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A Box-Behnken Design and Response Surface Approach for the Simultaneous Determination of Chromium (III) and (VI) Using Catalytic Differential Pulse Polarography

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The present paper describes an optimized Box-Behnken design using a catalytic-differential pulse polarograhic technique for the simultaneous determination of chromium (III) and (VI) in wastewater samples using ammonium piperidine dithiocarbamate as a complexing agent. The optimization strategy was carried out using a two level full factorial design. The results of the experimental design were based on an analysis of variance and demonstrated that only the pH, concentrations of the buffer and the complexing agent were statistically significant throughout the experiment. The optimal values for the three variables were 8.0, 0.2 mM and 5.0 mM for pH, concentrations of the buffer and the complexing agent respectively. Under optimum experimental conditions the detection limit of the proposed method was found to be 0.0841 µg L^{-1} while the linear range was 1.0-10.0 and 0.5-25.0 µg L^{-1} for chromium (III) and (VI) respectively. The present method was also applied for the simultaneous determination of chromium in the presence of some foreign ions with satisfactory analytical responses.

Keywords: Box-Bahnken design, Catalytic-differential pulse polarography, Speciation, Chromium (III) and chromium (VI), Ammonium piperidine dithiocarbamte (APDC), Wastewater samples

1. INTRODUCTION

Due to the adverse effects of chromium compounds, speciation plays a significant role in toxicological studies. However, chromium in its trivalent form does not occur freely in nature, yet it is a dietary requirement for a number of organisms. On the other hand the hexavalent chromium is very toxic to flora and fauna, but does not pose a threat to the environment. The discharging of the untreated chromium polluted wastewater into rivers has caused environmental disasters in the past.

Several compounds of chromium are relatively water insoluble, but chromium (III) compounds such as the oxides and hydroxides are soluble. These compounds are largely bound to the floating particles in water. The trivalent chromium is mainly discharged by the metal industry while the hexavalent chromium in industrial wastewater mainly originates from tanning and paint industries. Chromium compounds are applied as pigments and 90% of the leather is tanned by means of chromium compounds. Particularly in wastewater samples, chromium (VI) is generally present in relatively small amounts compared with chromium (III) [1]. Furthermore, the toxicity level of chromium (III) in the living cells is about 1000 times less than that of chromium (VI) [2]. Spectrophotometric techniques have been reported since the 19th century for the speciation and determination of chromium. Generally, these methods are conducted sequentially where the total chromium content is firstly determined followed by the compound being determined after extraction, complexation or enrichment. It's well understood that direct determination of total chromium concentration cannot always provide the right answer, hence the speciation analysis is required instead.

Different separation methods include Liquid–liquid extraction [3], Solid phase extraction [4-6], Co-precipitation [7], Cloud point extraction [8-11], Ion-exchange [12] and Membrane filtration [13] have been reported in the literature for the speciation and determination of chromium in various environmental matrices. Additionally other widely used techniques for the speciation of chromium includes Adsorptive stripping voltammetry [14-20], Differential pulse polarography [21], DC polarography [22], Capillary electrophoresis [23], High performance liquid chromatography [11,24-26], Flame atomic absorption spectrometry [7, 8, 27-38], Graphite furnace atomic absorption spectrometry [39, 40], Electrochemical atomic absorption spectrometry [41-44], Inductively coupled plasma atomic emission spectrometry [45], Inductively coupled plasma mass spectrometry [46-48], Spectrophotometry [49-55], Spectrofluorimetry [56, 57], X-ray absorption spectrometry [58] and X-ray fluorescence spectrometry [59]. Nevertheless, chromium (VI) detection limits are frequently substandard using chromatographic and spectroscopic methods. In addition, some of these procedures do not meet the minimum requirements of routine analysis, principally because of their complicated design and cost. Electrochemical techniques, especially catalytic-differential pulse polarography appears to be a suitable alternative for the speciation analysis of chromium due to its low cost, low detection limit and in-situ determination of chromium species with a simple cleaning procedure [60-62].

A good experiment is achieved more effectively with the help of statistical design to monitor the traceability, validation and optimization process in all stages of the experiment [63]. Experimental design plays a pivotal role in the improvement of the analytical data process for method development and validation [64, 65]. In particular, during method optimization some performance parameters should also be investigated in order to avoid serious problems during the validation step. These performance parameters include accuracy, precision and robustness which are evaluated during the pre-validation stage [66-68]. Moreover, experimental design helps to verify changes in factor values in relation to the observed response by a well-defined mathematical model. Hence, experimental design is considered to be a valuable tool for the validation and optimization of variable parameters in the multivariate experimental design systems [69]. Strategies used for the experimental optimization studies include method precision (repeatability), Taguchi approach [70], Box-Meyer approach [71], artificial neural networks [72], Box-Behnken design [73].

In this paper, we present a multivariate optimization methodology for the speciation and simultaneous determination of chromium (III) and (VI) with ammonium piperidine dithiocarbamate (APDC) using catalytic-differential pulse polarography (CDPP). To the best of our knowledge this study is the first of its kind to apply the Box Behnken experimental design to the catalytic-differential pulse polarographic technique for the speciation and simultaneous determination of chromium (III) and (VI) in different wastewater samples. Accordingly, we designed a simple, sensitive, rapid and robust method for the speciation of chromium (III) and (VI) with APDC resulting in catalytic hydrogen currents generated at the multimode electrode.

2. EXPERIMENTAL

2.1. Apparatus

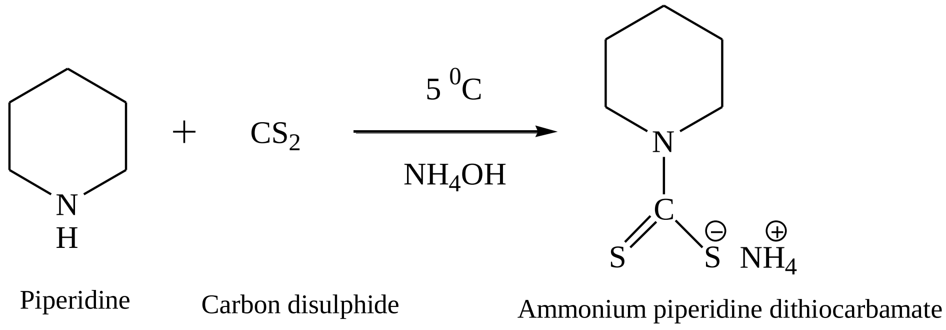
A Metrohm model 797 VA Computrace three electrode system (Metrohm Herisau, Switzerland) consisting of the multimode electrode (MME) while the dropping mercury electrode (DME) mode was selected for the working electrode to obtain all the polarographic waves. The reference electrode was Ag/AgCl and platinum wire was used as the counter electrode. All pH measurements were carried out with Crison micro pH model 2000 pH meter.

2.2. Chemicals and reagents

All the experiments were performed at 25 ^{o}C using freshly prepared solutions. Double distilled mercury was supplied by Metrohm (Durban, South Africa (SA)) and deionized water generated from an aqua MAX^{TM} – Basic 360 series water purification system from the TRILAB SUPPORT (Durban, SA). The dissolved oxygen in the solutions was removed by passing 99.8% pure nitrogen gas (AFROX Durban, SA) for 15 minutes. Stock solutions of chromium (III) and (VI) were prepared by dissolving appropriate amounts of $Cr(NO_3)_3$ and $K_2Cr_2O_7$ (Merck Laboratory Suppliers Pty Ltd, SA) in double distilled water. A 1.0 M ammonium chloride was prepared by weighing approximately 53.50 g and dissolving into 1.0 L with deionised water. Subsequently the pH was adjusted as per experimental design (see Table 1) with 5% (v/v) ammonium hydroxide and 1% (v/v) hydrochloric acid solutions. Carbon disulphide and piperidine were also purchased from Merck Laboratory Suppliers Pty Ltd, SA.

2.3. Synthesis of ammonium piperidine dithiocarbamate (APDC)

Carbon disulphide (80 g) was carefully added to the piperidine solution (85 g) and dissolved to 25 mL with distilled water at 5 ^{0}C under constant stirring, followed by the addition of 20% (v/v) ammonium hydroxide for neutralization. The resulting reaction mixture was warmed at room temperature and washed repeatedly with acetone of 99.5% purity. The product was purified by recrystallization in acetone with melting points ranging from 196-199 °C at 740 mm pressure [74-77] as shown in Scheme 1.



Scheme 1. Preparation method of ammonium piperidine dithiocarbamate

2.4. Polarographic measurements

The developed procedure was examined by using standard solutions. At optimum pH, 5 mL of 3.0 µg of chromium (VI) and 3.0 mm APDC was added into the electrochemical cell followed by 5 mL of 0.1 M ammonium chloride-ammonium hydroxide buffer. The dissolved oxygen was expelled by bubbling pure nitrogen through the measuring solution for 15 min. At selected pH chromium (VI) undergoes the reduction process and results in formation of chromium (III) -APDC complex. Polarogram of the solution were recorded using DPP against SCE in ammonium chloride-ammonium hydroxide medium. APDC or simple metal ions in the medium do not give any current signal at the potential mentioned. According to the reported method in the literature [78], total chromium was determined after oxidizing Chromium (III) to (VI) by the addition of $K_2S_2O_8$ in an acidic medium as follows: 1.0 mL of $K_2S_2O_8$ (1%, w/v), one drop of $AgNO_3$ (0.01%, w/v) and 1.0 mL concentrated H_2SO_4 were added into a 50 mL beaker containing 25 mL of the standard solution of 3.0 µg mL^{-1} Cr(III). The beaker was kept in a sonicator for 5 min for proper dissolution and the mixture was heated on a hot plate at 60 °C for 30 min to facilitate oxidation and then boiled to reduce excess of $K_2S_2O_8$. Then the mixture was cooled to room temperature and transferred into the polarographic cell for electrochemical investigation. The concentration of chromium (III) was calculated by subtracting the concentration of chromium (VI) from total chromium concentration.

2.5. Preparation of water samples

Wastewater samples collected from different industrial areas around Durban, South Africa were filtered through a 0.45 µm cellulose nitrate filter. In case of chromium (VI) determination; 10 mL of wastewater was diluted 10-fold with double distilled water, then acidified with 5.0 mL of concentrated H_2SO_4 then allowed to cool to room temperature in 30 min. The speciation procedure was followed as mentioned in the section.

2.6. Experimental design

A Box-Behnken design with fifteen runs, three independent variables (pH, concentration of NH_4Cl-NH_4OH and APDC) and three replicates at a centre point was used in this study. The experiment was randomized to reduce confounding variables by equalizing the three independent variables that have not been accounted for in the experimental design. Table 1 shows the experimental design of the three factors; pH, concentration of NH_4Cl-NH_4OH and APDC. The maximum current signals and their respective potentials due to chromium species were taken as the response of the designed experiments per APDC for the three replicates. The statistical and correlation analysis of the response of the model was performed with analysis of variance (ANOVA), pareto charts, interaction plots and surface responses. Interestingly, the design enabled us to evaluate the responses with respect to the simultaneous factor variations in all the experimental regions studied and to optimize the experimental conditions for the speciation and simultaneous determination of chromium (III) and (VI), taking into account randomization for even distribution (Figure 1). The concentrations of APDC and buffer were varied from 2.6 to 5.0 and 0.2 to 1.0 Mol/L, respectively while the pH was varied from 6 to 8.

Table 1. Box-Behnken experimental design of 15 runs and 3 parameters.

Run No.	pH	[NH4Cl-NH4OH] mol/L	[APDC] mol/L	*Potential V	*Current nA
1	7	0.2	5.0	1.38	46.37
2	7	0.6	2.6	1.37	10.68
3	7	0.6	2.6	1.38	12.06
4	8	1.0	2.6	1.39	51.71
5	7	1.0	5.0	1.28	1.20
6	8	0.2	2.6	1.39	48.40
7	6	0.6	0.3	1.52	63.79
8	7	0.6	2.6	1.41	11.90
9	6	0.6	5.0	1.36	8.24
10	8	0.6	0.3	1.37	10.41
11	7	0.2	0.3	1.40	11.78
12	7	1.0	0.3	1.40	6.41
13	8	0.6	5.0	1.38	6.43
14	6	1.0	2.6	1.38	11.28
15	6	0.2	2.6	1.39	1.34

**Average current response and potential of three replicates*

2.7. Data analysis

STATGRAPHICS *Plus* version 5.1 and Microsoft excel® 2013 were used for data evaluation and preparation of the experimental design. Peak evaluation was performed with 797 PC Software 1.3.1® 2008.

3. RESULTS AND DISCUSSION

3.1. Analysis of variance on analyte response

In order to account for lurking variables, the order of the experiments was fully randomized. The current was recorded as an analytical response, whereas the potential tells us how the parameters affect the reduction process at the electrode surface. The current response is the average of the two replicates. Analysis of the current for the overall experiment can be summarized by comparing the standardization effect as shown by the pareto chart illustrated in Figure 1a.

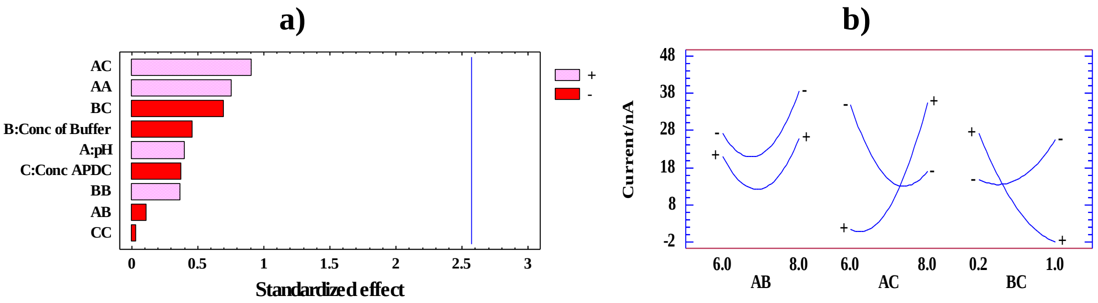


Figure 1. a) Standardized pareto chart for current response, **b**) Interaction plot for current of two parameters

The pareto charts show a *t*-statistical test for each effect, where each bar represents the standardized effect. The standard effect is compared to a *t*-critical value shown by a vertical line just above 2.5. Fortunately, no bar extends closer nor beyond the *t*-critical value, suggesting that all the variables studied were statistically insignificant. The bars indicated with dotted lines correspond to positive effects, whereas red bars correspond to negative effects. The estimated effects presented in Figure 1b for current shows that AC and AA were the most influential experimental factors with high sum of squares as shown in Table 2. Both variables presented positive effects on I_p resulting in an increase in pH related to the decrease in concentration of the ligand, APDC. On the other hand, the interactions of AB and CC were of less importance, suggesting a negative impact on *IP*.

Table 2. Analysis of Variance (ANOVA) for Current at the 95.0% confidence level

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
A:pH	130.411	1	130.411	0.16	0.7046
B:C*	173.818	1	173.818	0.21	0.6624
C:C#	113.628	1	113.628	0.14	0.7231
AA	463.715	1	463.715	0.57	0.4830
AB	10.989	1	10.9892	0.01	0.9117
AC	664.866	1	664.866	0.82	0.4061
BB	108.834	1	108.834	0.13	0.7287
BC	396.010	1	396. 010	0.49	0.5152
CC	1.060	1	1.060	0.00	0.9725

Df: Degree of freedom; C* *is the concentration of* NH_4Cl-NH_4OH;
C$^\#$ *is the concentration of APDC*

The statistical significance shown in Table 2 of each effect predicted by comparing the mean square against an estimate of the experimental error shows that none of the effects have *P*-values less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level. This also indicates that there is a possible serial correlation based on the order in which they occur in our data. Therefore, the Durbin-Watson (DW) statistic testing of the residuals was performed to determine if there were any significant correlations resulting to the matrix shown in Table 3.

Table 3. Correlation matrix showing the extent of the confounding amongst the effects included.

	1	2	3	4	5	6	7	8	9	10
Ave	1.00	0.00	0.00	0.00	-0.56	0.00	0.00	-0.56	0.00	-0.56
A:pH	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B:C^*	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C:$C^\#$	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
AA	-0.56	0.00	0.00	0.00	1.00	0.00	0.00	0.08	0.00	0.08
AB	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00
AC	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
BB	-0.56	0.00	0.00	0.00	0.08	0.00	0.00	1.00	0.00	0.08
BC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00
CC	-0.56	0.00	0.00	0.00	0.08	0.00	0.00	0.08	0.00	1.00

C^* *is the concentration of* NH_4Cl-NH_4OH, $C^\#$ *is the concentration of APDC*

The *R*-squared statistic indicated that the model is fitted explains 33.56% of the variability in the current response. In addition, the correlation matrix in Table 3 shows the extent of the confounding amongst the effects. This is a perfectly orthogonal design showing a diagonal matrix with 1's on the diagonal and 0's off on the diagonal. All the non-zero terms of the diagonal imply that the estimates of the effects corresponding to rows and columns were correlated. Herein there are three pairs of effects with non-zero correlations; however they are less than 0.5. Furthermore, using the main effect plot, we evaluated the individual variables relative to current as it was more susceptible to changes in any of the selected experimental factors. Overall, the analysis depicted in Figure 2a shows a higher response at pH 8.0, whereas the 5.0 mM concentration of APDC shows the least response.

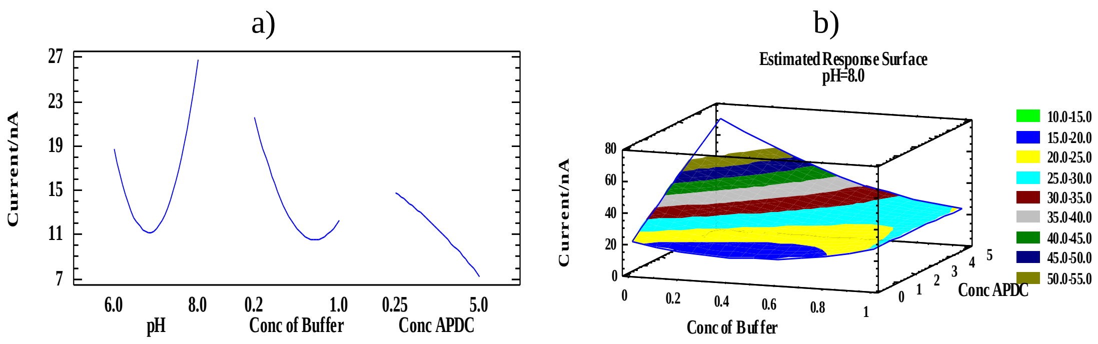


Figure 2. (a) Effect of individual parameters to current response; **(b)** Surface plot of current response at pH 8.0 and simultaneous variation of concentration of the NH_4Cl-NH_4OH and APDC.

On the other hand, the current response of the surface plot, shown in Figure 2b, is significantly affected by the concentration of the NH_4Cl-NH_4OH and APDC. Therefore, current response due to chromium changed between 23 to 70 nA as a result of varying the concentration of APDC. Interestingly, the highest response was obtained at minimal concentration of the NH_4Cl-NH_4OH and the highest concentration of the APDC for the selected range. For this purpose, the optimum values obtained over the selected range were found to be; pH 8.0, 0.2 mM NH_4Cl-NH_4OH and 5.0 mM APDC.

3.2. Foreign ion effect

To test the selectivity of the proposed method, a possible effect of the presence of other ions in the sample solution was investigated. Therefore, in order to discriminate the interferences from other foreign species under optimized experimental conditions, chromium (III) and (VI) concentrations were fixed at 0.5 and 0.05 µM respectively by changing the amount of different foreign ions. A closer inspection of Table 4 suggests that a large number of ions have no considerable effect on the speciation and simultaneous determination of the chromium (III) and (VI) ions in the wastewater samples.

Table 4. Tolerance levels of foreign ions for the speciation and determination of chromium (III) and (VI) under optimized experimental conditions

Index	Foreign Ions	Tolerance limits (nmol L^{-1})	Index	Foreign Ions	Tolerance limits (nmol L^{-1})
1	$Pb(NO_3)_2$	1×10^6	13	Mg^{2+}	15×10^4
2	$MnSO_4$	1×10^6	14	Ni^{2+}	10×10^4
3	$FeCl_3$	9×10^5	15	Cu^{2+}	30×10^4
4	$CuCl_2$	7×10^5	16	K^+	25×10^5
5	$CaCl_2$	1×10^5	17	Na^+	15×10^5
6	$Co(NO_3)_2$	1×10^6	18	Pb^{2+}	20×10^5
7	KCl	5×10^6	19	Ni^{2+}	25×10^5
8	$Ni(NO)_3$	7×10^5	20	Ca^{2+}	5×10^4
9	NaCl	1×10^6	21	Ba^{2+}	25×10^5
10	NH_4VO_3	1×10^5	22	Al^{3+}	10×10^5
11	Zn^{2+}	25×10^4	23	Ag^+	15×10^4
12	Fe^{3+}	25×10^4			

3.3. Figures of merit

Chromium (III) and (VI) were speciated and simultaneously determined under the optimized experimental conditions presented in Table 5. The regression equations and the correlation coefficients were $y = 0.8818x-0.695$, $y = 1.1081x-0.43$ and 0.9997, 0.9997 for chromium (III) and (VI) respectively. The LOD and LOQ were found to be 0.0841 and 0.0276 µg L^{-1} respectively.

To obtain good reproducibility of the developed method in terms of signals, five successive runs with APDC/NH_4Cl-NH_4OH buffer of pH 8.0 were carried out with the same solutions. The repeatability was evaluated by performing five determinations with the same solution resulting in the percentage relative standard deviations (%RSD n = 5) of ±1.24.

Table 5. Optimum parameters obtained from the Box-Behnken experimental design over the indicated region

Factor	Low	High	Optimum
pH	6.0	8.0	7.94
C^*	0.2	1.0	0.20
$C^\#$	0.25	5.0	5.0 0

C is the concentration of NH4Cl-NH4OH;*
C# is the concentration of APDC

3.4. Method validation

The developed method was validated using synthetic samples prepared by spiking 20 mL of laboratory tap water with solutions of chromium (III) and (VI) solution (1.0 and 4.0 μg L^{-1}). From the prepared water sample, 5.0 mL was then transferred into the polarographic cell containing 10.0 mL of deionised water, 3.0 mL of NH_4Cl-NH_4OH buffer (0.2 mM, pH 8.0) and 2.0 μL of mM APDC, and the determination of chromium (VI) was carried out using catalytic differential pulse polarography. The concentrations of chromium (III) and (VI) were determined as per the procedure described in section 2.4. Figure 3 shows the differential pulse polarograms for the speciation and determination of chromium (VI).

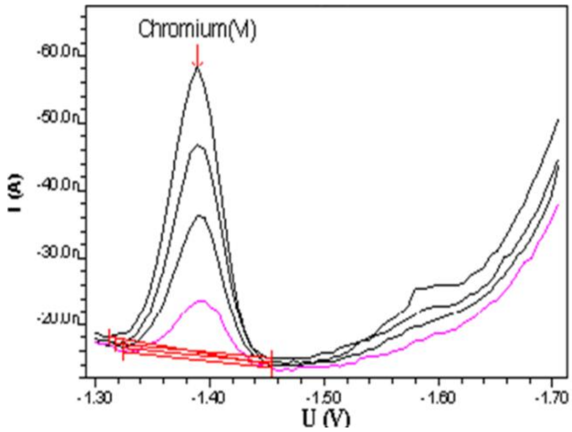


Figure 3. Voltammogram for the speciation and determination of chromium (III) and (VI) (Conditions= pH: 8.0, NH_4Cl-NH_4OH: 5 mM, APDC: 0.2 mM)

3.5. Analytical applications

Accuracy studies were performed using the proposed procedure, by collecting wastewater samples from different industrial areas around Durban, South Africa. Recovery studies of chromium

(III) and (VI) were investigated using standard addition method, due to low concentration (below detection limits of the instrument) of analyte in the wastewater samples.

Table 6. Analytical data for the simultaneous determination of chromium (III) and (VI) in wastewater samples spiked with chromium (III) and (VI)

Sample		Chromium(III)		Chromium(VI)	
	Added (µg L^{-1})	Found (µg L^{-1})	Recovery±RSD*	Found (µg L^{-1})	Recovery±RSD*
Site-1	5.0	4.84	96.80±1.85	4.95	99.0±1.33
Site-2	10.0	9.45	94.50±1.47	9.79	97.90±1.24
Site-3	15.0	14.59	97.26±1.65	14.90	99.30±1.50

**Relative standard deviation for five individual determinations*

In the case of chromium (VI) analysis, the standard addition method was based on the measurements obtained from the same solution before and after addition of chromium (VI). Where as in the case of chromium (III) determination, it was added to the new portion of analyzing samples and voltammogram was recorded as depicted in Table 6. As can be seen, the percentage recoveries and reproducibility of chromium (III) and (VI) reveal the acceptable accuracy and precision. This method was also found to be sensitive, selective, specific, reliable and rapid and may be successfully applied for the speciation and simultaneous determination of chromium (III) and (VI) APDC as complexing agent using Box-Behnken design coupled with catalytic-differential pulse polarographic technique in various water samples of environmental importance as compared to the other techniques reported in the literature (see Table 7).

It is interesting to highlight the fact that reference materials for chromium speciation obtainable up to now have considered very simple matrix and the procedure validated using such material does not include chemomatrics approach. It is also clear that most of the work done doesn't focus particularly on speciation with electrochemical measurements.

Table 7. Brief review of electrochemical methods reported on the simultaneous determination of chromium (III) and chromium (VI)

Method	Ligand	Detection Limits		Matrix	Citation
		Cr(III)	Cr(VI)		
DPAdSV	PCV and HEDTA	NR	NR	Mineral, Tap and tannery wastewater	[14]
CAdSV	DTPA	NR	80.0 µM	Tannery wastewater	[15]
AdSV	PGR	0.00096 µM	0.00096 µM	Sea water	[17]
DPP	-	0.3 µM	1.0 µM	River water	[21]

IP	di(2-hydroxy phenylimino) ethane	[§]0.14 µM [¢]0.54 µM	NR	Soil extract, Waste waters and CMR	[79]

Table 7. Continues ……..

Method	Ligand	Detection Limits		Matrix	Citation
		Cr(III)	Cr(VI)		
Chrono-amperometric	Tyrosinase /$SPC_{TTF}E$ and GOx/ $SPC_{Pt}E$	[π]2.0 ±0.2 µM	90.5 - 7.6 nM	Tap and Waste water	[80]
DPV	NaNO+ MES + DTPA	NR	0.2 nM	Spiked river water and soil samples	[81]
DPV	NR	NR	0.3 nM	Unspiked synthetic sea water and soil samples	[82]
CAdSV	DTPA	-	0.07 nM	CRM and Spiked river water	[83]
DPCSV	-	-	[€] 80.0 nM [₱]40.0 nM [₴]10.0 nM	Black tea	[84]
Box-Behnken design -CDPP	APDC	1.617 nM	1.617 nM	Wastewater samples	Present work

Analysis was performed in [§] *static mode and* [¢] *on-line mode in citation [79].* [π] *in citation [80] was obtained in the presence of Cr (VI) as a matrix. In the citation [84], the LODs were obtained for different buffers: € for Septonex®, ₱ for CTAB and ₴ for CPB.*

NR=Not reported, CAdSV=Catalytic adsorptive stripping voltammetry, AdSV=Adsorptive Stripping Voltammetry, DPAdSV=Differential pulse adsorptive stripping voltammetry, DPCSV=Differential pulse cathodic stripping voltammetric, DPV= Differential pulse voltammetry, DTPA=Diethylenetriaminepentaacetic acid, PCV=Pyrocatechol violet, HEDTA= N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid, PGR= Pyrogallol red, DHPE= di(2-hydroxyphenylimino)ethane, IP=Injection potentiometry, GOx=Glucose oxidase, SPC=Screen printed electrode, TTF=Tetrathiafulvalene, MES= Morpholinoethanesulfonic acid, APDC= Ammonium piperidine dithiocarbamate, CMR= Certified reference materials

4. CONCLUSION

The developed catalytic differential pulse polarographic technique using APDC as complexing agents was successfully employed for the speciation and simultaneous determination of chromium (III) and (VI), without a separate step. A significant aspect of this method is that it makes the speciation analysis much easier, lowers its costs and shortens analysis time. In this regard experimental design was convenient to evaluate the statistical significance of the variation in current responses due to factor variations and optimization of the related parameters. In contrast to chromium (III), lower detection limits for chromium (VI) along with the wide linear range offers greater opportunity for real and routine speciation applications.

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