

General Research

## Voltammetric quantification of 4-nitrophenol using a multivariate optimized plated bismuth film electrode.

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7 **Voltammetric quantification of 4-nitrophenol**  
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11 **using a multivariate optimized plated bismuth**  
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15 **film electrode.**  
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32 **KEYWORDS:** Bismuth film electrode; Box–Behnken design; Response Surface Methodology;  
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34 Nitrophenol  
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42 **ABSTRACT:** In this study the multivariate optimization of bismuth film deposition (BiFE) on  
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44 glassy carbon electrode, together with square wave voltametry (SWV) parameters for  
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46 nitrophenol quantification is described for the first time. For this purpose response surface  
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48 methodology (RSM) and desirability function (DF) were applied to a Box–Behnken design  
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50 (BBD). For the BiFE deposition procedure, the relationship between the reduction current peak  
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52 ( $i_p$ ) and standard deviation (SD) values with time, potential deposition and Bi (III) concentration  
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54 were analyzed, meanwhile for frequency, step and amplitude only the relationship with  $i_p$  was  
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3 considered. The pH influence in nitrophenol determination was carried on by univariate analysis.  
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5 After parameters optimization a LOD of  $3.4 \times 10^{-6}$  mol L<sup>-1</sup> was observed.  
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## 9 **1. Introduction**

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11 Application of Bismuth-film electrodes (BiFEs) was described around years 2000,  
12 showing several interesting properties such as simple preparation, high sensitivity, low toxicity,  
13 and well-defined electrochemical peaks <sup>1-4</sup>. In addition, due to the portability of the new  
14 potentiostats and accessories it is possible to carry out on site analysis with in situ plated BiFEs  
15 associated with an electroanalytical technique. Metals <sup>5-14</sup> and organics <sup>15,16,25,26,17-24</sup>  
16 quantifications have been proposed by using BiFEs. In these articles, different procedures for  
17 obtaining the bismuth film are described; where it is varied not only the composition of the  
18 solution but also the potential and time for film deposition.  
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31 Different industries, for instance, explosives, pharmaceuticals and pesticides use aromatic  
32 nitro compounds and some of them can be toxic or harmful to humans or the environment. In the  
33 particular cases of organic compounds determination, nitrophenol (NP) is one of the selected as  
34 test analyte, since it has a reducible group that allows a good evaluation of this type of films  
35 <sup>2,16,27-30</sup>. In the ex situ BiFE deposition different conditions <sup>2,12,35-38,16,27,28,30-34</sup> have been  
36 proposed, starting from the media pH which can be either acidic or acetate buffer with pH values  
37 between 4 and 5, furthermore, deposition potential is varied between -0.500 to -1.400 V,  
38 deposition time between 60 to 600 seconds and from  $2.5 \times 10^{-4}$  mol L<sup>-1</sup> (100 ppm) to  $2.0 \times 10^{-6}$   
39 mol L<sup>-1</sup> (900 ppb) Bi(III) concentration. Some authors claim that they have optimized the  
40 deposition method, however, as far as we know, the experimental parameters influence on the ex  
41 situ bismuth film electrodes obtained for nitrophenol current peak determination has been limited  
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3 to single factor analysis <sup>2,27-29</sup>, no papers about multifactor interaction effects of Bi(III)  
4 concentration, time and deposition potential has been reported by now.  
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9 Some methods using statistical designs of experiments, have been employed in the  
10 optimization of electrochemical sensors <sup>39-45</sup>, as they provide advantages allowing to set the  
11 optimum conditions using lower numbers of experiments. Using adequate experimental design a  
12 regression equation can be obtained were the importance of each independent variable in the  
13 global process can be obtained, furthermore the possibility of considering interaction effects  
14 between the variables can be detected, being an advantage over the classical method <sup>46</sup>. In the  
15 literature there are some reports describing multivariate optimization of the voltammetric  
16 response <sup>47-52</sup>. Furthermore, none of before mentioned articles describe NP determination.  
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28 Square wave voltammetry (SWV) is a rapid and sensitive electroanalytical technique <sup>53</sup>.  
29 In general SWV optimization has been performed by one-factor-at-a-time (OFAT) approach.  
30 <sup>50,51,54,55</sup>. However, we believe that all the analytical process must be optimized taking into  
31 account all the factors at once. We have already performed SWV parameters optimization for  
32 other compounds <sup>47,48,52</sup> and we will continue optimizing analytical techniques by using design  
33 experiments methods.  
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42 This research reports for the first time, the multifactor optimization affecting bismuth  
43 film growth on glassy carbon electrode and SWV parameters for nitrophenol quantification by  
44 using RSM a statistical and graphical technique. Particularly, 4-nitrophenol was used as  
45 reference compound, in general with electrochemical techniques related compounds show  
46 similar behaviors so the technique can be generalized to other nitrocompounds and the idea is to  
47 quantify all the nitrocompounds present in the samples. With regard to the optimization, for  
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3 BiFE deposition, two different solutions traditionally reported were analyzed together with Bi  
4 (III) concentration, time and deposition potential. While, SWV step, amplitude and frequency  
5 parameters were considered. Furthermore, figures of merit were established for 4-nitrophenol  
6 determination and the practicality and feasibility of the sensor was verified by NP analysis in  
7 water samples.  
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## 18 **2. Experimental**

### 19 **2.1. Apparatus, reagents and solutions**

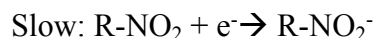
20 All the electrochemical experiments (Cyclic voltammetry (CV), SWV and potentiostatic  
21 measurements, were described elsewhere <sup>56,57</sup>.  
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28 All reagents are analytical grade and used without further purification. Solution were  
29 prepared with Milli-Q Millipore purified water. 4-Nitrophenol (NP) and  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  were  
30 obtained from Sigma–Aldrich (St. Louis, MS, USA). All the chemical compounds used as  
31 supporting electrolyte, sodium acetate, potassium hydroxide, acetic, phosphoric, boric and  
32 hydrochloric acid were from J.T. Baker. Solution of Britton Robinson buffer (BRBS) at different  
33 pH values were prepared by mixing acetic, phosphoric and boric acid to a final concentration of  
34 each component of  $0.04 \text{ mol L}^{-1}$ . The electrodeposition solution was prepared with  $1 \times 10^{-3} \text{ mol}$   
35  $\text{L}^{-1} \text{ Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  either in acetate buffer solution (ABS) pH 4.50 or  $0.10 \text{ mol L}^{-1} \text{ HCl}$ .  
36 Accuracy and applicability of the optimized method was analyzed using water samples, from Rio  
37 Tercero river, San Roque Lake, and drinking water, all samples from Córdoba, Argentina. Water  
38 samples were filtered using a Micropore membrane of  $0.45 \mu\text{m}$  and stored at  $4 \text{ }^\circ\text{C}$  in glass  
39 containers until they were studied. Each analysis was performed by adding a 2.5 mL aliquot to a  
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3 10.0 mL calibrated flask, after that a NP standard solution aliquot was added to a  $20 \times 10^{-6}$  mol  
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5  $L^{-1}$  concentration and completed with buffer solution.  
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## 10 **2.2. Experimental procedure**

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12 Nitrophenol (NP) determination can be electrochemically carried out by the reduction of  
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14 the nitro group that proceeds by the following two steps mechanism <sup>27-29</sup>:  
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24 here, NP determination was evaluated by CV and SWV, in both cases a negative current peak  
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26 was observed. Optimization procedures were carried on to BiFE preparation deposited by CV,  
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28 and for SWV parameters for nitrophenol determination, in both cases NP concentration was 10  
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30  $mgL^{-1}$ .  
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34 The electrode modification under the optimized conditions established in the present  
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36 article are the following: prior to modification, the GCE was polished with 0.05  $\mu m$  alumina  
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38 slurry and then ultrasonically washed with Milli-Q water. A pretreated GCE was immersed into  
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40 pH 4.50, 0.1 mol  $L^{-1}$  acetate buffer containing 2.7  $gL^{-1}$  Bi(III). The Bi film was potentiostatically  
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42 electrodeposited onto the GCE at  $-0.64$  V (vs Ag/AgCl) for 67 s to form BiFE/GCE. The  
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44 bismuth film is removed by applying for 60 s a  $+0.5$  V potential step in a BRBS solution, after  
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46 that a new film is deposited.  
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51 All the experimental condition (instrumental and chemical) were analyzed and the optimized  
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53 SWV procedure was: a 10 mL aliquot of BRBS 0.05 mol  $L^{-1}$ , a 5 s equilibration time at open  
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55 circuit potential, and finally a cathodic potential scan from  $-0.200$  to  $-1.000$  V versus Ag/AgCl  
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(3.0 mol L<sup>-1</sup> KCl) at a frequency of 45 Hz, an amplitude of 0.02 V and a step potential of 0.008 V. All measurements were performed three times (n = 3) for each concentration.

### 2.3. Data analysis

Design-Expert 7.0.0 software was used for experimental designs, statistical analyses, and regression models. Analysis of variance (ANOVA) was used to test the significance of each term in the equation and goodness of fit of the obtained regression model. R-squared (R<sup>2</sup>), adjusted R-squared (R<sup>2</sup><sub>adj</sub>) and predicted R-squared (R<sup>2</sup><sub>pre</sub>), were used to check the fitness of the developed models. The significant levels are given as probability values less than 0.05.

### 2.4. Experimental design

Response Surface Methodology (RSM) were used to find the optimized variables values for film deposition and instrumental SWV parameters. Optimization of the BiFE/GCE preparation and SWV instrument parameters were performed by following a three-level-three-factor full factorial Box–Behnken design (BBD) as already described elsewhere<sup>48,52,58</sup>. In the optimization study for BiFE preparation, deposition time (A), Bi (III) concentration (B) and deposition potential (C) were selected as independent variables, while the evaluated response was the cathodic current peak (i<sub>p</sub>) and its standard deviation (SD) (Table S1). By the other side, for SWV instrumental variables optimization, Step (A), Amplitude (B) and Frequency (C) were selected as independent variables and current peak (i<sub>p</sub>) as response (Table S2). Data from BBD was subjected to a multiple regression methodology to obtain the parameter estimation of the mathematical model by the quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} x_i x_j + \varepsilon$$

where  $Y$  is the predicted response,  $\beta_0$  is an offset term,  $\beta_i$  is the coefficient of linear effect,  $\beta_{ii}$  is the coefficient of squared effect,  $\beta_{ij}$  is the coefficient of interaction effect, and  $\varepsilon$  is the random error. This equation may be a linear one, but it may be unsuitable to explain fully the wide range included in the factor space or to describe completely the influence of all the variables and their interactions.

On the other hand, the Derringer function (DF) as described elsewhere<sup>48,52,58</sup> was used to find optimal compromises between the total number of responses taken into account. As already mentioned, this function allows to determine the levels of the predictor variables that produces the most desirable response on the dependent variables. It considers desirability 1 for maximum and 0 for minimum by the desirability profile inspection.

### 3. Results and discussion

#### 3.1. Comparison between GCE and BiFE

Fig. 1 shows three successively negative scans for 10 mg L<sup>-1</sup> nitrophenol reduction on glassy carbon (A) and BiFE (B) electrodes obtained in 0.05 mol L<sup>-1</sup> ABS pH 4.5 at 0.100 Vs<sup>-1</sup>, the solution was stirred between each scan. As can be observed, both electrodes exhibit a cathodic current peak; however with a different current intensity for the same conditions. Mainly, current peak values for BiFE electrodes are around 23  $\mu$ A and almost constant for the three successive scans (Fig. 1 B), while on GCE the first scan presents a current value similar to that obtained with BiFE electrode, however on the successive scans a remarkable signal decay is obtained (Fig. 1



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3 A). All this suggest that BiFE electrodes are more suitable for NP quantification in successively  
4 determination as compared to GCE and will be used from now on.  
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### 10 **3. 2. Experimental design for BiFE preparation optimization**

11  
12 In order to get the best analytical response for NP determination and reproducibility,  
13 BiFE deposition was optimized using the cathodic current peak and standard deviation (SD) as  
14 dependant variables; the measurements were performed in the two traditional solutions reported  
15 in the literature (ABS and HCl solutions). The purpose is to obtain a film that can be used several  
16 times. With the proposed design independent variables significance and interactions, on the  
17 negative current peak and SD for the successive scans can be explained; the design considers  
18 current peak maximization and standard deviation minimization.  
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29 In Table S1 the dependence of NP cathodic current peak values ( $i_p$ ) after blank correction  
30 with the corresponding SD, for each BBD run, both in 0.05 mol L<sup>-1</sup> ABS pH 4.5 and 1.0 mol L<sup>-1</sup>  
31 HCl are presented. The analytical results were adjusted by using the least squared methodology  
32 to a polynomial function of second order. The significance for the adjustment of the multiple  
33 regression was carried on by the ANOVA test and the lack of fit (LOF) with a 0.05 significance  
34 level. The ANOVA test demonstrated that results obtained in HCl are not significant for all the  
35 independent variables analyzed, probably due to the irreproducibility of the current values  
36 obtained. So the analysis will be performed only with data obtained in acetate buffer.  
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48 The significant terms for the cathodic current peak ( $i_p$ ) and SD optimization, different R-  
49 Squared values ( $R^2$ ,  $R^2_{adj}$  and  $R^2_{pred}$ ), LOF p values and fitted model equations for semi-empirical  
50 expressions are presented in Table S3. It can be observed that leave out, the significant terms for  
51 BiFE deposition optimizations were all the three variables studied, deposition time (A), Bi(III)  
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3 concentration (B) and deposition potential (C), but also the quadratic terms for A and B ( $A^2$  and  
4  $B^2$ ). On the other side, interactions between variables or the quadratic term for C were not  
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6 significant. The LOF p-value of 0.987 indicates that the analysis is not significant with respect to  
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8 the pure error. Furthermore, the present model explains 98.6 % of the total variability ( $R^2_{adj}$   
9 0.986) and 98.4 % of new data variability ( $R^2_{pred}$  0.984). As the difference between both values is  
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11 less than 0.2 that indicates a good model agreement.  
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18 In Fig. 2, 3D response surface plots are presented, which was adequate to determine the  
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20 dependence of dependent variables on independent ones. Being the cathodic current peak the  
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22 dependent variable these surface plots shows the dependence between Bi (III) concentration and  
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24 deposition potential (Fig. 2A), Bi(III) concentration and deposition time (Fig. 2B) and potential  
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26 and time deposition (Fig. 2C). In Fig. 2A there is a maximum (in absolute value) for intermediate  
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28 Bi (III) concentration values while the current peak value increases with decreasing deposition  
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30 potential at a constant 60 s deposition time. Fig. 2B shows a maximum in the response for  
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32 intermediate Bi (III) concentration values and deposition time at a constant  $-0.76$  V deposition  
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34 potential. Finally, Fig. 2C shows the behavior between potential and time deposition at a  
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36 constant  $2.75$  g  $L^{-1}$  Bi (III) concentration. The surface plot describes a similar shape as Fig. 2A  
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38 with a maximum response at intermediate deposition time values. Response surface analysis  
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40 show the significance of the quadratic terms corresponding to Bi(III) concentration and  
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42 deposition time.  
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49 On the other hand, when analyzing the ANOVA results for SD (Table S3) only A and C  
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51 are significant while the LOF has a p-value of 0.9836. Data of SD were transformed to base 10  
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53 log and the R-square values were 0.697 ( $R^2$ ), 0.646 ( $R^2_{adj}$ ) and 0.605 ( $R^2_{pred}$ ), as already  
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55 mentioned the difference between the last two values is less than 0.2. Although in the case of SD  
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3 the model explains 60.5 % of the total variability and 64.6 % of the new data variability, the  
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5 results are considered satisfactory.  
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9 Fig. 3 shows 3D response surface plot for SD vs potential and time deposition at a  
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11 constant  $2.75 \text{ mg L}^{-1}$  Bi (III) concentration. As it can be observed, there is a SD decreases as  
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13 both time and deposition potential increase.  
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17 Considering that only, deposition and time potential are significant for SD model and  
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19 comparing Fig 2C and Fig 3, where it can be seen that optimum conditions for  $i_p$  maximization  
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21 and SD minimization are opposed, other tools must be used. To achieve this purpose the  
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23 desirability function (DF) was obtained by maximizing current peak values and minimizing SD  
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25 for the all three independent variables. To obtain the best operational conditions, cathodic  
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27 current peak values (as absolute value) maximization with a +++++ (5 of 5) significance and SD  
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29 minimization with a +++ (3 of 5) significance for the three responses each with a new BiFE were  
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31 obtained.  
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36 Fig. 4 presents the desirability function for the three analyzed variables, deposition time (Fig.  
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38 4A), Bi (III) concentration (Fig 4B) and deposition potential (Fig. 4C). As it can be observed, in  
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40 Figs 4 A and B, maximum DF values are obtained for intermediate deposition time and Bi (III)  
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42 concentration values. While, in the case of deposition potential (Fig. 4C) a slight variation of DF  
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44 is observed. From the DF analysis, the highest desirability score obtained was 0.851 for 67 s  
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46 deposition time,  $2.7 \text{ mg L}^{-1}$  Bi (III) concentration and 0.600 V deposition potential. This set of  
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48 conditions was determined to be the optimum and to confirm the optimized condition validity.  
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50 To compared experimental results with model predicted values different experiments were  
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52 performed and a  $-19.97 \mu\text{A}$  NP reduction current peak was obtained with a prediction interval of  
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3 95 % between  $-20.43 \mu\text{A}$  and  $-19.52 \mu\text{A}$ . Furthermore, using three different BiFEs for  $10 \text{ mg L}^{-1}$   
4 NP a current value of  $-19.7 \pm 0.4 \mu\text{A}$  was obtained. The results of the comparison between the  
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6 obtained mean values with the predicted one indicates that the developed quadratic model is  
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8 adequate.  
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### 11 12 13 14 **3. 3. Optimization of the pH value determination**

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16 To obtain the best pH value for NP quantification, BRBS at different pH values were used.  
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18 Fig. 5 presents the negative scans for  $10 \text{ mg L}^{-1}$  NP at  $0.100 \text{ Vs}^{-1}$  in BRBS at the following pH 3,  
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20 5, 7 and 9. As already reported, better signals are obtained at acid pH in agreement with the  
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22 proposed mechanism<sup>27-29</sup>. Particularly, in this case as demonstrated by Fig. 5, a well defined  
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24 current peak is obtained for pH values lower than 7 and the maximum reduction current peak is  
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26 defined at pH 5. For alkaline solutions (pH 9), the signal is much lower and even two different  
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28 peaks can be detected, indicating that different process are taking place. For that reason, pH 5  
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30 was chosen as the working value.  
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### 38 **3. 4. Optimization of instrumental variables**

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40 Nitrophenol quantification was performed by an electrochemical method, consisting on  
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42 an equilibration time followed by SWV using a BiFE modified glassy carbon electrode. In the  
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44 present study, the optimization of SWV variables such as amplitude, frequency and step potential  
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46 using three independent factors and the current reduction peak as response variable was carried  
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48 on. The Box–Behnken design is presented in Table S2 along with experimental data, all current  
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50 peak values ( $i_p$ ) were blank corrected, and the experiments were performed in  $0.05 \text{ mol L}^{-1}$   
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52 BRBS pH = 5.0. It is worth noting that reduction current peak values for SWV are somehow  
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3 smaller than those for CV, as the studied electrochemical process is irreversible. However, SWV  
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5 is a faster technique.  
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9 The variables analyzed were step potential (A), amplitude (B) and frequency (C), and as  
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11 previously reported an ANOVA test was performed. The significant terms for the cathodic  
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13 current peak ( $i_p$ ) and SD optimization, different R-Squared values ( $R^2$ ,  $R^2_{adj}$  and  $R^2_{pred}$ ), LOF p  
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15 values and fitted model equations for semi-empirical expressions are presented in Table S3,  
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17 together with the already discussed analysis for BiFE formation. As it can be observed from  
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19 Table S3, all the three variables studied were significant, and also the quadratic term for step  
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21 potential ( $A^2$ ). On the other hand interactions between variables or the quadratic terms for B and  
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23 C were not significant. The LOF p-value of 0.964 indicates that the analysis is not significant  
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25 with respect to the pure error. Furthermore, the  $R^2_{adj}$  value (0.981) and  $R^2_{pred}$  (0.978) demonstrate  
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27 that 98.1 % of the model variability and 97.8 % of new data variability can be explained by the  
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29 model. Again there is a correspondence between both values as they present difference less than  
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31 0.2 indicating a reasonable agreement.  
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37 Fig. 6 presents 3D plots of the polynomial equation (Table S3) for the dependence  $i_p$  as a  
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39 function of the significant variables. Fig. 6A shows the variation of  $i_p$  as a function of the  
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41 amplitude and step potential at 50 Hz frequency, as it can be observed there is a current increase  
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43 as the amplitude increases, while for the step potential there is an increase up to intermediate  
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45 values and then remains practically constant. In Fig. 6B the dependant variable is represented vs  
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47 SWV frequency and amplitude at a 0.008 V constant step, in this case there is a clear current  
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49 increase as the amplitude increases while there is slight current increase as the frequency  
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51 decreases. Finally, when analyzing the effects of frequency and step potential on the reduction  
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53 current peak with constant 0.02 V amplitude (Fig. 6C), the same behavior previously described  
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3 for both variables is observed. The quadratic term for the step potential ( $A^2$ ) although significant,  
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5 does not have a notorious influence on the results (Figs. 6A and C).  
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9 As already mentioned before the dependent variable value ( $i_p$ ) was maximized to 1.0 with a  
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11 +++++ (5 of 5) significance. On the basis of these calculations, the optimized  $i_p$  reduction was  
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13  $8.9 \pm 0.5 \mu A$  with a 95 % of confidence, obtained using the proposed model and the following  
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15 instrumental values 45.0 Hz frequency, 0.008 V step potential and 0.02 V amplitude. In order to  
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17 check the validity of the model different experiments were performed by triplicate and results  
18  
19 compared to predicted values. Under the optimal condition the cathodic current peak of  $10 \text{ mg L}^{-1}$   
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21 NP was  $8.4 \pm 0.3 \mu A$ . The results of the comparison between the obtained mean values with the  
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23 predicted one indicate that the developed quadratic equation is adequate.  
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### 29 30 **3. 5. Method performance**

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32 The method linearity was tested in the  $(1.0\text{--}100) \times 10^{-6} \text{ mol L}^{-1}$  NP concentration range by  
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34 measuring triplicate samples, prepared at five concentration levels. Data of  $i_p$  vs. concentration  
35  
36 were fitted by least-squares obtaining a  $R^2$  value greater than 0.999. The detection limit (LOD)  
37  
38 and quantification limit (LOQ) were calculated as described elsewhere <sup>59</sup>, the obtained values  
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40 were  $3.4 \times 10^{-6} \text{ mol L}^{-1}$  and  $1.0 \times 10^{-5} \text{ mol L}^{-1}$  respectively, and the resulting equation is:  
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$$44 \quad i_p(\mu A) = (1.34 \pm 0.03) + (5.99 \pm 0.08) \times 10^4 C_{NP}(\mu A \text{ mol}^{-1} L^{-1})$$

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48 Repeatability (intra-assay precision studies) was analyzed, by the same analyst, under the  
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50 same conditions, and on the same day using three different levels of concentration ( $5.00 \times 10^{-5}$ ,  
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52  $7.00 \times 10^{-5}$ , and  $9.00 \times 10^{-5} \text{ mol L}^{-1}$ ) with six replicate samples. Table 1 shows the coefficient of  
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54 variation (CV %) values for different NP concentrations and results at intermediate precision for  
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3 current peaks values under different conditions. In general it can be observed that, CV values are  
4 close to 5% being an indication of an excellent precision. Intermediate precision was also  
5 analyzed by considering three replicates samples at  $5.00 \times 10^{-5} \text{ mol L}^{-1}$  by the same analyst,  
6 under the same conditions, on three different days (day 1, 2 and 3) for three different BiFE (I, II  
7 and III). The ANOVA test presented in Table 1 shows that there are no significant differences at  
8 95 % confidence, between the calculated averages ( $p = 0.398$ ).  
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18 The accuracy was obtained by analyzing samples with known concentrations at three  
19 levels and comparing the experimental concentration with the true value. In Table 1 real and  
20 averages concentration values and Student's t-test  $p$  values are presented, and at 95 % confidence  
21 level no statistical differences were observed.  
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28 The analysis method presented in this paper was used to NP detection in water samples to  
29 validate the application potential in practical analysis. Water samples were prepared as described  
30 in Experimental section, and determinations were carried on by the standard addition method. In  
31 Table 2, NP concentration added and found, SD and recovery % are presented, the obtained  
32 results are satisfactory with a 103 % average recovery, indicating that the developed method is  
33 adequate. It is worthy to note that recovery analysis in different samples and matrixes (Table 1  
34 and Table 2) with results rounding 100% are an indication that no interferences are present in the  
35 sample.  
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#### 49 **4. Conclusion**

50 This article described the development of an alternative electroanalytical method, using  
51 bismuth film electrodes, for nitrophenol quantification. The proposed methodology included a  
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3 previous bismuth film preparation and then SWV determination. The experimental variables  
4 were studied and optimized by BBD together with RSM and DF. The proposed model allows the  
5 analysis of the different effects of independent variables, such as Bi(III) concentration and  
6 electrolyte pH solution, and time and deposition potential for BiFE preparation and SWV  
7 instrumental variables for a better understanding on NP detection. The quadratic response  
8 surface models is in very well agreement with data at different conditions. From the statistical  
9 analysis optimal conditions for the analytical method for NP determination was developed. To  
10 the best of our knowledge, the use of experimental design for *ex situ* BiFE preparation together  
11 with NP quantification by SWV is reported for the first time, good linearity range together with  
12 acceptable LOD and LOQ were obtained making the method a good candidate for a reference  
13 one. Finally, the application of the method to NP detection in natural water samples has been  
14 successful.

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##### 48 49 **Author Contributions**

50  
51 The manuscript was written through contributions of all authors. All authors have given approval  
52 to the final version of the manuscript.  
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**Supporting Information**

This file presents Tables containing coded and actual levels of variables considered for Matrix of variables for bismuth film deposition (S1) and variables for SWV (S2). Also Box-Behnken design for bismuth deposition variables (S1), instrumental SWV variables (S2) and the respective responses. Finally, a Table with significant terms and statistics summary of response surface models for bismuth film deposition and SWV optimizations is included (S3).

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**FIGURES**

**Figure 1.** Successive linear scan for 10 mg L<sup>-1</sup> NP in acetate buffer pH 4.5 (black) of 1<sup>st</sup> scan (red); 2<sup>nd</sup> scan (green) and 3<sup>rd</sup> scan (blue) at (A) GCE and (B) BiFE electrodes,  $\nu = 0.100 \text{ V s}^{-1}$ .

**Figure 2.** Response surface of the current peak for the Box Behnken designs of data in Table S1: (A) Deposition potential (V) vs Bi (III) concentration (mg L<sup>-1</sup>), (B) Bi (III) concentration (mg L<sup>-1</sup>) vs Deposition time (s), (C) Deposition potential (V) vs Deposition time (V).

**Figure 3.** Response surface of the Standard Deviation for the Box Behnken designs of data in Table S1: Deposition potential (V) vs Deposition time (V).

**Figure 4.** Desirability function profiles for (A) Deposition Time, (B) Bi (III) concentration and (C) Deposition potential.

**Figure 5.** Linear scan obtained in supporting electrolyte (dashed line) and 10 mg L<sup>-1</sup> NP (solid line) in 0.05 mol L<sup>-1</sup> of different pH 3.0 (black); 5.0 (red); 7.0 (blue) and 9.0 (dark cyan) at a BiFE electrode,  $\nu = 0.100 \text{ V s}^{-1}$ .

**Figure 6.** Response surface of the current peak for the Box Behnken of data in Table S2: (A) Amplitude (V) vs Step potential (V), (B) Frequency (Hz) vs Amplitude (V) and (C) Frequency (Hz) vs Step potential (V).

## TABLES

**Table 1.** Analysis of known concentration samples

Concentration					
Real (x 10 <sup>-5</sup> mol L <sup>-1</sup> )	Obtained (x 10 <sup>-5</sup> mol L <sup>-1</sup> ) ± SD <sup>a</sup>		CV <sup>b</sup> (%)	<i>p</i> -value	
5,00	4,9 ± 0,2		3,50	0,2892	
7,00	7,0 ± 0,1		1,74	0,5301	
9,00	8,7 ± 0,4		3,72	0,6443	
Current peak (μA)					
	Day 1	Day 2	Day 3	<i>p</i> -value	
BiFE I	4,129	4,340	4,152	0,398	
BiFE II	4,286	4,473	4,377		
BiFE III	4,364	4,295	4,226		

<sup>a</sup>Mean values and standard deviation of six determinations. <sup>b</sup>CV: coefficient of variation.

**Table 2.** Results for the detection of NP in several water samples.

Sample	Added ( $\times 10^{-6}$ mol L <sup>-1</sup> )	Found ( $\times 10^{-6}$ mol L <sup>-1</sup> ) <sup>a</sup>	SD	Recovery (%)
San Roque lake	20	20	4	102
Rio Tercero river	20	19.5	0.7	98
Córdoba city drinking water	20	21	1	106

<sup>a</sup>SWV measurements were repeated five times (n = 3).

Figure 1

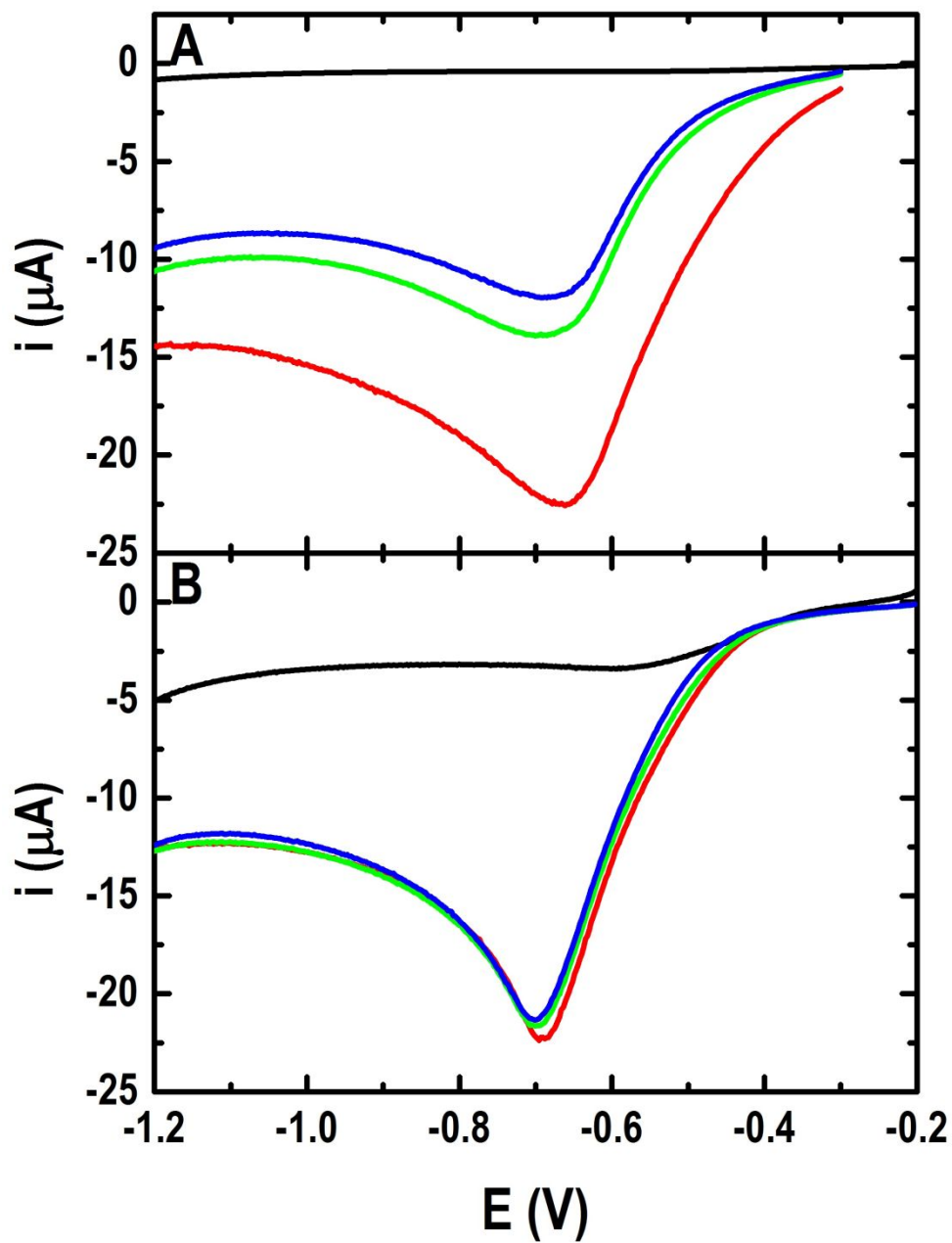


Figure 2

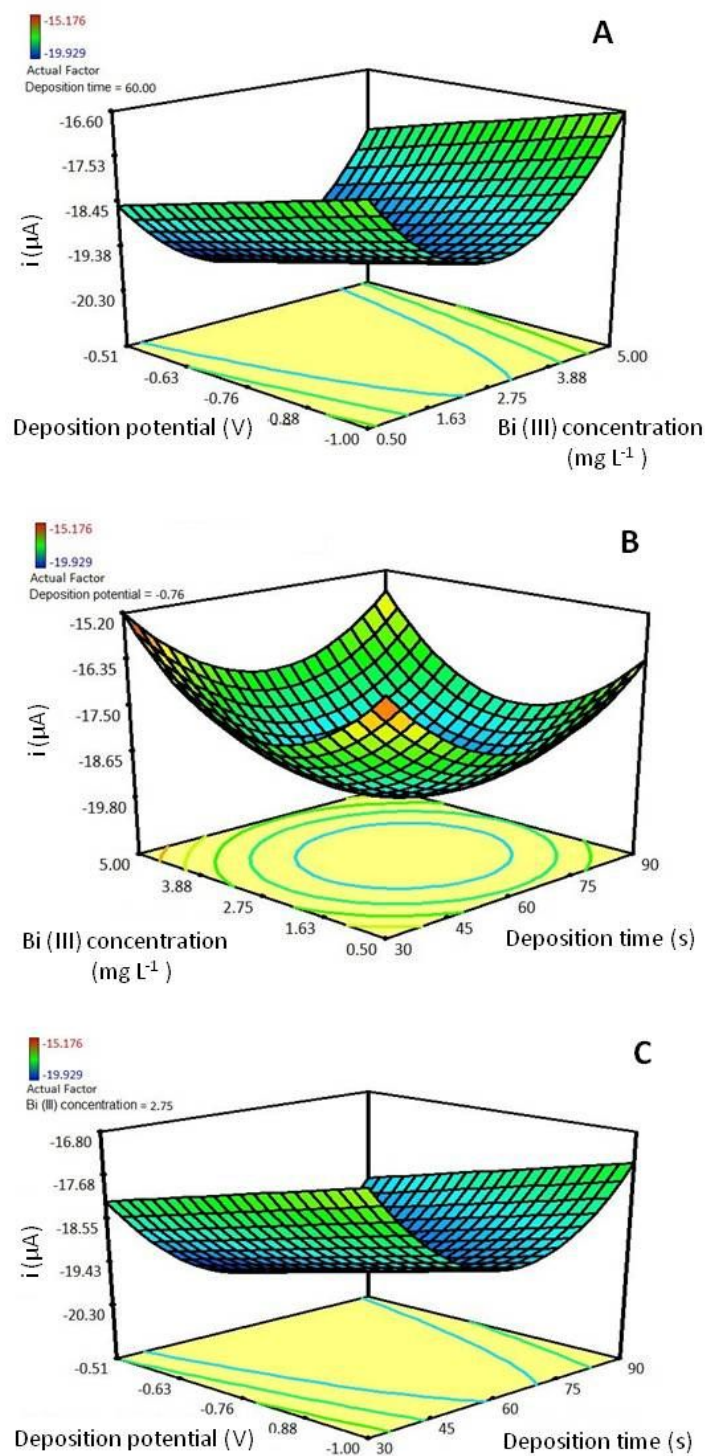


Figure 3

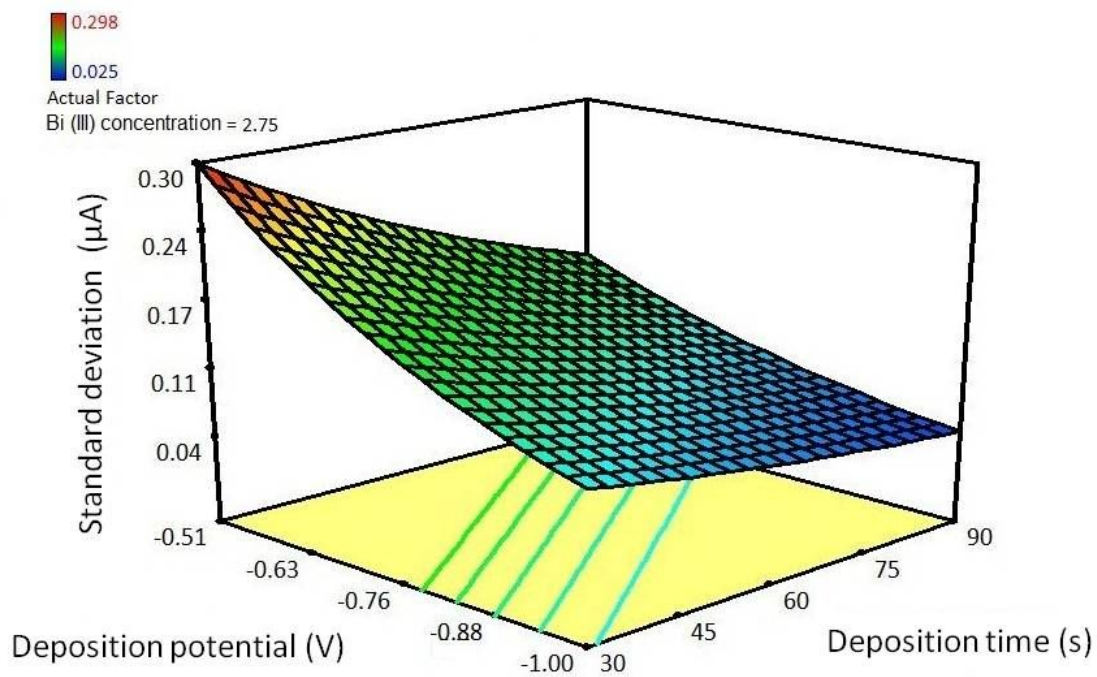


Figure 4

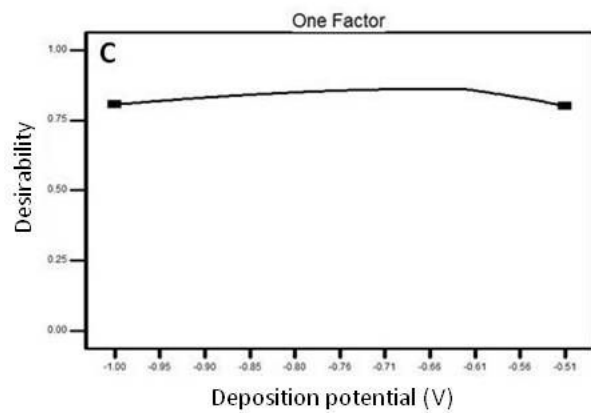
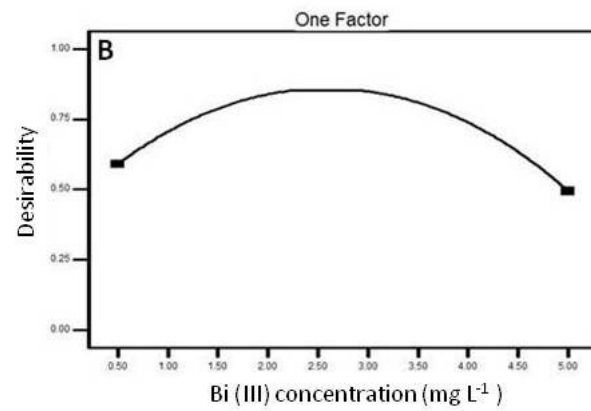
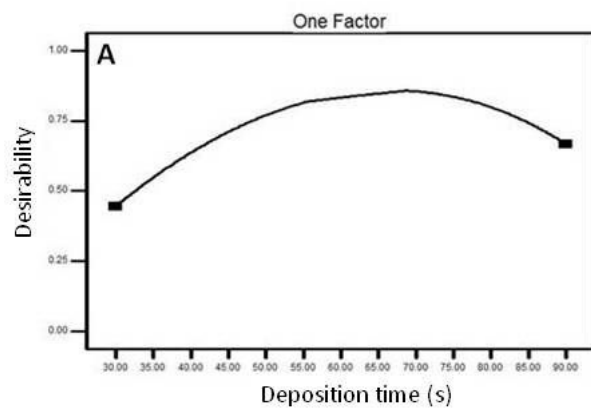




Figure 5

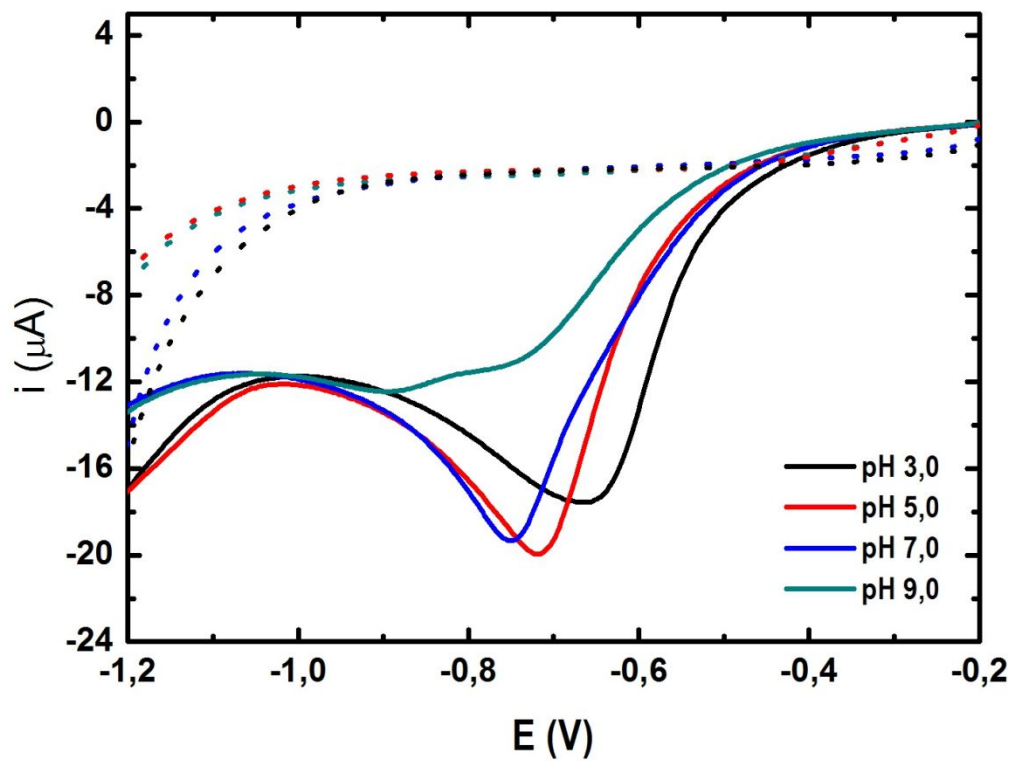
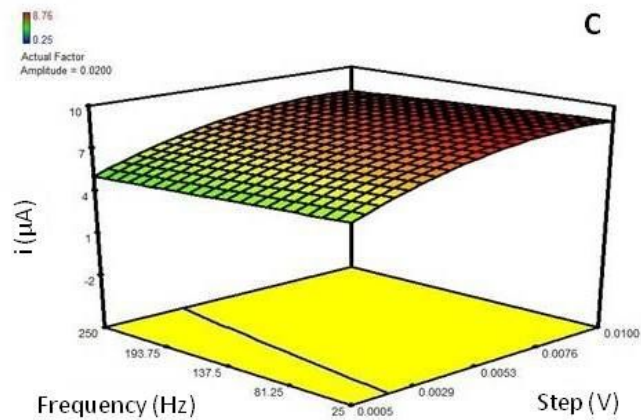
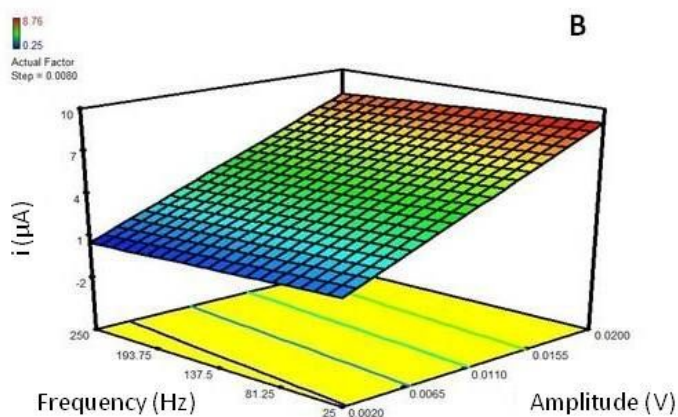
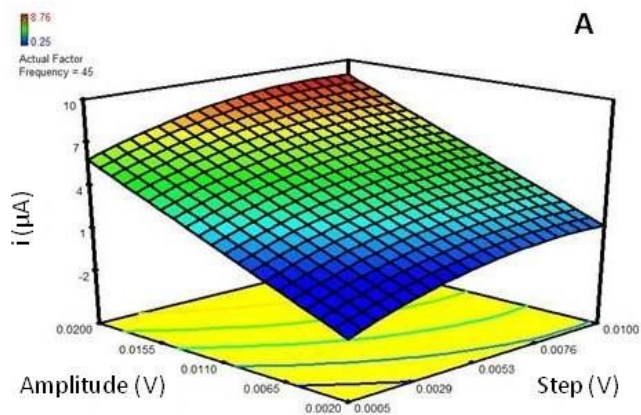


Figure 6



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