



Application of multi-factorial experimental design to successfully model and optimize inorganic chromium speciation by square wave voltammetry



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ARTICLE INFO

Article history:

Received 18 May 2015

Received in revised form 28 July 2015

Accepted 30 July 2015

Available online 8 August 2015

Keywords:

Chromium speciation

Square wave voltammetry

Experimental design

Desirability profile

ABSTRACT

Response surface methodology (RSM) was used to optimize voltammetric signal of hanging mercury drop electrode (HMDE) for inorganic chromium determination and speciation. Initial solution pH, DTPA (Diethylenetriaminepentaacetic acid) concentration, SWV frequency, pulse height, step potential, accumulation time and accumulation potential were considered as important operating factors through experimental design methodology for Cr(VI) quantification. The central composite and a Box–Behnken designs as response surface design coupled with numerical optimization technique was applied for obtaining the optimum chemical and SWV instrumental conditions as well as the maximum height of chromium reduction peak. Optimum conditions were set as follow: pH 6.5, DTPA concentration 5.5 mmol L⁻¹, SWV parameters: frequency 500 Hz, step potential 0.005 V, pulse height 0.1 V, accumulation time 120 s and accumulation potential -1.10 V. Speciation is performed in two separate samples, in the first one Cr(VI) is measured after 40 min and DTPA addition. While in the second one, Ce(IV) is added and all Cr(III) is oxidized to Cr(VI) allowing total chromium determination, finally Cr(III) is obtained by difference. Under the optimal experimental conditions, the current reduction peak was proportional to chromium(VI) concentration in the 5–105 nmol L⁻¹ range, with detection and quantification limits of 3.77 and 8.47 nmol L⁻¹, respectively. Using the proposed method, inorganic chromium speciation was successfully determined in water samples with standard addition method, suggesting that this method can be applied to the quantification of inorganic chromium speciation in water samples.

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1. Introduction

Chromium (Cr) presence in the environment mainly owing to industrial production became an important threat for plant productivity and food safety [1,2]. It exists in two stable forms, as trivalent (Cr(III)) and hexavalent (Cr(VI)) species, but various intermediate states have also been detected [3–5]. Generally reduction of Cr(VI) to Cr(III) is favored in nature and in plants by naturally present antioxidants [6,7] but its re-oxidation may take place by the action of microorganisms [8]. In this view, Cr redox changes are dynamic process which do not allow for distinguishing between toxicity of individual chemical forms [1].

The measurement of the total Cr content in environmental samples does not provide sufficient information about possible health hazards as toxicity and bioavailability of the chromium species depend on their oxidation states and complexes composition [9]. A detailed knowledge of the concentration of chromium at different oxidation states in environment is important for the following reasons: (i) the geochemical behavior and consequently the transport pathways are different for Cr(III) and Cr(VI) [10]; (ii) impact of chromium on living organisms depends strongly on its oxidation state; Cr(VI) is reported as toxic for

mammals due to its solubility and reactivity, however, high concentrations of Cr(III) can also adversely affect water organisms [11]; (iii) the limits of the Cr(III) and Cr(VI) contents are separated in all the legislations, such as World Health Organization (WHO) that establish for Cr(VI) in groundwater a limit of 50 ppb [12].

Many analytical procedures using different techniques have been reported in the literature to quantify chromium species, atomic absorption spectroscopy (AAS) and inductive coupled plasma mass spectrometry (ICP-MS) being the most frequently employed [13]. In general, previous treatment of samples involving preconcentration and separation schemes is required, such as chromatography [14], coprecipitation [15], extraction [16], X-ray fluorescence spectroscopy [17] or ion-exchange resins [18]. However, these methods are not profitable enough to be adapted to routine analysis, since they are somewhat complicated, and usually involve expensive instrumentation.

Electrochemical methods appear very attractive for analytical purposes because they are reliable, sensitive and require less expensive equipment [19]; for chromium in particular, they allow direct redox speciation with no separation step [20–27]. While Cr(VI) has been successfully determined with detection limits below nanomolar [28–30], the determination of Cr(III) is not such an easy task, since a continuous decrease of the voltammetric signal has been observed [20–27]. Using diethylenetriaminepentaacetic acid (DTPA) as a complexing agent, Li

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Table 1
Central Composite design for chemical variables and obtained response.

Runs	Variables		ip (μA)
	Buffer pH	[DTPA] (mmol L^{-1})	
1	6.5	5.5	1.596
2 (C)	5.75	3.5	1.402
3	6.5	1.5	1.187
4	4.69	3.5	0.300
5	5.75	3.5	1.420
6	5	1.5	0.470
7	5.75	0.67	0.779
8	5.75	3.5	1.097
9	5.75	3.5	1.048
10	6.81	3.5	1.158
11	5.75	3.5	1.317
12	5	5.5	0.480
13	5.75	6.33	1.279

and Xue [23] developed an entire protocol for chromium speciation at subnanomolar level, which was applied to natural samples. In other works [20–27], reaction schemes were proposed for the complexation and redox reactions of Cr(III) and Cr(VI) with DTPA in the presence of nitrate at mercury electrodes, under catalytic adsorptive stripping voltammetric (CAdSV) electrode surface where it is rapidly reduced to Cr(III) at potentials as low as -0.05 V [23]. An interesting review presented not only the mechanisms for Cr(III) and Cr(VI) reduction but also indicate the use of other electrode surfaces [27]. A Cr(III)–DTPA complex is then formed and adsorbed; its reduction to a Cr(II)–DTPA species takes place, originating a well-defined reduction peak. On the other hand, Cr(III) is easily complexed in bulk solution with DTPA; the complex diffuses to the electrode surface where it is weakly adsorbed and then reduced. However, this process is hindered because only a small fraction of the complex reaches the electrode; during its transport, the conversion to a non-electroactive complex species occurs.

Square wave voltammetry (SWV) is rapid, sensitive and consume less analyte than other pulsed techniques [30]. Most of the optimization of SWV also deals with the traditional one-factor-at-a-time (OFAT) approach, examining SWV parameters such as frequency, step potential and pulse height [31–33]. 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). Recently, some statistical designs of experiment methods have been employed in electrochemical sensors optimization [34–36]. Using statistical design of experiment imparts advantages, as it allows obtaining the optimum conditions through relatively smaller numbers of experiments. A proper design matrix can lead to obtain a regression equation which highlights effect of individual factors and their relative importance in given operation process. The possibility of evaluating the interaction effect between the variables on the signal can also be known which are not readily

possible in a classical method [37]. Until now, a few reports of the optimization of SWV signal using response surface methodology (RSM) have been published [38–40]. Nevertheless, there is lack of reports on optimization of voltammetric signal for chromium determination using RSM. In an attempt to overcome these problems a new approach is proposed in this research, where the speciation is still carried out in two steps, but in a different way. Influence of important variables was investigated and optimized by experimental design. We focused on the enhancement of the intensity of chromium current by response surface experimental designs as improved optimization methodology. Derringer's desirability function, was used for the evaluation the SWV parameters.

2. Experimental

2.1. Equipment

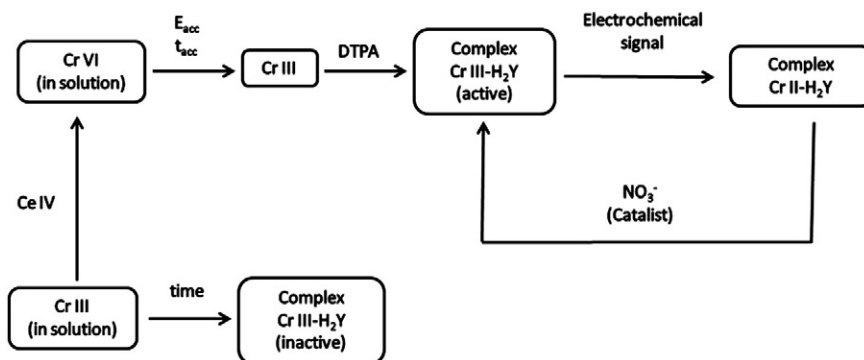
Cataytic cathodic square wave stripping voltammetry (CCSSWV) measurements were performed with an Autolab (Eco-Chemie, Utrecht, Netherlands) equipped with a PSTAT 20 potentiostat and the GPES 4.8 software package. A static mercury drop (VA663 Metrohm, Switzerland) with a small drop having a 4.0 mm^2 surface area was used as the working electrode, in hanging mercury drop electrode (HMDE) mode. A glassy carbon rod was the counter electrode and all potentials in the text are referred to Ag/AgCl (3 mol L^{-1} KCl) reference electrode. Each scan was done on a separate drop at room temperature.

2.2. Data analysis

The STATISTICA statistical package software version 7.0 (StatSoft Inc., Tulsa, USA) was used for experimental design analysis and data processing. The adequacy of the developed models were tested by performing coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}) and predicted coefficient of determination (R^2_{pre}) analysis, 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. Chemicals and solutions

Acetic buffer (0.5 mol L^{-1} , pH 6.5) was prepared by mixing the corresponding amounts of acetic acid and NaOH (both Suprapure®, Cicarelli). Diethylenetriaminepentaacetic acid (DTPA) was purchased from Sigma. A DTPA solution (20 mmol L^{-1}) was prepared by dissolving the appropriate amount of the reagent and the addition of 25% ammonia (Suprapur®, Merck) until pH 6.0. KNO_3 (0.5 mol L^{-1}) was prepared by dissolving the corresponding amount of the salt (Sigma) in deionized water. Solution of Ce(IV) was prepared by dissolving $\text{Ce}(\text{SO}_4)_2$ salt (Anedra) (3 mmol L^{-1}) and adjusted to pH 2 by H_2SO_4 (Cicarelli) addition. Stock solution of Cr(III) was prepared by dissolving Chromium potassium sulfate (Mallinckrodt) while stock solution of Cr(VI) by



Scheme 1. Proposed reactions in bulk solution and at the electrode interface to describe the electrochemical Cr(VI) and Cr(III) speciation and quantification in the presence of DTPA and Ce(IV).

dissolving potassium dichromate (Cicarelli) both in deionized water. All other chemicals were analytical grade.

2.4. Experimental procedure for Cr speciation

All the experiments are performed by Cr(VI) quantification, the ion is reduced to Cr(III) at a fixed potential for a certain time, after the addition of DTPA and 0.5 mol L⁻¹ KNO₃ in 0.5 mol L⁻¹ acetic buffer, Cr(III) complex with DTPA and finally is stripped to Cr(II), giving the electrochemical signal. So, two different samples (A and B) were prepared. Sample A, Cr(VI) is measured by CCSSWV after 40 min since the addition of DTPA and 0.5 mol L⁻¹ KNO₃ in 0.5 mol L⁻¹ acetic buffer. In the meantime the voltammetric response of Cr(III) disappears, as an inactive complex is formed. While sample B is treated with 0.3 mmol L⁻¹ Ce(IV) left 30 min at 50 °C, and then Cr(VI) is measured, and the total Cr content in the form of Cr(VI) is determined. The concentration of Cr(III) is calculated from the difference between total chromium and Cr(VI) concentration.

Blank solution consisted on 0.5 mol L⁻¹ KNO₃, DTPA and 0.5 mol L⁻¹ acetic buffer. Concentration of DTPA and acetic buffer pH were determined by experimental designs as instrumental parameters of SWV (step potential, amplitude and frequency). After recording the voltammetric signal, the solution was purged for 10 min with high purity and water saturated N₂. A new mercury drop let grow after four drops were discarded, followed by adsorption with stirring at E = -1.1 V for 120 s. Then, the stirring was stopped and a quiescence time of 5 s was allowed. All voltammograms were recorded in the cathodic direction from -0.95 to -1.50 V.

2.5. Experimental design

The RSM consists of a group of empirical techniques devoted to the evaluation of existing relationship between a cluster of controlled experimental factors and measured responses according to one or more selected criteria. Several superimposed designs were gathered to construct the central composite design (CCD); a full factorial design (2^k), a star or axial points design (at some value α and -α on each axis) and replicates at center points C₀, where k denotes number of factors and C₀ is the replicates at center points. The number of axial points is always twice the number of factors (2k). Therefore total number of experiments (N) at CCD equals to N = 2k + 2k + C₀. Central composite design inherently has both orthogonality and rotatability resources [41–43]. Orthogonality refers to a matrix design where its elements are orthogonal with each other (correlation coefficients between them are zero except for interactions) [43]. However rotatability, which implies confidence in the predictions, depends only on the distance from the center of design pattern. Since the rotatability depends only on the value of α, it should be equal to (2^k)^{1/4}.

The Box–Behnken design (BBD) was specifically selected since it requires fewer runs than a central composite design (CCD) in the cases of three or four variables. Box–Behnken is a spherical, revolving response surface methodology (RSM) design that consists of a central point and with the middle points of the edges of the cube circumscribed on the sphere. BBD are a class of rotatable or nearly rotatable second-order designs based on three-level, incomplete factorial designs [44]. The number of experiments (N) required for BBD development is defined as N = 2k(k - 1) + C₀, where k is the number of factors and C₀ is the number of central points [45]. The design is represented as a cube; however, the experimental points are at the midpoints of the cube edges rather than at the corners and centers of the faces. An advantage of BBD is that it does not contain combinations for which all factors are simultaneously at their highest or lowest levels. Therefore, these designs are useful in avoiding experiments that would be performed under extreme conditions, for which unsatisfactory results might occur [44].

According to CCD and BBD matrix design (Tables 1 and 3) for predicting the mathematical relationship between independent factors

Table 2
ANOVA results related to Central Composite design.

Source	Sum of squares	df	Mean square	F value	p-value
Model	1.82814003	5	0.36562801	13.8393662	0.0016
X ₁ -pH	1.16088492	1	1.16088492	43.940593	0.0003
X ₂ -[DTPA]	0.15882951	1	0.15882951	6.01184728	0.0440
X ₁ X ₂	0.04010006	1	0.04010006	1.51782533	0.2577
X ₁ ²	0.4360248	1	0.4360248	16.5039514	0.0048
X ₂ ²	0.0698638	1	0.0698638	2.64441089	0.1479
Residual	0.18493593	7	0.02641942		
Lack of Fit	0.0644973	3	0.0214991	0.71402675	0.5928
Pure Error	0.12043863	4	0.03010966		
Cor Total	2.01307596	12			

and dependent response, a second order polynomial model was fitted to experimental results [45]. During optimization process, relationship of response, main variables, and interactions can be formulated as a quadratic model which also includes the linear terms:

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

where Y is predicted response, β₀ is offset term, β_i is coefficient of linear effect, β_{ii} is coefficient of squared effect, β_{ij} is coefficient of interaction effect, and ε is random error.

In this study, optimization process of chemical variables of chromium determination was carried out using central composite design (CCD), totally 13 experiments were performed consisting 4 factorial points (coded to the usual ±1 notation), 4 axial points (±α, 0, 0), (0, ±α, 0), (0, 0, ±α), and 5 replicates at the center points (0, 0, 0) in order to allow the estimation of pure error (Table 1). While a Box–Behnken design for SWV instrumental variables optimization, consisting of 37 factorial points and 6 replicates at the center points in order to allow the estimation of pure error was employed (Table 3), indicating that altogether 43 experiments were required.

2.6. Desirability function

Desirability function (DF) is a common and established technique to concurrently determine of input variables that can give the optimum

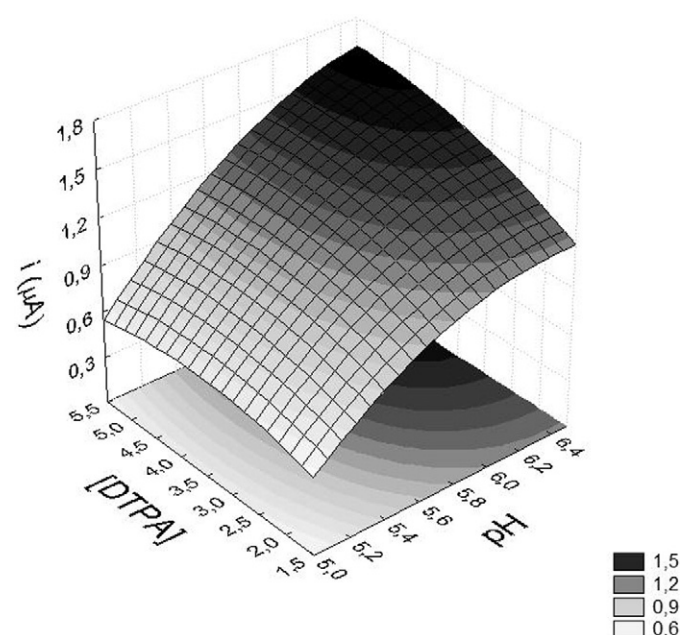


Fig. 1. Response surface for the central composite designs of data in Table 1.

performance levels for one or more responses. Harrington first developed the DF, and it was later optimized by Derringer and Suich [45] 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 3
Box–Behnken designs for SWV instrumental variables and obtained response.

Runs	Variables					
	Frequency (Hz)	Step potential (V)	Pulse height (V)	Accumulation time (s)	Accumulation potential (V)	ip (μA)
1	255	0.01	0.1	65	-1.05	11.79
2	10	0.0005	0.055	65	-1.05	0.7967
3	255	0.0005	0.055	120	-1.05	9.097
4	255	0.00525	0.055	120	-1.15	7.329
5	255	0.01	0.01	65	-1.05	1.804
6	255	0.00525	0.01	65	-1.15	1.105
7	255	0.00525	0.1	65	-1.15	6.494
8	10	0.00525	0.055	65	-0.95	2.566
9	255	0.00525	0.01	10	-1.05	0.3619
10	255	0.01	0.055	65	-1.15	5.315
11	255	0.00525	0.055	65	-1.05	8.257
12	500	0.01	0.055	65	-1.05	8.87
13	255	0.00525	0.1	10	-1.05	2.009
14	10	0.00525	0.055	65	-1.15	2.558
15	255	0.00525	0.055	120	-0.95	9.049
16	255	0.00525	0.055	10	-0.95	1.154
17	255	0.00525	0.01	120	-1.05	3.073
18	10	0.00525	0.1	65	-1.05	4.029
19	255	0.0005	0.01	65	-1.05	1.367
20	255	0.0005	0.055	10	-1.05	1.039
21	255	0.00525	0.055	65	-1.05	7.709
22	10	0.01	0.055	65	-1.05	4.326
23	500	0.00525	0.1	65	-1.05	10.95
24	500	0.0005	0.055	65	-1.05	6.206
25	255	0.00525	0.1	120	-1.05	16.64
26	10	0.00525	0.01	65	-1.05	1.026
27	500	0.00525	0.01	65	-1.05	1.821
28	255	0.0005	0.055	65	-0.95	3.528
29	500	0.00525	0.055	10	-1.05	1.617
30	255	0.00525	0.055	65	-1.05	7.879
31	255	0.00525	0.1	65	-0.95	6.821
32	500	0.00525	0.055	120	-1.05	13.31
33	500	0.00525	0.055	65	-1.15	13.29
34	10	0.00525	0.055	10	-1.05	0.8157
35	255	0.01	0.055	120	-1.05	14.16
36	255	0.01	0.055	65	-0.95	5.842
37	255	0.00525	0.055	65	-1.05	8.839
38	255	0.00525	0.01	65	-0.95	1.266
39	255	0.00525	0.055	65	-1.05	8.51
40	255	0.01	0.055	10	-1.05	1.658
41	255	0.00525	0.055	10	-1.15	1.423
42	500	0.00525	0.055	65	-0.95	5.772
43	255	0.00525	0.055	65	-1.05	8.266
44	255	0.0005	0.055	65	-1.15	3.485
45	10	0.00525	0.055	120	-1.05	6.226
46	255	0.0005	0.1	65	-1.05	6.388

Table 4
ANOVA results related to SWV instrumental variables and obtained response.

Source	Sum of squares	df	Mean square	F value	p-value
Model	686.44506	15	45.763004	89.6224259	<0.0001
X ₁ -Frequency	58.5413595	1	58.5413595	114.647602	<0.0001
X ₂ -Step potential	29.3615838	1	29.3615838	57.5018277	<0.0001
X ₃ -Pulse height	177.536304	1	177.536304	347.687714	<0.0001
X ₄ -Accumulation time	254.266047	1	254.266047	497.955509	<0.0001
X ₅ -Accumulation potential	0.3992978	1	0.3992978	0.78198619	0.3843
X ₁ X ₃	9.381969	1	9.381969	18.3736806	0.0002
X ₁ X ₄	9.86807982	1	9.86807982	19.3256818	0.0002
X ₂ X ₃	6.16280625	1	6.16280625	12.0692611	0.0017
X ₂ X ₄	1.81993885	1	1.81993885	3.56417457	0.0698
X ₃ X ₄	35.521004	1	35.521004	69.5644575	<0.0001
X ₁ ²	27.3405155	1	27.3405155	53.5437604	<0.0001
X ₂ ²	7.98358107	1	7.98358107	15.6350729	0.0005
X ₃ ²	27.4548161	1	27.4548161	53.7676071	<0.0001
X ₄ ²	4.28444118	1	4.28444118	8.39066448	0.0074
X ₅ ²	47.5170166	1	47.5170166	93.0574902	<0.0001
Residual	13.7867403	27	0.51062001		
Lack of Fit	13.0271923	23	0.56639966	2.98282486	0.1486
Pure Error	0.759548	4	0.189887		
Cor Total	700.2318	42			

3. Results and discussion

The speciation of Cr(III) and Cr(VI) by means of the CCSSWV is based on the difference in the voltammetric response in the solutions containing DTPA and NO₃⁻ ions. As it was reported previously [23] for the same concentration of Cr(III) and Cr(VI) the time decay of the Cr(VI) signal was only ca. 15% h⁻¹, whereas Cr(III) signal almost completely diminished after ca. 40 min. Because of the fast fall of Cr(III)-DTPA response in time, the accurate determination of Cr(VI) in the presence of some excess of Cr(III), occurring in the form of inorganic complexes, is still possible when the CCSSWV measurements are performed with 40 min delay after addition of the DTPA to the analyzed solution. However, this method has some complications and is not very reproducible, so we propose a Cr(III) oxidation to Cr(VI) with Ce(IV), according to Scheme 1.

3.1. Optimization of chemical variables by central composite design

In optimization step, a central composite design with three replicates at the center point was used to identify the effect of individual

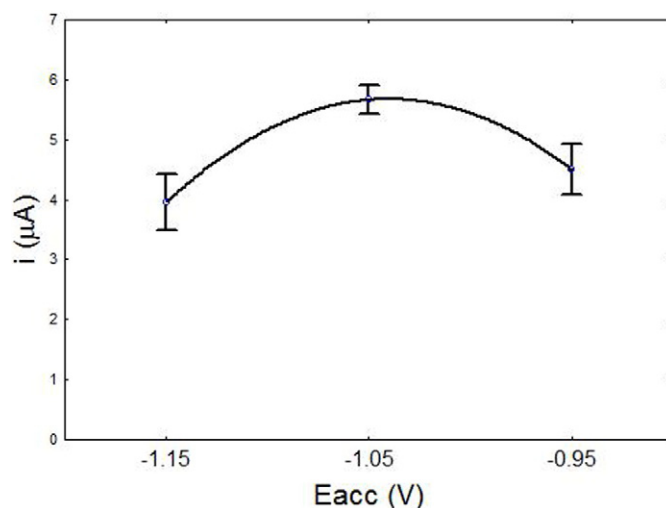


Fig. 2. Plot of marginal means and confidence limits (95%) of accumulation potential (E_{acc}) for data in Table 3.

variables and their interactions on response. Selection of extreme levels of factors in experimental design was performed by previous experiences and bibliography reports [46]. Responses are presented in Table 1. Two factors, buffer pH and DTPA concentration, were selected as operational (independent) variables, while reduction peak height of the square wave voltammogram of chromium (300 nmol L⁻¹) was considered as response. By applying regression analysis on experimental data, the results of CCD were fitted with a polynomial equation. An empirical relationship between response and input variables after

removing insignificant effects in coded values was expressed by the following quadratic models:

$$i (\mu\text{A}) = 1.25688 + 0.38093387x_1 + 0.14090312x_2 + 0.100125x_1x_2 + 0.25035688x_1^2 + 0.10021438x_2^2 \quad (2)$$

where x_1 is buffer pH and x_2 DTPA concentration.

In order to verify whether the main effects are significant, an analysis of variance ($\alpha = 5\%$) was conducted [37]. Significance of each

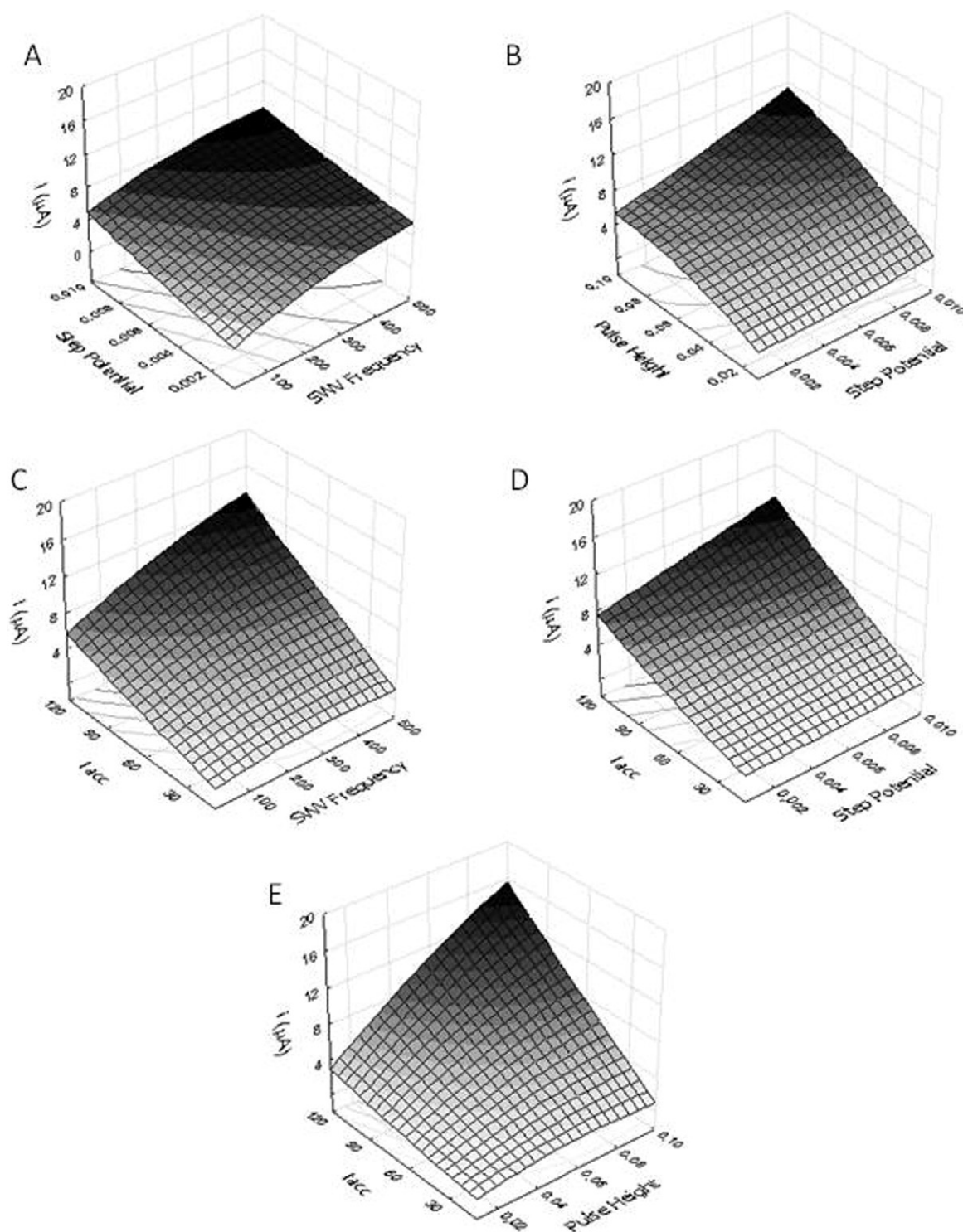


Fig. 3. Response surface for the Box Behnken designs of data in Table 3: (A) Step potential (V)–Frequency (Hz), (B) Step potential (V)–Pulse height (V), (C) Accumulation time (s)–Frequency (Hz), (D) Accumulation time (s)–Step potential (V), (E) Accumulation time (s)–Pulse height (V).

coefficient of Eq. (2) determined by applying *t*-test along with *p*-values of each, are listed in Table 2. Since the *p*-values of all the coefficients are $p < 0.05$, it implies that they are significant. The lack of fit *p*-value is reported to be 0.5928 which shows an appropriate fitting between data. The goodness of model can be checked by the coefficient of determination (R^2) as well. R^2 is a measure of the amount of variation around the mean explained by the complete model and the R^2_{adj} is a measure of the amount of variation around the mean explained by the model, adjusted for the number of terms include in it. The R^2_{adj} decreases as the number of terms in the model increases if those additional terms do not add value to the model. On the other hand, R^2_{pred} is a measure of the amount of variation in new data explained by the model. In general, it is accepted that the R^2_{pred} and the R^2_{adj} should be within 0.20 of each other to be in reasonable agreement. Otherwise there may be a problem with either the data or the model. The R^2 value of 0.908 is in reasonable agreement with the experimental results, indicating that 90.8% of the variability can be revealed by the model. A high value of R^2_{adj} (0.842) is also an indication for high significance of the proposed model. The model shows R^2_{pre} values of 0.679 for which are high and advocate a high correlation between the observed and the predicted values. Fig. 1 shows the response surface plot for relationship between buffer pH values and DTPA concentrations. On the basis of the coefficients in Eq. (2) and Fig. 1, it can be determined that the peak height increases for chromium determination, with increasing buffer pH and DTPA concentration in the studied region and reflects the quadratic term of buffer pH according with ANOVA results (Table 2). Based on Fig. 1, it can be established that the optimal values for chemical variables were: buffer pH 6.5 and DTPA concentration 5.50 mmol L⁻¹.

3.2. Optimization of instrumental variables by Box–Behnken design

In the present study, SWV variables such as frequency, pulse height, step potential and accumulation time and potential were chosen by borrowing methodology as five independent factors and the current reduction peak was selected as response variable. A five-factor with three level experimental design of Box–Behnken method at random order was used to find the maximum of the current reduction peak and is

presented in Table 3 along with experimental data. In order to evaluate the effects of independent variables on current reduction peak (Y_1) experiment data in Table 3 were subjected to regression analysis and the obtained equation was:

$$i(\mu\text{A}) = 8.19 + 1.9912699x_1 + 1.43720328x_2 + 3.33106875x_3 + 4.22934047x_4 + 0.16445504x_5 + 1.5315x_1x_2 + 1.5315x_1x_3 + 1.570675x_1x_4 + 1.24125x_2x_3 + 0.82676186x_2x_4 + 2.979975x_3x_4 - 1.88028114x_1^2 - 1.02515337x_2^2 - 1.84713275x_3^2 - 0.7509950x_4^2 - 2.47881447x_5^2 \quad (3)$$

where x_1 is SWV frequency, x_2 step potential, x_3 pulse height, x_4 accumulation time and x_5 accumulation potential.

The ANOVA analysis (Table 4) showed the linear, interactive and quadratic relationship between the effects of independent variables on their dependent variables. The significance of each term was assessed according to their *p*-values. The analysis of variance concluded that, the models were highly significant at probability level $p < 0.0001$ for the response. The statistical significance of the second-order model, Eq. (3), was checked by *F*-test (Table 4). The analytical results show that the model is highly significant as the value of probability is less than 0.05. The values of R^2 were calculated to be 0.9803, which implies that 98% of experimental data were compatible. The value of R^2_{adj} (0.969) is also high and advocate a high significance of the model. The model shows an R^2_{pre} value of 0.945 which is high and advocates a high correlation between the observed and the predicted values. The whole analysis shows that the form of the chosen model has a good correlation between the factors and the response.

The significant model terms ($p < 0.05$) are, x_1 (SWV frequency), x_2 (step potential), x_3 (pulse height) and x_4 (accumulation time), as well as the quadratic term of the total instrumental variables. The lineal model term of accumulation potential are not significant ($p > 0.05$), implying that it has only quadratic dependence on response, whereas it is still considered in Eq. (3) because quadratic term of accumulation potential is included in the model. Fig. 2 shows the accumulation potential effect on the current reduction peak. As it can be observed, a nonlinear

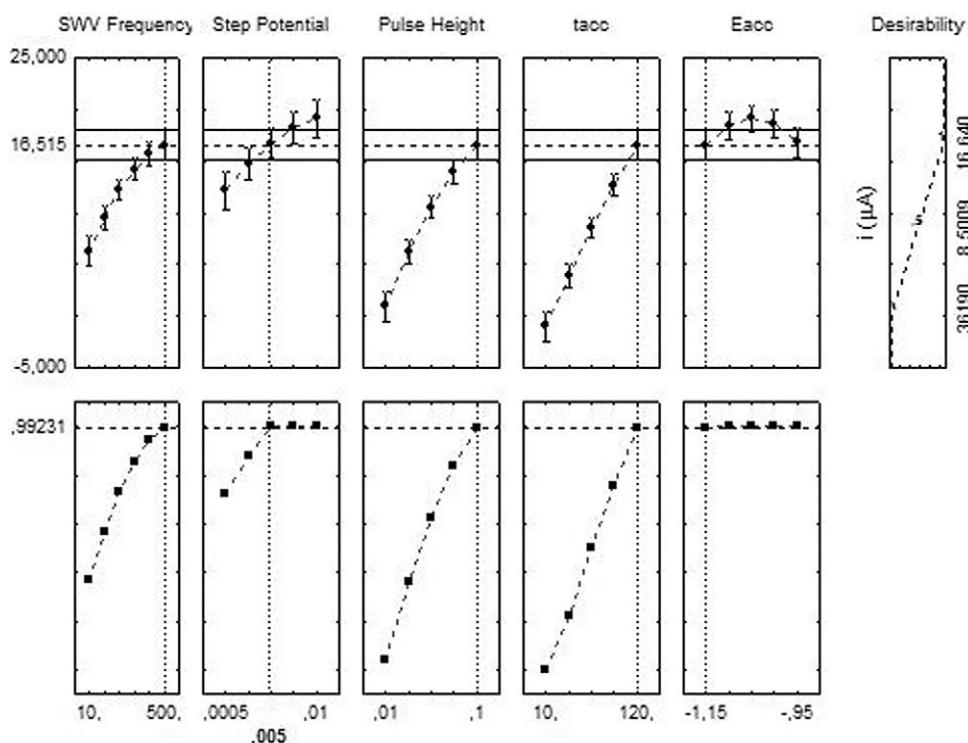


Fig. 4. Profiles of predicted values and desirability function for the i_p (μA). Dotted lines indicate optimization values.

response is obtained indicating that the quadratic term is important. Other model terms, as well as the second order effect of x_1x_3 , x_1x_4 , x_2x_3 , x_2x_4 and x_3x_4 are significant ($p < 0.05$), implying that they had impact on the response.

The overall significant interaction effects are displayed in Fig. 3; a 3D representation of the polynomial (Eq. (3)) obtained from the experimental data, depicting the surface plots of i_p versus the significant variables. Fig. 3 shows that the response increases with mean values of accumulation potential and with increasing SWV frequency, pulse height, step potential and accumulation time values. Also, the quadratic term of the factors is significant on all surfaces response involved, being for all the factors statistically significant the quadratic terms (Table 4).

3.3. Optimization of BBD by desirability function

The profile for predicted values and desirability option in the STATISTICA 7.0 software is used for the optimization process (Fig. 4). 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 3 represented the maximum (16.640 μA) and minimum (0.3619 μA) i_p of Cr(III) reduction. According to these values, DF settings for each dependent variable of i_p are depicted at the right hand side of Fig. 4; desirability of 1.0 was assigned for maximum i_p (16.640 μA), 0.0 for minimum (0.3619 μA) and 0.5 for middle (8.5009 μA). On the left hand side of Fig. 4 (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. 4. 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 0.992, i_p for chromium reduction was optimized at 16.640 μA by calculating the optimized model variables of 500 Hz SWV frequency, 0.005 V SWV step potential, 0.100 V SWV pulse height, 120 s accumulation time and -1.10 V accumulation potential. This set of conditions was determined to be optimum by the RSM optimization approach and to confirm the validity of the optimized conditions; experiments were carried out to compare the experimental results with the predicted values of the responses using the model equation. The reduction current peak of chromium, using these optimum conditions, was found to be 18.46 μA and the experimental value was $18.21 \pm 0.06 \mu\text{A}$. The mean value obtained was compared with the predicted values and indicated the suitability of the developed quadratic models. The percentage deviation of the experimental and theoretical results was found as 1.35%. The results obtained through confirmation experiments indicate the suitability of the developed quadratic model and it may be noted that these optimal values are valid within the specified range of process parameters.

3.4. Chromium speciation

As already mentioned speciation of Cr(III) and Cr(VI) was performed by means of the CCSSWV based on Cr(VI) reduction and accumulation to Cr(III) and then stripped to Cr(II) in solutions containing DPTA and NO_3^- ions. Hence, to perform the speciation of Cr the investigated samples must be divided into two parts:

- In sample A, Cr(VI) is measured after 40 min since the addition of 5.5 mmol L^{-1} DPTA and 0.5 mol L^{-1} KNO_3 at pH = 6.5 to the sample by CCSSWV. In the meantime the voltammetric response of Cr(III) disappeared.
- In sample B, 3 mmol L^{-1} Ce(IV) is added and left at 50 °C for 30 min. Then the solution is brought to 5.5 mmol L^{-1} DPTA and 0.5 mol L^{-1} KNO_3 at pH = 6.5 and the total Cr content in the form of Cr(VI) is determined by CCSSWV.

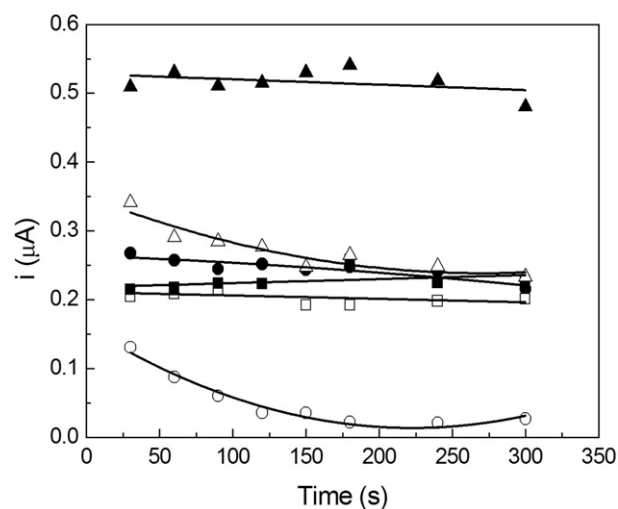


Fig. 5. Electrochemical CCSSWV response with time, for different 50 nmol L^{-1} chromium solutions in 0.5 mol L^{-1} acetate buffer pH = 6.5, with 0.5 mol L^{-1} KNO_3 + 5.5 mmol L^{-1} DPTA addition and with and without 0.3 mmol L^{-1} Ce(IV). (○) Cr(III), (●) Cr(III) + Ce(IV), (□) Cr(VI), (■) Cr(VI) + Ce(IV), (△) Cr(III) + Cr(VI), (▲) Cr(III) + Cr(VI) + Ce(IV). CCSSWV parameters Step potential = 5 mV, amplitude = 100 mV, frequency = 500 Hz, $E_{\text{acc}} = -1.1$ V, $t_{\text{acc}} = 120$ s.

The concentration of Cr(III) is calculated from the difference between total chromium and Cr(VI) concentration.

In order to demonstrate that electrochemical signal for Cr(III) solutions decay with time and that the use of Ce(IV) overcame this situation studies of Cr(III) and Cr(VI) with and without Ce(IV) addition were performed. Solutions of Cr(III) and Cr(VI), containing only KNO_3 and sodium acetate, produce a reduction peak at -1.35 V at pH 6.5 in the presence of 5.5 mmol L^{-1} DPTA. In Fig. 5 the reduction peak behavior with time for Cr(VI), Cr(III) and a Cr(VI)/(III) mixture solutions at pH 6.5, with DPTA addition, using the optimized conditions are presented; also curves with and without Ce^{4+} solution addition (3 mmol L^{-1}) are included. As it can be observed, current peaks for all Cr(VI) solutions, with and without Ce(IV) and Cr(III) and the mixture Cr(VI)/(III) with Ce(IV) addition are stable with time. By the other side, current peaks for Cr(III) and Cr(VI)/(III) mixture without Ce(IV) addition, gradually decrease over time (Fig. 5). Furthermore, when calibration curves for Cr(VI) are performed with and without Ce(IV) addition no significant differences are observed (Fig. 6). These results allow us to use Ce(IV) as an oxidant agent for chromium speciation.

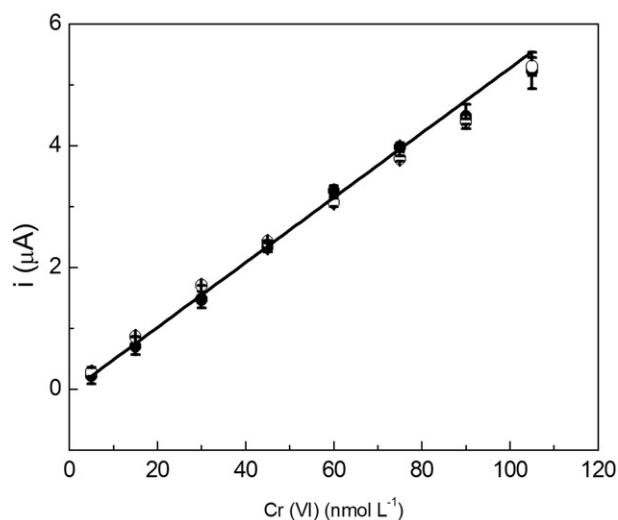


Fig. 6. Cr(VI) calibration curve with (○) and without (●) 0.3 mmol L^{-1} Ce(IV) addition. Other experimental conditions as in Fig. 5.

Table 5
Recoveries experiments for different Cr (III)/(VI) mixtures in nmol L⁻¹.

Mixture	Added amounts			Obtained amounts			% Recovery		
	[Cr III]	[Cr VI]	[CrTotal]	[Cr III]	[Cr VI]	[CrTotal]	[Cr III]	[Cr VI]	[CrTotal]
A	30	30	60	35.8 ± 0.8	25 ± 1	61 ± 2	119.33	83.33	101.67
B	15	30	45	15 ± 3	30 ± 6	44 ± 3	100	100	97.78
C	30	15	45	35 ± 2	14 ± 5	48 ± 5	116.67	93.33	108.89

3.5. Calibration plots and stability

In order to verify the method linearity within a concentration range of (5–105) nmol L⁻¹ of Cr(VI), three replicates were prepared at five concentration levels and subjected to the analytical procedure. A least-square fitting was performed with the obtained data (*i_p*) and the results present a coefficient of determination (*R*²) higher than 0.998; the computed LOD and LOQ were 3.77 and 8.47 nmol L⁻¹ respectively.

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

$$i_p(A) = (52 \pm 2) \times C_{Cr(VI)} (A/mol L^{-1}) + (9 \pm 4) \times 10^{-9} (A) \quad (4)$$

3.6. Speciation of chromium(III)/(VI) in synthetic samples

Standard solutions of Cr(III), Cr(VI) and Cr(III)/(VI) mixtures, in the 15 to 60 nmol L⁻¹ concentration range were used for recoveries experiments. Three replicate experiments were performed following the whole analysis process. Table 5 presents the obtained results. As it can be observed, recuperation percentages (% R) for standard solutions ranged from 93% to 119% and the relative standard deviations (RSD) were between 4 and 5% respectively. These results indicate that the proposed method presents reasonable figure of merits.

4. Conclusions

In this study, a CCD and BBD response surface design were carried out to optimize and to study individual and interactive effects of chemical variables (DTPA concentration and buffer pH) and instrumental variables (SWV frequency, pulse height, step potential and accumulation time and potential) on Cr(III) and Cr(VI) speciation and quantification. Second order polynomial models were developed for predicting reduction current signal and gave a satisfactory description of the experimental data. The response surface plots were constructed and used for estimating the interaction effect between four independent variables (SWV frequency, SWV step potential, SWV pulse height, accumulation time and accumulation potential) on the responses. An optimized condition for Cr(VI) quantification by CCSSWV in DTPA and nitrate solutions was determined. Under the optimized conditions the experimental values of Cr(VI) synthetic solution agreed closely with the predicted values. This study can be used for Cr speciation in water.

Acknowledgments

Authors wish to acknowledge the assistance of the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) and the Universidad Nacional de Córdoba, both of which support facilities used in this investigation. This work was financed by Argentina's SECYT-UNC (Res. 203/14), Mincyt-Córdoba (PID 2008), FONCYT (PICT-2011-0718) and CONICET (PIP 112-201101-00579). V. Pfaffen and P. Ortiz, are members of CICYT in Argentina's CONICET. M. Cuéllar thanks CONICET for the fellowship granted.

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