of the linear regression line with the y-axis, as described in the literature.[1] The results obtained are presented in Table 3 and an example of the analytical curves obtained by linear regression with the calculated detection limit is shown in Fig. 5.

As observed in Table 3, the detection limits calculated by the IUPAC proposed methodology are orders of magnitude lower than those obtained using the analytical curves method. This difference is strictly related to the standard deviation of the blank calculation, at the same potential value as for the peak current for paraquat reduction. In this way, the calculated detection limit is not given by the capability of the analytical methodology to recognize a signal significantly different from the blank, since the mean current value observed for the blank solution by MWSV is -0.65 V is -0.025 μA, which using the corresponding regression curve equation yields a paraquat concentration value of 0.027 μmol L^{-1}, higher than the detection limit itself, and defies the definition of detection limit (as defined in the Introduction section). The same calculations performed with the statistical method proposed yield a detection limit value of 0.12 μmol L^{-1}, with an analytical signal of -0.030 μA higher than the blank signal. The same discussion holds for all the other methodologies tested in this work.

In conclusion, the statistical methodology proposed by Miller and Miller[1] is more accurate in the determination of detection limit (within the well-defined statistical meaning) and we suggest it replaces that recommended by IUPAC for electroanalytical work.

D. Conclusions

In this work, the results obtained for the electrochemical determination of paraquat in electrolytes made with either purified laboratory or natural water, using well-established analytical procedures, allows an useful comparison of methods employed to calculate detection limits.

The resulting values, using two different calculation procedures, *i.e.*, one that emphasizes the blank measurements (IUPAC) and another that uses analytical curves (statistical methodology) show significant differences. The most commonly used method (IUPAC) yielded detection limit values that, for all analytical curves, resulted in current values smaller than responses obtained for the blank, which contradicts the definition of detection limit. This inconsistent behavior is not observed when one uses the whole analytical curve for the calculations (statistical method). In this way, it is considered that the statistical method is more consistent than the more commonly used IUPAC method and should therefore be employed as the standard.

Moreover, by using the statistical method, the confidence intervals of slopes and intercepts values can be calculated and, following statistical concepts, no quantitative results are of value unless they are accompanied by some estimate of the inherent errors.[1] Thus, in electroanalysis, as well as other analytical procedures statistical treatment is fundamental.

We conclude that calculations of important parameters like the detection limit (or quantification) in trace or ultratrace analyses is being performed in a controversial way and a more comprehensive discussion of such determinations is imperative.

Acknowledgements

One of the authors (OBS) thanks the *Secretaria de Educação do Estado de São Paulo* under the scholarship (Programa de Bolsa

de Mestrado). Thanks are also given to *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for financial support.

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