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Multivariate optimization of square wave voltammetry using bismuth film electrode to determine atrazine

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ABSTRACT

A rapid and simple square wave voltammetry method (SWV) for atrazine determination with bismuth film electrode using chemometrics was developed. Different variables such as buffer pH and SWV frequency, pulse height and step potential were screened in a 2^4 full factorial design. The significant variables were optimized by using a central composite design (CCD) combined with desirability function (DF). Optimum conditions were set as follow: buffer pH 2.3, frequency 300 Hz, pulse height 0.09 V and step potential -0.0024 V. Under these optimum conditions the method has a linear response over $(1.8–7.5) \times 10^{-6}$ mol L^{-1} with detection and quantification limits of 5.9×10^{-7} and 1.8×10^{-6} mol L^{-1}, respectively.

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1. Introduction

Environmental monitoring has become an increasingly demanding field in recent years, due to the fact that both, governments and consumers are becoming more aware of the damage that pollution can do, caused by industrial and agricultural practices. Therefore, guidelines have been set on the maximum permissible levels of certain pollutants, such as pesticides. Atrazine (ATZ) (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is a member of *s*-triazine family of herbicide and is one of the most commonly used herbicide [1], and its structure is presented in Scheme 1. Due to the widespread use of atrazine to control annual grasses and broad-leaved weeds in crops like sugar cane, maize, soybean, citrus fruits, etc. in the railways, roadside verges and golf courses, great attention has been given to its environmental impacts and monitoring [2–4]. Although the toxicological effects of atrazine and other triazines on humans is weaker than those reported for chlorinated and organophosphorus pesticides, severe environmental problems can result from their persistence in soils and sediments, as well as their run-off to surface and ground waters. Due to its low reactivity, water solubility and slow degradation, ATZ is reasonably stable, mobile and persistent in the environment for a long period of time (half-life varying among 20–100 days) [5]. In very small quantities, it may act as a carcinogen of type C and an endocrine disruptor of the hormonal system [6].

A literature research shows many reports describing various analytical methods for the determination of ATZ and other triazine herbicides. These particularly involve spectrophotometric [7] and especially chromatographic methods such as high performance liquid chromatography (HPLC) [8,9] and gas chromatography (GC) [10]. The latter have become the most widely used for the determination of trace amounts of triazine herbicides, because of their absolute best sensitivity and selectivity. However, these techniques require the complicated sample preparation usually involving the pre-concentration step prior to the analysis. In addition, they are expensive, time consuming, and the solvent waste-production requires highly skilled personnel that often restricts their use in routine analytical practice.

The electrochemical methods are especially suitable for the environmental monitoring of heavy metals and electroactive organic pollutants. They are simple, inexpensive, sufficiently sensitive as well as they can represent an independent alternative to so far dominant spectrophotometric and chromatographic techniques [11]. Regarding the electrochemical determination of ATZ, enzyme biosensors and immunosensors have been used [12] or mercury electrodes, such as hanging mercury drop electrode (HMDE) [13–15], static mercury drop electrode (SMDE) [16] and mercury film electrode (MFE) [17,18] are mostly exploited. These methods are most sensitive due to the excellent electrochemical properties of mercury. On the other hand, the highly toxic nature of mercury insists on the search for alternative electrode materials. Therefore,
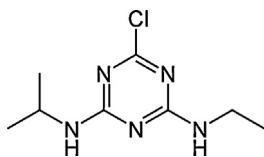
Scheme 1. Atrazine structure.

the novel, simple and environmentally acceptable electrochemical tool for the sensitive environmental monitoring of pesticide residues is of great importance.

Recently, a significant step forward is the introduction and development of bismuth [19] and antimony-based electrodes [20,21]. Bismuth film electrodes (BiFEs) consist of an electrochemically generated thin bismuth film, deposited mainly on carbon-based substrates, such as glassy carbon [22], carbon fiber [23], or screen-printed carbon [24]. The bismuth film is mechanically stable, exhibiting high electroanalytical performance for trace metal analysis, well comparable to the mercury electrodes. Most of the articles presented in the literature are dedicated to the application of BiFE to determine metallic cations [25–29]. However, few papers described the use of BiFE for organic compounds, such as drugs [30,31] and pesticides [32–35] determination.

The square wave voltammetry (SWV) is rapid, sensitive and consumed less analyte than the other pulsed techniques [36]. Most of the optimization of SWV also deal with the traditional one-factor-at-a-time (OFAT) approach, examine the SWV parameters such as frequency, step potential and pulse height [13,14,17]. If the factors are independent (which is rarely the situation), the most common practice is OFAT while holding all others parameters constant. However, the result of this univariate analysis shows inadequate optimization toward response(s). Moreover, OFAT approach is costly in sense of time and reagents. There is now increasing recognition that hereditary malpractice ought to be replaced by soundly based reliable methods, such as response surface methodology (RSM) based on statistical design of experiments (DOEs). Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes [37] that is dedicated to the evaluation of relations existing between a group of controlled experimental factors and the observed results of one or more selected criteria. Such statistical analyses are more efficient, since they account for interaction effects between the studied variables and determine more accurately the combination of levels that produces the optimum of the process. The importance, theoretical concepts and application behind the optimization through experimental design, as well as RSM in research and development efforts have been thoroughly discussed in a number of informative articles [37–40]. Furthermore, a study has been done on the SWV optimization by using statistical experimental design [41]. Thus, RSM provides a predicted model, considering the response over the whole domain. By using this predicted model the optimum response can be determined.

In the present study, SWV using bismuth film electrode was employed for atrazine determination. Influence of important variables was investigated and optimized by experimental design. We focused on the enhancement of the intensity of atrazine current by using screening and response surface experimental designs as improved optimization methodology. Derringer's desirability function, was used for the evaluation the SWV parameters.

2. Experimental

2.1. Apparatus

Square wave voltammetry measurements were performed with Autolab (PGSTAT 101 Eco-Chemie) potentiostat. A conventional three-electrode system was used where glassy carbon (GCE, 3 mm 75 diameter), a platinum wire, and a Ag/AgCl (3 M KCl) were used as working, counter, and reference electrodes, respectively. All potentials are referred to this reference electrode. All electrochemical experiments were performed at room temperature.

2.2. Data analysis

The STATISTICA statistical package software version 7.0 (Stat Soft Inc., Tulsa, USA) was used for experimental design analysis and data processing. The quality of the fit of the polynomial model equation was expressed by the coefficient of regression R^2, and its statistical significance was checked by a Fisher F-test. The level of significance was given as values of the probability less than 0.05.

2.3. Reagents and solutions

All reagents were obtained as analytical grade and used without further purification. Purified water obtained from a Milli-Q water purifying system (18 MΩ cm^{-1}) was used for all experiments. Atrazine and Bi(NO$_3$)$_3$·5H$_2$O were purchased from Sigma–Aldrich (St. Louis, MS, USA). K$_2$HPO$_4$, KH$_2$PO$_4$, acetic acid, sodium acetate, sulfuric acid and potassium hydroxide were J.T. Baker. 0.05 mol L^{-1} Phosphate buffer solution (PBS) was prepared by adjusting a mixture of 0.05 mol L^{-1} K$_2$HPO$_4$/KH$_2$PO$_4$ to the required pH value by adding either 1.0 mol L^{-1} hydrochloric acid (HCl) or potassium hydroxide (KOH) solution.

The electrodeposition solution was prepared with 1×10^{-3} mol L^{-1} Bi(NO$_3$)$_3$·5H$_2$O in 0.1 mol L^{-1} H$_2$SO$_4$.

To evaluate the accuracy and applicability of the proposed method, the optimized procedure for different water samples was performed. River water samples were taken from different field areas of the Los Espinillos River at Córdoba, Argentina (November 2012). The collected water samples were filtered through a 0.45 μm micropore membrane and were maintained in glass containers, then stored at a temperature of 4 °C until their analysis time. For each sample, an aliquot of 2.5 mL was transferred to three different calibration flasks. After this, an aliquot of 3.8×10^{-4} mol L^{-1} ATZ standard solution was added to the samples and filled with buffer solution up to 10 mL and homogenized.

2.4. Preparation of BiFE

The glassy carbon electrode was mechanically polished with 0.05 μm alumina/water slurry (Buehler, USA) on a polishing cloth to a mirror-like finish followed by sonication, rinsed with distilled water, dried in air and inserted into the electrochemical cell. The bismuth film electrodeposition onto the GCE was carried out chronoamperometric by applying a deposition potential of −1.00 V for 360 s in the electrodeposition solution.

To remove the bismuth film, a potential step of +0.5 V for 60 s in a PBS buffer solution was applied, after which the deposition of a fresh film was performed.

2.5. Experimental procedure

The determination of pesticides such as ATZ (Scheme 1) can be obtained by electrochemical reduction which corresponds to the reduction of the carbon−chloride bond of the triazine ring with the previous protonation equilibrium of atrazine as proposed by De Souza et al. [42]. In this work, ATZ determinations were evaluated by square wave voltammetry (SWV), and a cathodic peak was obtained. The SWV parameters and the best chemical conditions (chemical buffer and pH) were evaluated. The following optimized SWV procedure was used: 10 mL of phosphate buffer 0.05 mol L^{-1}

were transferred into the voltammetric cell and deaerated by bubbling oxygen-free nitrogen for 10 min; the nitrogen stream was then kept over the solution. After equilibrium time (5 s), a cathodic potential scan was applied between −0.6 and −1.0 V versus Ag/AgCl (3.0 mol L^{-1} KCl) with the following settings: frequency 300 Hz, pulse height 0.09 V and step potential −0.0024 V. All measurements were carried out in triplicate (n = 3) for each concentration.

2.6. Experimental design

The most popular first-order design is the two-level full (or fractional) factorial, in which each factor is experimentally studied at only two levels that are expressed in coded form: −1 for low level and +1 for high level. The full factorial design consists of a 2^k experiment (k factors, each experiment at two levels), which is very useful for either preliminary studies or in initial optimization steps, while fractional designs are almost mandatory when the problem involves a large number of factors [43]. In this work, the full factorial design was applied to evaluate the main effects of the following four factors: buffer pH, SWV frequency, pulse height and step potential. Table 1 depicts the variables and their corresponding levels used in the experimental design as well as the factorial design matrix and cathodic peak current (i_p) measured in each factorial experiment. Each factor was examined at two levels: −1 for low level and +1 for high level, and a center point was run to evaluate the linear and curvature effects of the variables. The experimental design included 16 experiments plus 2 central points in order to estimate the experimental error (in total 36 runs with replicates). The low and high levels for the factors were selected according to some preliminary experiments. The order in which the experiments were made was randomized to avoid systematic errors. The main effects and interactions between factors were determined. Then, the significant variables were optimized by using a central composite design (CCD) and a quadratic model was built between the dependent and the independent variables. The CCD is a better alternative to the full factorial, three-level design as it demands a smaller number of experiments while providing comparable results to fit quadratic models and was first described by Box and Wilson [44].

Therefore, this has been the most accepted experimental design for second-order models. A variation of the central composite design is sometime used because it requires only three levels of each factor, and in practice it is frequently difficult to change factor levels [45]. The total number of design points needed (N) is determined by the following equation:

$$N = 2^f + 2f + N_0 \tag{1}$$

where f and N_0 are the number of variables and center points (f = 4, N_0 = 2), respectively [37,45,46]. Therefore, totally 31 experiments (in total 26 with 5 center point) had to be run for the face-centered central composite design CCD (see Table 2).

2.7. Desirability function

Desirability function (DF) is a common and established technique to concurrently determine of input variables that can give the optimum performance levels for one or more responses. Harrington first developed the DF, and it was later optimized by Derringer and Suich [47] for specifying the relationship between predicted responses on a dependent variables and the desirability of the responses. The DF have three "inflection" points (0, 0.5 and 1) and desirability procedure involves three steps: (1) predicting responses on the dependent variable by fitting the observed responses using an equation, based on the levels of the independent variables, (2) finding the levels of the independent variables that simultaneously produce the most desirable predicted responses on the dependent variables and (3) maximize the overall desirability with respect to the controllable variables. Therefore, the main advantages of using desirability functions are to obtain qualitative and quantitative responses by the simple and quick transformation of different responses for one measurement. First, the response is converted into a particular desirability function that varies from 0 to 1. The desirability 1 is for maximum and desirability 0 is for non-desirable situations or minimum. Inspecting the desirability profile, it determines which levels of the predictor variables produce the most desirable predicted response on the dependent variables.

Table 1
Full factorial experimental design matrix of important variables for atrazine detection and obtained response.

Runs	Variables				i_p (µA)	
	Buffer pH (1.50[a], 2.30[b] and 3.10[c])	Frequency (Hz) (75.0[a], 187.5[b] and 300.0[c])	Pulse height (V) (0.02[a], 0.090[b] and 0.160[c])	Step potential (V) (0.00050[a], 0.00525[b] and 0.01000[c])	Replication	
					1	2
1	−	−	−	−	0.90	0.52
2	+	−	−	−	2.98	1.21
3	−	+	−	−	9.92	10.54
4	+	+	−	−	5.69	6.44
5	−	−	+	−	0.00	0.00
6	+	−	+	−	22.98	20.46
7	−	+	+	−	71.09	58.72
8	+	+	+	−	67.78	73.13
9	−	−	−	+	0.72	0.30
10	+	−	−	+	1.93	1.32
11	−	+	−	+	5.92	8.12
12	+	+	−	+	4.58	4.17
13	−	−	+	+	0.00	0.00
14	+	−	+	+	12.30	12.34
15	−	+	+	+	33.22	27.82
16	+	+	+	+	54.97	48.23
17	0	0	0	0	45.77	48.29
18	0	0	0	0	50.26	51.55

[a] − level.
[b] 0 center point.
[c] + level.

Table 2
Central composite design for four variables and obtained response.

Runs	Variables				
	Buffer pH	Frequency (Hz)	Pulse height (V)	Step potential (V)	i_p (μA)
1	1.50	75.0	0.020	0.00050	0.22
2	1.50	75.0	0.020	0.01000	0.00
3	1.50	300.0	0.020	0.00050	10.90
4	1.50	300.0	0.020	0.01000	6.73
5	1.50	75.0	0.090	0.00050	0.00
6	1.50	75.0	0.090	0.01000	0.00
7	1.50	300.0	0.090	0.00050	37.07
8	1.50	300.0	0.090	0.01000	7.68
9	3.10	75.0	0.020	0.00050	0.59
10	3.10	75.0	0.020	0.01000	0.00
11	3.10	300.0	0.020	0.00050	6.45
12	3.10	300.0	0.020	0.01000	2.81
13	3.10	75.0	0.090	0.00050	10.12
14	3.10	75.0	0.090	0.01000	0.00
15	3.10	300.0	0.090	0.00050	30.98
16	3.10	300.0	0.090	0.01000	18.53
17	1.50	187.5	0.055	0.00525	6.56
18	3.10	187.5	0.055	0.00525	14.29
19	2.30	187.5	0.020	0.00525	7.66
20	2.30	187.5	0.090	0.00525	35.94
21	2.30	75.0	0.055	0.00525	1.37
22	2.30	300.0	0.055	0.00525	19.85
23	2.30	187.5	0.055	0.00050	22.20
24	2.30	187.5	0.055	0.01000	17.85
25 (C)	2.30	187.5	0.055	0.00525	22.11
26 (C)	2.30	187.5	0.055	0.00525	20.68
27 (C)	2.30	187.5	0.055	0.00525	15.24
28 (C)	2.30	187.5	0.055	0.00525	17.72
29 (C)	2.30	187.5	0.055	0.00525	12.93
30 (C)	2.30	187.5	0.055	0.00525	12.30
31 (C)	2.30	187.5	0.055	0.00525	11.30

C: Center point.

Fig. 1. Square wave voltammograms obtained for 2×10^{-5} mol L^{-1} ATZ in 0.05 mol L^{-1} phosphate buffer solution (pH 2.3) at 300 Hz SWV frequency, 0.09 V SWV pulse height, and -0.0024 V step potential. (a) Glassy carbon electrode; (b) Bismuth film electrode.

the BiFE is suitable for determining atrazine when compared to unmodified GCE.

3.2. Optimization of the atrazine determination

The choice of supporting electrolyte in voltammetry, requires its selection among many solutions, thus this factor cannot be easily included in an experimental plan together with other important factors. For this reason the selection of the optimum supporting electrolyte was made in a classical way starting from a set of candidate solutions of different kind, pH and ionic strength. Atrazine determinations was reported in sulfuric acid [34] and acetate buffer [35] as supporting electrolytes with a bismuth film electrode. Therefore, the atrazine's response was evaluated in a pH range between 1.5 and 4.5 using sulfuric acid, phosphate and acetate buffer 0.05 mol L^{-1}. Fig. 2 shows the voltammograms of 2×10^{-5} mol L^{-1} of atrazine using BiFE in: (A) acetate buffer pH 4.5, (B) phosphate buffer pH 2.3 and (C) sulfuric acid pH 1.5. As can be seen, atrazine signal in the first two solutions and particularly in phosphate buffer give the relative maximum peak height at a relatively lower potential, so it was chosen as supporting electrolyte.

The optimization of atrazine determination by SWV using experimental design include buffer pH and SWV instrumental parameters such as frequency; step potential and pulse height. In order to select the initial factor levels, previous experiments and studies information were used (not shown). The low and high levels in the screening for effectiveness of factors were buffer pH

3. Results and discussion

3.1. Comparison between GCE and BiFE

For comparison of the performance of glassy carbon and BiFE electrodes, a 0.05 mol L^{-1} phosphate buffer was adjusted to pH 2.3 and the instrumental was fixed at the following SWV parameters, frequency 300 Hz, pulse height 0.09 V and step potential -0.0024 V. Fig. 1 shows the SWV voltammograms of 2×10^{-5} mol L^{-1} atrazine solution for GCE (a) and BiFE (b). As can be seen, both electrodes exhibit remarkable differences on the herbicide response, clearly appreciated by the absence of a signal in the GCE, while for a BiFE electrode under the same conditions, a reduction atrazine's signal was obtained at -0.83 V. Therefore, these features suggest that
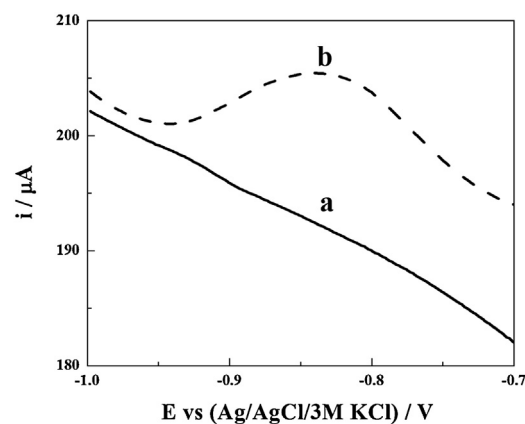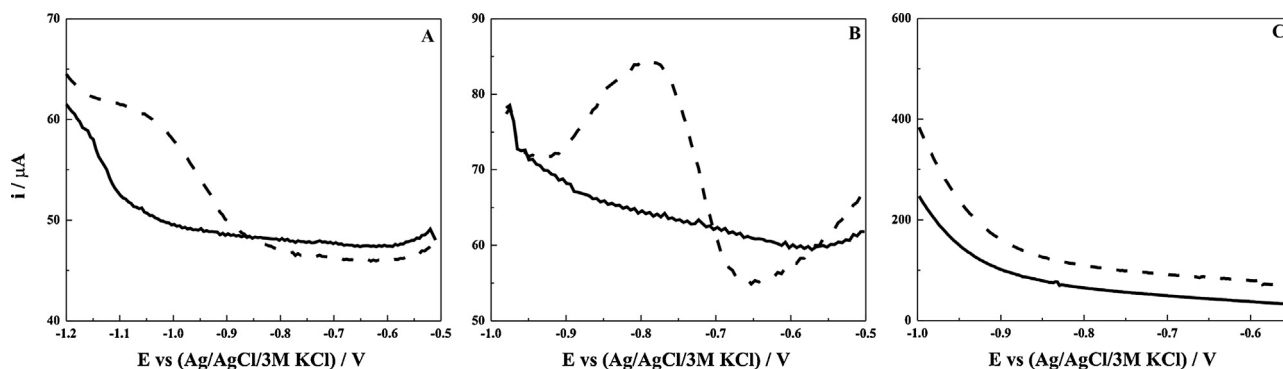

Fig. 2. Square wave voltammograms obtained in supporting electrolyte (solid line) and 2×10^{-5} mol L^{-1} ATZ (dashed line) in 0.05 mol L^{-1} of different supporting electrolyte solutions at 250 Hz SWV frequency, 0.02 V SWV pulse height and -0.005 V step potential. (A) Acetate buffer solution, pH = 4.5 (B) Phosphate buffer solution, pH = 2.3 and (C) sulfuric acid solution, pH = 1.5.
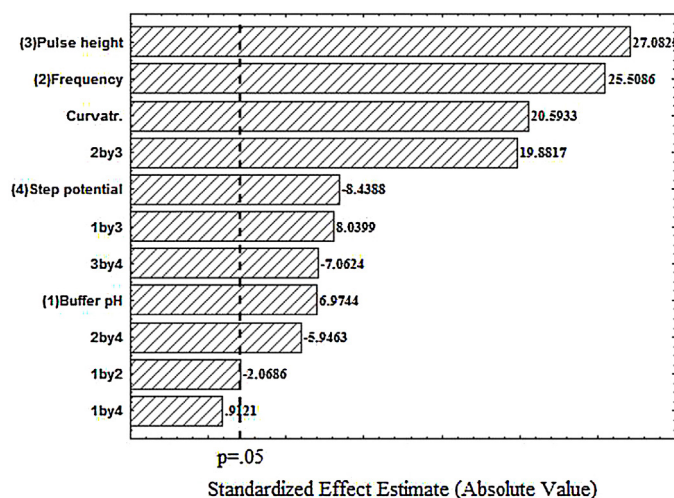
Fig. 3. Standardized main effect Pareto chart for the full factorial design of screening experiment. The vertical dashed line indicates the significance level at $p = 0.05$.

(1.5–3.1), frequency (75–300 Hz), pulse height (0.02–0.160 V), step potential (0.0005–0.01 V).

3.2.1. Full Factorial factors screening experiment

Analysis of variance (ANOVA) was performed to examine whether the studied experimental factors (Table 3) were significant in the performance of the proposed method. An effect was considered significant when it was above the standard error at the 95% confidence level ($p < 0.05$), indicated by the vertical line in the Pareto diagram (Fig. 3). In this chart, it can also be observed the relative importance of the main effects and their interactions (Fig. 3). A student's t-test was performed to determine whether the calculated effects were significantly different from zero, these values for each effect are shown in the Pareto chart by horizontal columns [45]. According to Fig. 3, the main factors (1) buffer pH, (2) frequency, (3) pulse height, (4) step potential and their interactions (2 by 3, 1 by 3, 3 by 4, 2 by 4 and 1 by 2) that extend beyond the reference line were significant at the level of 0.05. The initial pulse height represented the most significant effect on i_p. The pulse height, frequency, curvature and pulse height–frequency interaction had greater effects on atrazine response, except for the interaction effect between buffer pH and step potential (1 by 4). All other factors and their interactions had less effect but were statistically significant at 95% confidence. From the analysis of data (Table 3), in the studied experimental domain, it can be seen that all variables have significant impact ($p < 0.05$) on atrazine response. The lack of fit (LOF) is the variation of the data around the fitted model. LOF is a special investigative test for adequacy of a model fit, because the effects of the additional higher-order terms are removed from the error. Three central points are used for calculating the pure error. If the model does not fit the data well, this will be significant. In this design, the LOF value was also significant; indicating that a higher-order model or response surface study is needed in order to uncover the behavior of the significant factors and their interaction effects. According to the obtained results, all variables were evaluated in the CCD for further assessment.

3.2.2. Central composite design

According to the initial results (full factorial design) we selected all factors for response surface experimental design in order to complete the optimization process employing a central composite design (CCD) (Table 2). With respect to the screening design results, the low and high levels of parameters in the response surface design were changed (Table 2). The main effects, interaction

effects, and quadratic effects were optimized and evaluated in this design. The most important effects and interactions were calculated by the analysis of variance using STATISTICA 7.0 (Table 4). A p value less than 0.05 in the ANOVA table indicates the statistical significance of an effect at 95% confidence level. The LOF p value of 0.279693 implies that is not significant relative to the pure error. The model regression coefficient (Adj R^2) of 0.7736 is in reasonable agreement with the experimental results, indicating 77.36% of the variability can be revealed by the model. In the next step of the design, a response surface model was developed by considering all the significant interactions in the CCD. Data analysis gave a semi-empirical expression of peak current (i_p) with the following equation:

$$i_p = -71.1 - 11.6x_1{}^2 - 276.0x_2 + 0.3x_3 - 0.0006x_3{}^2 - 34x_4$$
$$+ 0.9x_2x_3 - 16295.6x_2x_4 \tag{2}$$

where x_1 is buffer pH, x_2 pulse height, x_3 frequency and x_4 step potential.

The overall interaction effects are displayed in Fig. 4; a 3D representation of the polynomial (Eq. (2)) obtained from the experimental data, depicting the surface plots of i_p versus the significant variables. Fig. 4 shows that the response increases with mean values of pH and with increasing frequency and amplitude values. While for the potential step these effect is obtained at decreasing values. Also, the quadratic term of the factors is significant on all surfaces response involving the frequency and pH, being for these factors statistically significant the quadratic terms (Table 4).

3.2.3. Optimization of CCD by desirability function

The profile for predicted values and desirability option in the STATISTICA 7.0 software is used for the optimization process (Fig. 5). Profiling the desirability of responses involves specifying the DF for each dependent variable (i_p), by assigning predicted values in a scale ranging from 0.0 (undesirable) to 1.0 (very desirable). The CCD design matrix results from Table 2 represented the maximum (37.067 μA) and minimum (0 μA) i_p of atrazine. According to these values, DF settings for each dependent variable of i_p are depicted at the right hand side of Fig. 5: desirability of 1.0 was assigned for maximum i_p (37.067 μA), 0.0 for minimum (0 μA) and 0.5 for middle (18.534 μA). On the left, hand side of Fig. 5 (bottom), the individual desirability scores are illustrated, respectively, for the i_p. Since desirability 1.0 was selected as the target value, the overall response (i_p) obtained from these plots with the current level of each variable in the model are depicted at the top (left) of Fig. 5. These figures allow seeing at a glance how changes in the level of each variable affect not only the response (i_p) but also the overall desirability of the responses. On the basis of these calculations and desirability score of 1.0, i_p of atrazine was optimized at 37.067 μA with calculating the optimized model variables of 2.30 buffer pH, 0.09 V pulse height, 300 Hz frequency and −0.0024 V step potential.

3.3. Method performance

3.3.1. Linearity and related figures of merit

In order to verify the method linearity within a concentration range of $(1.8–7.5) \times 10^{-6}$ mol L^{-1} of atrazine, three replicates were prepared at five concentration levels and subjected to the analytical procedure. A least-squares fitting was performed with the obtained data (i_p) and the results present a coefficient of determination (R^2) greater than 0.999. The limit of detection (LOD) was measured as the lowest amount of the analyte that may be detected to produce a response which is significantly different from that of a blank. The limit of detection and quantification were calculated on the basis of the residual standard deviation (δ) and
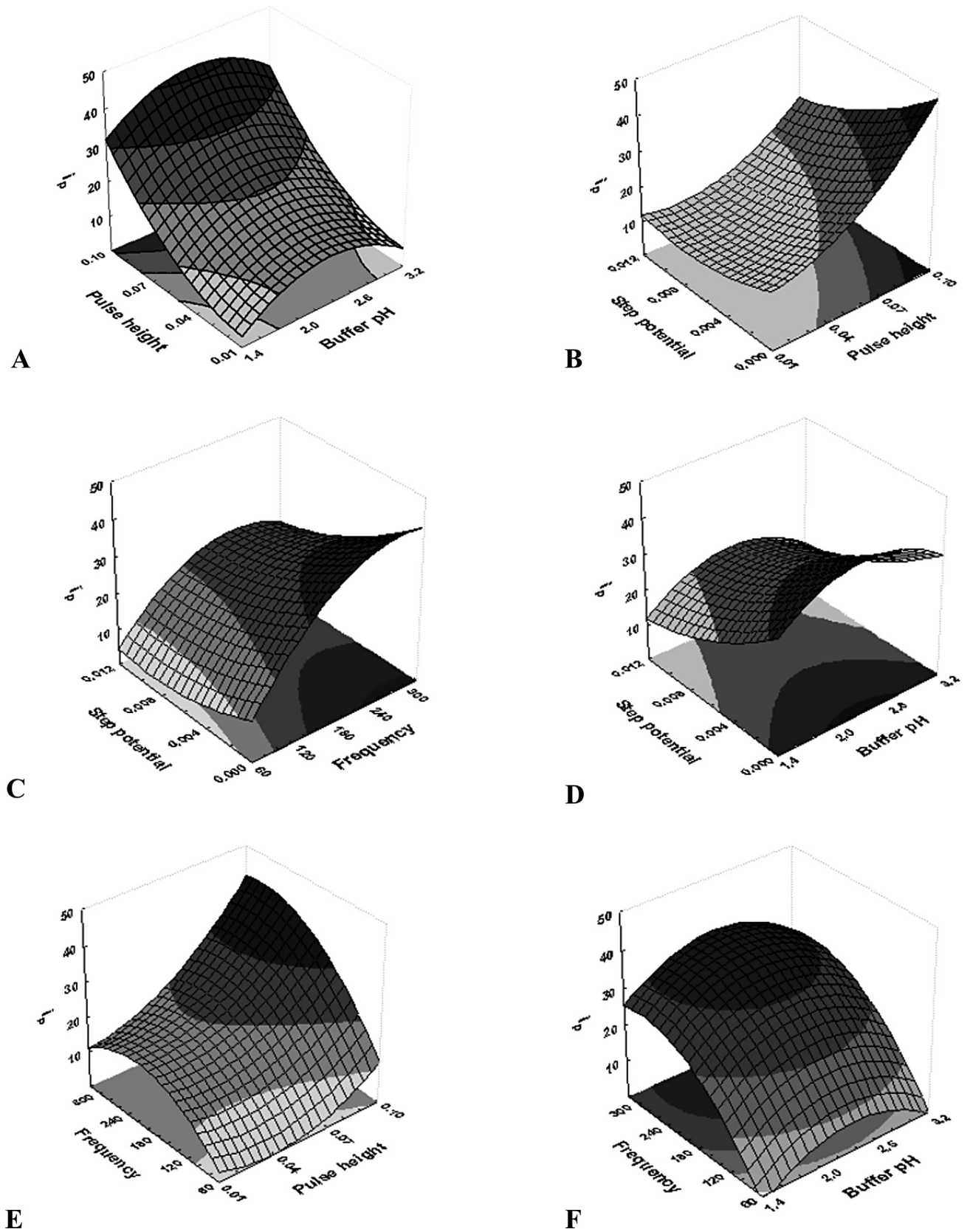
Fig. 4. Response surface for the central composite designs of data in Table 2: (A) pulse height–buffer pH, (B) step potential–pulse height, (C) step potential–frequency, (D) step potential–buffer pH, (E) frequency–pulse height, and (F) frequency–buffer pH.

Table 3
ANOVA results related to full factorial design.

	Sum of squares	d.f.	Means square	F ratio	p value
Curvature	3462.92	1	3462.917	424.0851	0.000000[a]
(1) Buffer pH	397.20	1	397.198	48.6428	0.000001[a]
(2) Frequency	5313.28	1	5313.278	650.6890	0.000000[a]
(3) Pulse height	5989.30	1	5989.300	733.4778	0.000000[a]
(4) Step potential	581.50	1	581.495	71.2127	0.000000[a]
1 by 2	34.94	1	34.941	4.2790	0.052471
1 by 3	527.83	1	527.831	64.6407	0.000000[a]
1 by 4	6.79	1	6.794	0.8320	0.373128
2 by 3	3227.72	1	3227.724	395.2823	0.000000[a]
2 by 4	288.72	1	288.724	35.3585	0.000010[a]
3 by 4	407.28	1	407.276	49.8769	0.000001[a]
Lack of Fit	355.69	5	71.139	8.7120	0.000198[a]
Pure Error	155.15	19	8.166		
Total SS	20,748.32	35			

[a] Significant at $p \leq 0.05$.

Table 4
ANOVA results related to central composite design.

	Sum of squares	d.f.	Means square	F ratio	p value
(1) pH (L)	11.875	1	11.8745	0.65968	0.447689
pH (Q)	142.670	1	142.6697	7.92596	0.030542[a]
(2) Amplitude (L)	611.853	1	611.8530	33.99128	0.001121[a]
Amplitude (Q)	40.652	1	40.6520	2.25841	0.183579
(3) Frequency (L)	920.000	1	919.9997	51.11026	0.000378[a]
Frequency (Q)	135.615	1	135.6152	7.53405	0.033521[a]
(4) Step (L)	234.247	1	234.2472	13.01352	0.011266[a]
Step (Q)	12.462	1	12.4615	0.69230	0.437240
2L by 3L	210.556	1	210.5560	11.69736	0.014141[a]
2L by 4L	117.432	1	117.4320	6.52389	0.043246[a]
3L by 4L	93.665	1	93.6647	5.20351	0.062703
Lack of fit	384.986	13	29.6143	1.64521	0.279693
Pure error	108.002	6	18.0003		
Total SS	3348.344	30			

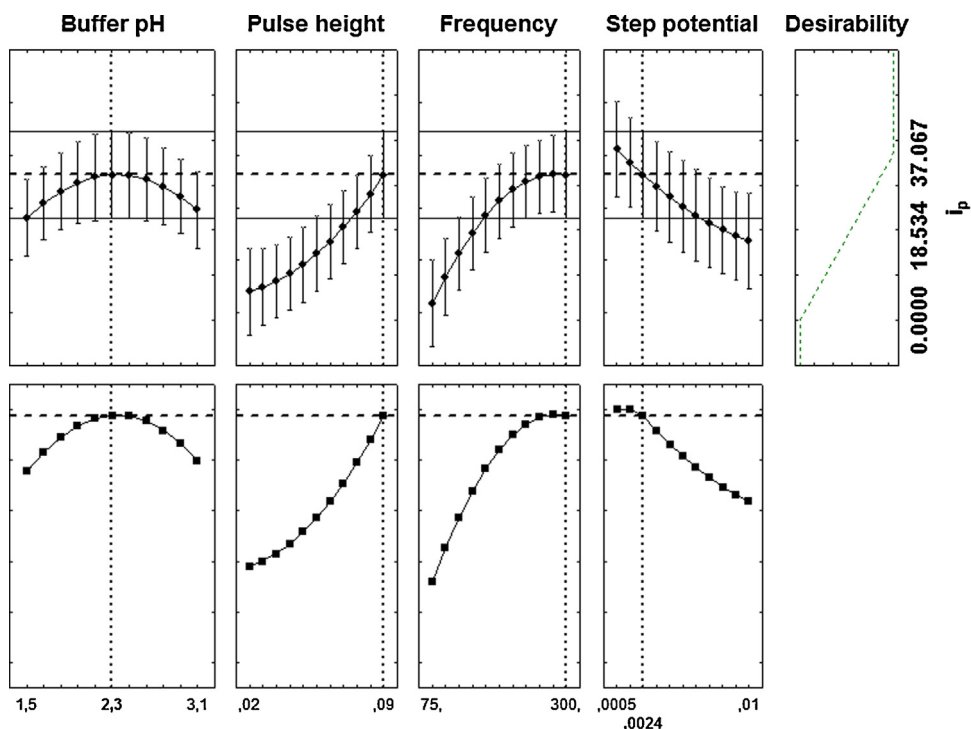[a] Significant at $p \leq 0.05$.

Fig. 5. Profiles of predicted values and desirability function for the i_p (μA). Dotted lines indicate optimization values.
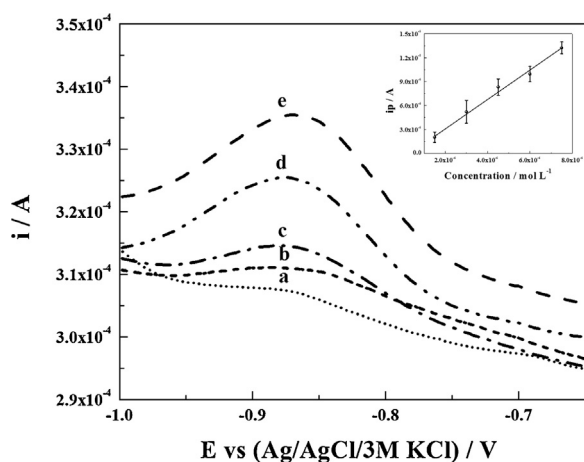
Fig. 6. Square wave voltammograms for different ATZ concentrations in 0.05 mol L^{-1} phosphate buffer solution (pH 2.3) at 300 Hz SWV frequency, 0.09 V SWV pulse height, and −0.0024 V step potential. (a) 1.5×10^{-6} mol L^{-1} (b) 3×10^{-6} mol L^{-1}, (c) 4.5×10^{-6} mol L^{-1}, (d) 6×10^{-6} mol L^{-1} and (e) 7.5×10^{-6} mol L^{-1} using a bismuth film electrode. Inset: Analytical curve.

the slope (S) of the calibration curves at the levels approaching the limits according to the equation LOD = $3.3(\delta/S)$ and LOQ = $10(\delta/S)$ [48], being the LOD 5.9×10^{-7} mol L^{-1} and LOQ 1.8×10^{-6} mol L^{-1}.

The average and standard deviation for the slope and the intercept of all the calibration curves were calculated and the obtained equation is

$$i_p(A) = (1.85 \pm 0.08)C_{AZ}(A/\text{mol L}^{-1}) + (6 \pm 4) \times 10^{-7}(A) \quad (3)$$

Fig. 6 shows the obtained voltammograms and the analytical curve for atrazine using the proposed BiFE.

3.3.2. Precision

The intra-assay precision (repeatability) was determined by the analysis of six replicate samples at three levels of concentration (3.00×10^{-6}, 4.50×10^{-6}, and 6.00×10^{-6} mol L^{-1}), under the same conditions, by the same analyst, and on the same day. The coefficient of variation (CV%) values obtained when computing the concentrations are shown in Table 5. As it can be seen, these values are near 5%, indicating an excellent precision for the present method. On the other hand, the intermediate precision was determined by the total analysis of six replicates samples at 4.50×10^{-6} mol L^{-1}, under the same conditions, by the same analyst, and on three different weeks (weeks 1, 2 and 3). An ANOVA test was applied to experimental data, and the results indicate not significant differences between the obtained averages in the 3 weeks ($p = 0.877$).

3.3.3. Accuracy

Accuracy was determined by analyzing a known sample, comparing the measured value with the true value at three levels of concentration. Averages obtained were compared through a Student's t-test that allowed us to conclude that no statistical

Table 5
Analysis of known concentration samples.

Theoretical concentration (mol L^{-1})	Experimental concentration (mol L^{-1})	CV[a] (%)	p value
3.00×10^{-6}	$3.0 (0.2) \times 10^{-6}$	5.62	0.258
4.50×10^{-6}	$4.5 (0.2) \times 10^{-6}$	5.36	0.917
6.00×10^{-6}	$6.0 (0.3) \times 10^{-6}$	5.41	0.941

Values between parenthesis correspond to the standard deviation.
[a] CV: coefficient of variation.

Table 6
Effect of several species for the determination of 4.00×10^{-6} mol L^{-1} atrazine reference solution.

Interferent	Concentration ($\times 10^{-6}$ mol L^{-1})	Interference (%)
Na^+	4.0	−0.1
Ca^{2+}	4.4	0.3
Fe^{3+}	4.0	−3.3
Al^{3+}	4.0	2.5
Mg^{2+}	4.1	−0.2
Ni^{2+}	1.2	0.2
Cu^{2+}	1.2	1.3
Pb^{2+}	1.2	3.8
Hg^{2+}	4.0	−2.8
Humic acid	18	−3.2
Prometryn	1.2	−4.7
Prometon	4.0	4.0

differences exist with 95% confidence between the results achieved by three concentrations (Table 5).

3.3.4. Interferents

The effect of possible interferents was investigated by the addition of such compounds in the 4.0×10^{-6} mol L^{-1} atrazine solution. The interferents were tested in different concentration ratios, adding each one separately. Table 6 shows the maximum concentration tolerated for each interferent. An excess of these concomitants had an influence of equal or less than 5.0% on the determination of ATZ.

3.3.5. Analysis of atrazine in real water samples

The water samples were pretreated as described in Section 2.3 and spiked with ATZ standards solution at concentration of 4.6×10^{-6} and 6.8×10^{-6} mol L^{-1}. Three replicate experiments with the whole analysis process were performed. Recuperation percent (R) for the spiked real samples ranged from to 95.3% and 102.7% and the relative standard deviations (RSD) were from 4 to 5%, respectively. The statistical calculations for the assay results showed good accuracy of the method according to the t test comparing the measured value with the true value (100%) (p values = 0.6257 and 0.8595 for 4.6×10^{-6} and 6.8×10^{-6} mol L^{-1} levels of ATZ, respectively). Thus, there are no significant differences between the results obtained by either procedure at a 95% confidence level, indicating that the BiFE can be used for ATZ determination in natural water samples.

4. Conclusions

Bismuth film electrode (BiFE) proved to be suitable for ATZ determination in water samples. The proposed method is inexpensive and simple, and also produces less toxic wastes than other methods presented in the literature. The analytical utility of multivariate chemometric techniques was used to obtain the optimum condition for atrazine's determination by square wave voltammetry. Chemometric procedure used first as a full factorial screening design to study the main variables that affect the ATZ current peak, and second the RSM in order to optimize the previous selected variables using the DF as maximize criterion of the response. Moreover, DF was used to identify optimum i_p by calculating specific variables optimization simultaneously. The proposed procedure also presents good sensitivity. Besides metallic cations, humic acid, and some pesticides with similar chemical structures do not interfere. Therefore, BiFE can be used for routine analysis and determination of ATZ in water samples.

Acknowledgements

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