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Statistical design-principal component analysis optimization of a multiple response procedure using cloud point extraction and simultaneous determination of metals by ICP OES

Marcos A. Bezerra [a,b], Roy E. Bruns [c], Sergio L.C. Ferreira [a,*]

[a] *Universidade Federal da Bahia, Instituto de Química, Grupo de Pesquisa em Química Analítica,*
Campus Universitário de Ondina, Salvador, Bahia 40170-290, Brazil
[b] *Universidade Estadual do Sudoeste da Bahia, Departamento de Química e Exatas,*
Laboratório de Química Analítica, Campus de Jequié, Jequié, Bahia 45200-190, Brazil
[c] *Universidade Estadual de Campinas, Instituto de Química, 13083-970 Campinas, São Paulo, Brazil*

Abstract

A procedure has been developed for the simultaneous determination of traces amounts of Cd, Cr, Cu, Mn, Ni and Pb from saline oil-refinery effluents and digested vegetable samples using inductively coupled plasma optical emission spectrometry (ICP OES). The procedure is based on cloud point extraction (CPE) of these metals as 2-(bromo-2-pyridylazo)-5-diethyl-amino-phenol (Br-PADAP) complexes into a micellar phase of octylphenoxypolyethoxyethanol (Triton X-114). Optimization of the procedure was performed by response surface methodology (RSM) using a Doehlert design. Principal components (PC) were used to simplify the multiple response analysis. A response surface for the first PC score is useful in determining the optimum conditions for the Cd, Cr, Cu, Mn and Pb determinations whereas the second PC is highly correlated with the Ni response. Improvement factors of 22, 36, 46, 25, 65 and 39, along with limits of detection ($3\sigma_B$) of 0.081, 0.79, 0.38, 0.83, 0.28 and 0.69 $\mu g\,L^{-1}$, and precision expressed as relative standard deviation (%R.S.D., $n = 8$, 20.0 $\mu g\,L^{-1}$) of 1.5, 2.2, 3.5, 2.6, 2.5 and 2.5 were achieved for Cd, Cr, Cu, Mn, Ni and Pb, respectively. The accuracy was evaluated by spike tests in oil-refinery effluent samples and analysis of a vegetable certified reference material (NIST 1571, orchard leaves). Results found were in agreement with certified values.
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1. Introduction

Cloud point extraction (CPE) is a separation and preconcentration procedure that has been extensively applied to trace metal ion determinations in several matrices. Major advantages are its low cost, simple experimental procedure, high preconcentration factors and environmental and personal safety characteristics. The CPE procedure is based on the following phenomenon: an aqueous solution of the surfactant separates into two isotropic phases if some condition such as temperature or pressure is changed or if an appropriate substance is added to the solution.

The surfactant solution separates because it attains the cloud point. At this point, the original surfactant solution separates into a surfactant phase of small volume, which is rich in the surfactant and contains the analyte trapped in micellar structures and a bulk diluted aqueous phase. CPE is an impressive alternative to conventional solvent extraction because it produces a high preconcentration factor when an analyte passes from a large volume of matrix solution to a reduced micellar phase volume [1,2].

Several atomic spectrometric techniques have been used for metal determination in micellar phase, such as, molecular absorption spectrophotometry [3] flame atomic absorption spectrometry (FAAS) [4–11], inductively coupled plasma mass spectrometry (ICP-MS) [12,13], graphite furnace atomic absorption spectrometry (GF AAS) [14–17] and inductively

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* Corresponding author. Fax: +55 71 3235 5166.
E-mail address: slcf@ufba.br (S.L.C. Ferreira).

coupled plasma optical emission spectrometry (ICP OES) [18–22].

It is relatively simple to find the optimum conditions for a single response using experimental designs. However, the researcher confronts more complex problems when trying to simultaneously optimize the determination of several responses.

The simplest strategy adopted for these cases is the visual inspection of the surfaces obtained for each response. If the number of significant factors allows the graphical visualization of the adjusted models and if the number of responses is not too large, the surfaces can be overlapped and the optimum conditions found by inspection [23]. However if the optimum conditions of each response differ a compromise solution must be found since changes in the levels of a factor that improves some responses will have negative and/or neutral effects on the others. Derringer and Suich [24] propose the use of a desirability function for simultaneous multiple response optimization that has been already applied to the solid-phase extraction of 18 organochlorine and nine organophosphorus pesticides [25]. An alternative approach using principal component analysis (PCA) is investigated here. PCA is a chemometric tool that has been extensively used for classification [26], pattern recognition [27] and multivariate calibration [28]. Principal component loadings identify responses whose variabilities as a function of the experimental factors being manipulated by the investigator are highly correlated. Principal component scores allow a significant reduction in the number of response surfaces to be analyzed. In this way a greater understanding of the optimization procedure can be attained. Here this chemometric technique and statistical design were used to optimize the simultaneous cloud point extraction of six metals.

An analytical procedure consisting of separation and preconcentration using cloud point extraction was developed to allow the simultaneous extraction and determination of trace amounts of Cd, Cr, Cu, Mn, Ni and Pb from saline oil-refinery effluents and digested vegetable samples using inductively coupled plasma optical emission spectrometry (ICP OES). The developed procedure is based on cloud point extraction of the studied metals as 2-(bromo-2-pyridylazo)-5-diethyl-amino-phenol (Br-PADAP) complexes into micellar media of octylphenoxypolyethoxy ethanol (Triton X-114) surfactant. Response surface methodology (RSM) using Doehlert designs was applied to optimize the procedure [29]. Principal component analysis was used to identify similar response surfaces and simplify the optimization procedure.

2. Experimental

2.1. Instrumentation

A Varian (Mulgrave, Australia) Vista simultaneous inductively coupled plasma optical emission spectrometry instrument with axial viewing and a charge coupled device (CCD) detector was used. The spectrometer was operated in the transient signal acquisition mode. A cyclonic spray chamber and a concentric nebulizer were used. The metal determinations in the micellar phase were carried out under manufacturer's recommended con-

ditions for power (1.2 kW), plasma gas flow (15.0 L min^{-1}), auxiliary gas flow (1.5 L min^{-1}), nebulizer gas flow (0.7 L min^{-1}), and also nebulizer pressure (200 kPa). Emission intensity scan duration was 60 s. The analytical wavelengths (nm) chosen were: Cd II (226.502), Cr II (267.7160), Cu I (327.395), Mn II (257.610), Ni II (230.299) and Pb II (220.353).

A system of sample introduction formed by a manual valve model Rheodyne 5041 (Cotati, CA, USA) connected to a peristaltic pump Alitea C-6 XV (Stockholm, Sweden), equipped with Tygon tubes was used to impel the micellar phase to a sampling loop of 100 μL. After filling the loop with the micellar phase, the sample was driven to the nebulizer of the spectrometer by the peristaltic pump. A Janetzki T 32 Centrifuge (Berlin, Germany) was used to accelerate the separation of the aqueous and micellar phases. Microwave equipment for domestic use (Panasonic, model "Inverter") was used for heating the solutions submitted to cloud point extraction. A Digimed DM20 (São Paulo) pHmeter was used to measure pH values.

2.2. Reagents and solutions

All reagents were of analytical grade unless otherwise stated. Ultrapure water was obtained from an EASY pure RF (Barnstedt, Dubuque, IA, USA). Nitric acid and ethanol were of Suprapur quality (Merck). Laboratory glassware was kept overnight in 10% nitric acid solution. Before use, the glassware was rinsed with deionized water and dried in a dust free environment.

Cadmium, chromium, copper, manganese, nickel and lead solutions were prepared by diluting 1000 μg mL^{-1} standard solutions (Merck) with 1% hydrochloric acid solution.

A 0.025% (w/v) solution of 5-Br-PADAP [2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol] in 3.2% (w/v) Triton X-114 solution was prepared by dissolving 0.025 g of the compound (Merck) in ethanol and 3.2 g of surfactant. The volume was completed to 100.0 mL with ethanol.

A buffer solution of pH 9 was prepared by mixing 25 mL of 0.4 mol L^{-1} TRIS [tris(hydroxymethylaminomethane)] and 2.5 mL of 0.4 mol L^{-1} HCl. Then the pH was adjusted with 0.5 mol L^{-1} HCl and the volume completed to 100 mL with distilled-deionized water.

A 5% (w/v) NaCl solution (Merck) obtained by dissolving 5 g of salt in 100.0 mL of distilled-deionized water, was used to facilitate the separation of the micellar phase from the aqueous phase.

A HNO$_3$ (1:1 v/v) diluent solution was used to decrease the micellar phase viscosity.

2.3. Sample treatment

2.3.1. Saline oil-refinery effluents

The samples of oil-refinery effluents were filtered using a membrane (pore diameter of 0.45 μm) through a vacuum system after sampling to remove suspended particulate material. Later, samples were acidified to pH 2 with nitric acid and stored at 6 °C.

2.3.2. Vegetable and certified samples

About 0.25 g of the material was treated with 4 mL 1:1 (v/v) HNO_3 and 0.1 mL H_2O_2 and maintained for 12 h in a Teflon container. Then the container was put in a pressurized system and thermal heating was carried out in an oven at 110 °C for 24 h. After cooling to room temperature, these solutions were adjusted until pH 9 with 3 mol L^{-1} NaOH before its volume was transferred to a 50.0 mL volumetric flask and its volume completed.

2.4. Optimized procedure for cloud point extraction and ICP OES determination

Buffer solution (0.55 mL, pH 9.0) and 0.5 mL of an alcoholic solution constituted by Triton X-114 3.2% (w/v) and Br-PADAP 0.025% (w/v) were added to the sample (50.0 mL). Then 1 mL of 5% NaCl solution (w/v) was added as a "salting out" agent. The solution was taken to a microwave oven at 10% maximum power to avoid the risk of sample loss. The separation of the micellar phase from aqueous phase was accelerated by centrifugation of the solution for 15 min at 2500 rpm. The solution was then placed in an ice bath for 15 min. The micellar phase becomes viscous and the aqueous phase must be discarded by inverting the container. In the last step, 0.4 mL of a HNO_3 (1:1 v/v) solution was added to the separated micellar phase. After addition of nitric acid, 100 μL of the micellar phase was introduced into the spectrometer through a sampling system and the analytical signals (emission intensities expressed as counts per second) was registered in the transient mode.

2.5. Optimization strategy

The analytical procedure was optimized using response surface methodology (RSM). A Doehlert experimental design for three variables was applied to the system with the objective of localizing the experimental conditions that provide the highest responses.

The pH and irradiation time (IT) variables of the solution for the microwave treatments were studied at five levels. The final concentration of the buffer (BC) was studied at three levels. Table 1 shows the design matrix corresponding to the necessary experiments for optimization of these variables with laboratory values along with coded ones in parentheses.

Thus, the pH variable was studied in the 3.0–9.0 range. The irradiation time of the sample solution for microwave treatment was studied from 2 to 10 min whereas the final concentration of the buffer was varied from 0.0004 to 0.0040 mol L^{-1}.

Experimental data were processed using the Statistica® program [30]. All experiments, necessary for the optimization process, were carried out with a standard solution for Cd, Cr, Cu, Mn, Ni and Pb concentrations of 5.0 μg L^{-1}.

To facilitate the simultaneous optimization of the six metal responses, principal component analysis (PCA) was used with the objective of reducing the number of responses to be analyzed.

3. Results and discussion

Table 1 also contains the emission intensities for the Cd, Cr, Cu, Mn, Ni and Pb metal ions. Two principal components explain 95% of the total variance of the six metal ion emission intensity data. The first principal component explains 78.4% of the total variance and is given by the expression

$$PC_1 = -0.83(Cd) - 0.98(Cr) - 0.98(Cu) - 0.93(Mn)$$
$$- 0.65(Ni) - 0.90(Pb)$$

where the individual metal intensities are indicated in parentheses. High metal intensities are seen to correspond to large negative PC_1 scores. The second PC accounts for 16.6% of the total variance and its scores are predominantly determined by the Ni ion intensities

$$PC_2 = -0.49(Cd) + 0.02(Cr) - 0.08(Cu)$$
$$- 0.33(Mn) + 0.71(Ni) + 0.35(Pb).$$

Table 1
Doehlert design and intensity responses for CPE ICP OES procedure optimization

| Experiment	IT (min)	pH	BC ($\times 10^{-4}$ mol L^{-1})	Emission intensity (counts per second)					
				Cd	Cr	Cu	Mn	Ni	Pb
1	10 (1)	6.0 (0)	22 (0)	2493.37	4170.19	4884.03	9664.98	**4524.69**	**1967.91**
2	8 (0.5)	4.5 (−0.5)	40 (0.707)	2335.56	3598.05	4408.24	8475.72	3604.95	1821.25
3	8 (0.5)	4.5 (−0.5)	4 (−0.707)	2326.83	3533.80	4308.91	8282.62	3451.96	1868.67
4	8 (0.5)	7.5 (0.5)	40 (0.707)	**4274.74**	**4489.56**	**5881.62**	18087.42	4122.62	**2039.05**
5	8 (0.5)	7.5 (0.5)	4 (−0.707)	**3989.24**	**4364.44**	**5805.71**	17931.94	4011.63	**2000.29**
6	6 (0)	3.0 (−1)	22 (0)	2203.06	3220.27	3362.42	6469.92	2533.04	1515.58
7A	6 (0)	6.0 (0)	22 (0)	2321.98	4049.24	4813.43	10907.44	**4228.27**	**2032.43**
7B	6 (0)	6.0 (0)	22 (0)	2531.62	4112.08	4828.07	10129.58	**4114.23**	2016.89
7C	6 (0)	6.0 (0)	22 (0)	2429.90	4072.67	4753.22	10894.94	**4145.50**	**2011.02**
8	6 (0)	9.0 (1)	22 (0)	**5155.81**	**4641.53**	**5929.67**	20045.43	3884.77	**2032.51**
9	4 (−0.5)	4.5 (−0.5)	40 (0.707)	2451.57	3413.32	4361.15	6608.74	3413.80	1754.59
10	4 (−0.5)	4.5 (−0.5)	4 (−0.707)	2410.91	3467.84	4272.82	6464.46	3260.67	1752.68
11	4 (−0.5)	7.5 (0.5)	40 (0.707)	**3248.73**	**4354.08**	**5375.44**	16304.62	3354.67	**2049.55**
12	4 (−0.5)	7.5 (0.5)	4 (−0.707)	**3197.34**	**4207.27**	**5217.73**	15843.54	3305.60	**1999.52**
13	2 (−1)	6.0 (0)	22 (0)	2319.66	3434.93	4479.25	6993.34	3495.73	1809.87
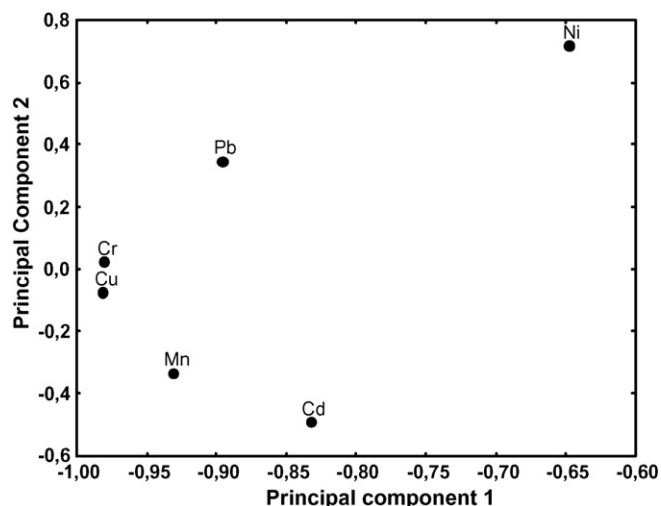
Fig. 1. Loading plot for first and second principal components for data of Table 1.

High Ni ion intensities result in large positive PC_2 scores with smaller negative score contributions from the Cd and Mn ion intensities and a smaller positive one from the Pb intensities. Fig. 1 shows the loading graph for these metal ion intensities. The Cd, Cr, Cu, Mn and Pb variables are grouped together indicating they probably have similar optimization characteristics. On the other hand, the Ni ion has significantly more positive loadings on both PCs indicating favorable operating conditions that are different from those of the other ions.

The principal component score graph is given in Fig. 2. Three groups of score points can be clearly seen. The group of points representing experiments 4, 5, 8, 11 and 12 fall in the lower left-hand corner of the graph. Since they have large negative PC_1 scores their experimental settings should be adequate for maximizing the Cd, Cr, Cu, Mn and Pb ion intensities. This is borne out by inspection of the values in Table 1. Results from these experiments are given in bold face for the Cd, Cr, Cu, Mn and Pb ions. For the first four of these metal ions all the bold face values are significantly much larger than the values obtained from all the other experiments. This trend is not so clear for Pb
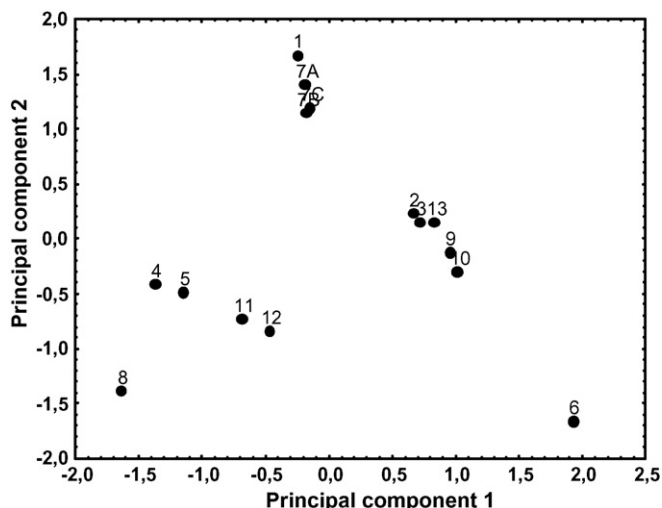
although results for experiments 4, 5, 8, 11 and 12 are among its highest intensities.

The second group is tightly clustered involving experiments 1, 7A, 7B and 7C. The scatter of points for experiments 7A, 7B and 7C provide a measure of experimental error since they represent triplicate experiments at the center point of the experimental design. These points have large positive scores on the second PC and their experimental settings should be appropriate for maximizing the Ni ion intensity. This can be verified in Table 1 where the bold face values for Ni from experiments 1, 7A, 7B and 7C are seen to be larger than all of the other values for Ni except the one from experiment 4. Its intensity value of 4123 is slightly larger than the reading for experiment 7B of 4114 but is smaller than the values for experiments 1, 7A and 7C. It should be remembered that the Pb intensity has a positive loading on the second PC. Hence the intensities of experiments 1, 7A, 7B and 7C can be expected to correspond to relatively large Pb intensities. This is indeed the case as can be seen in Table 1. Their intensities are very similar to those of the experiments in the lower left-hand group of Fig. 2 and are larger than the Pb intensities of those in lower right-hand cluster. This group of experiments has positive first PC scores and negative or close-to-zero scores on the second PC. These experiments result in clearly the lowest intensity values in Table 1 for all the metal ions.

The response surface model for the first PC score obtained from the regression analysis as a function of the coded factor levels is

$$PC_1 = - \underset{(\pm 0.01)}{0.18} - \underset{(\pm 0.01)}{0.51} (IT) - \underset{(\pm 0.01)}{1.77} (pH) - \underset{(\pm 0.01)}{0.10} (BC)$$
$$+ \underset{(\pm 0.02)}{0.46} (IT)^2 + \underset{(\pm 0.02)}{0.32} (pH)^2 - \underset{(\pm 0.02)}{0.13} (BC)^2$$
$$- \underset{(\pm 0.03)}{0.39} (IT)(pH) + \underset{(\pm 0.02)}{0.00} (IT)(BC) - \underset{(\pm 0.02)}{0.12} (pH)(BC)$$

Standard errors for the model coefficients are given in parentheses below the corresponding model coefficient. Although terms involving all three experimental factors are significant variations in IT and pH result in the largest changes in the score values.

This is confirmed in the analysis of variance (ANOVA) presented in Table 2. F values for terms involving only IT or pH are much larger than those for BC. Furthermore the calculated F value for lack of fit of 3.22 is much lower than the 95% confidence critical value showing that the model describes the experimental points adequately. Its R^2 value adjusted for numbers of degrees of freedom is 0.9992.

The response surface for the PC_1 scores as a function of IT and pH for the intermediate buffer level $(22 \times 10^{-4} \, M)$ is shown in Fig. 3a. Since the model cross terms involving the buffer concentration are small one can expect a similar shape for the response surfaces at other buffer levels. The response surface shows that the PC_1 scores and hence the intensities for Cd, Cr, Cu, Mn and Pb are very sensitive to changes in the pH. High pH values (7.5 and 9.0) result in low PC_1 scores and high intensity readings for these five metal ions. On the other hand the irradiation time is not seen to be so important although intermediate times appear to be more favorable owing to the positive

Fig. 2. Score plot for first and second principal components for data from Table 1.

Table 2
Analysis of variance (ANOVA) for (a) first and (b) second principal component scores

	SS	d.f.	MS	F	p
(a) First principal					
(1) IT (L)	1.04733	1	1.04733	3092.24	0.000323
IT (Q)	0.25876	1	0.25876	763.98	0.001306
(2) pH (L)	12.50715	1	12.50715	36927.22	0.000027
pH (Q)	0.12077	1	0.12077	356.58	0.002793
(3) BC (L)	0.03765	1	0.03765	111.17	0.008875
BC (Q)	0.01444	1	0.01444	42.65	0.022655
IT (L) by pH (L)	0.07633	1	0.07633	225.36	0.004408
IT (L) by BC (L)	0.00000	1	0.00000	0.01	0.928685
pH (L) by BC (L)	0.01357	1	0.01357	40.05	0.024071
Lack of fit	0.00328	3	0.00109	3.22	0.245669
Pure error	0.00068	2	0.00034		
SS total	14.00000	14			
(b) Second principal					
(1) IT (L)	1.26965	1	1.269652	66.9639	0.014607
IT (Q)	0.13702	1	0.137019	7.2267	0.114993
(2) pH (L)	0.22165	1	0.221648	11.6901	0.075928
pH (Q)	9.20576	1	9.205759	485.5296	0.002053
(3) BC (L)	0.02437	1	0.024375	1.2856	0.374477
BC (Q)	2.26260	1	2.262598	119.3338	0.008276
IT (L) by pH (L)	0.00237	1	0.002367	0.1249	0.757593
IT (L) by BC (L)	0.00217	1	0.002165	0.1142	0.767579
pH (L) by BC (L)	0.00066	1	0.000664	0.0350	0.868802
Lack of fit	0.70347	3	0.234490	12.3675	0.075732
Pure error	0.03792	2	0.018960		
SS total	14.00000	14			

SS, sum of squares; d.f., degree of freedom; MS, mean square.

significant curvature (0.46 ± 0.02) indicated by the squared IT term in the above model equation. Optimum conditions for the Cd, Cr, Cu and Mn are thus close to those for experiment 8, i.e. pH of 9.0, irradiation time of 6 min and a buffer concentration of 22×10^{-4} M. These conditions also seem favorable for the Pb ion intensities.

For the second PC score the response surface model is

$$PC_2 = \underset{(\pm 0.08)}{1.25} + \underset{(\pm 0.07)}{0.56\,(IT)} - \underset{(\pm 0.07)}{0.24\,(pH)} + \underset{(\pm 0.07)}{0.08\,(BC)}$$
$$- \underset{(\pm 0.13)}{0.34\,(IT)^2} - \underset{(\pm 0.13)}{2.77\,(pH)^2} - \underset{(\pm 0.14)}{1.57\,(BC)^2}$$
$$- \underset{(\pm 0.19)}{0.07\,(IT)(pH)} - \underset{(\pm 0.14)}{0.05\,(IT)(BC)} - \underset{(\pm 0.14)}{0.03\,(pH)(BC)}$$

Again the IT and pH terms appear to be the most important in the model although variations in the buffer concentrations are not negligible owing to its very significant squared term. The ANOVA presented in Table 2 shows that only the linear irradiation time and quadratic pH and BC terms are significant at the 95% confidence level with $p < 0.05$. The calculated F lack of fit value of 12.4 is less than the 95% confidence level critical value of 19.2 indicating that the model adequately describes the experimental data ($p > 0.05$). The fact that all the interaction term coefficients are insignificant at the 95% confidence level simplifies the analysis of the above model. The optimization of any one of the three factors does not depend on the levels of the other two. Since the linear irradiation time term
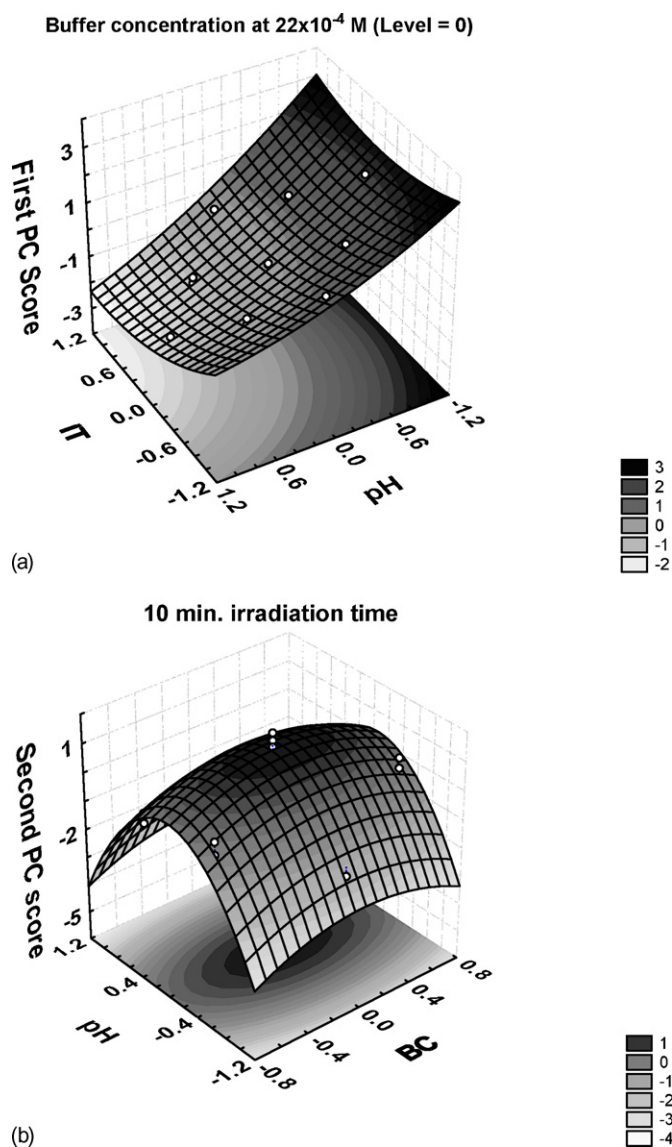

(a)

(b)

Fig. 3. Response surface for: (a) pH vs. IT of the first principal component scores and (b) pH vs. BC of the second principal component scores.

is positive this factor should be kept at its high level to maximize PC_2 and the Ni ion intensity. Also the negative quadratic terms for pH and BC indicate the existence of a maximum PC_2 value in the investigated region. A graph of the response surface as a function of coded pH and BC values indeed shows that a maximum exists close to the (0.0) pH and BC center point values (pH 6.0 and BC = 22×10^{-4} M). Maximum PC_2 scores and Ni ion intensities are those expected for irradiation times of 10 min, pH of 6.0 and a 22×10^{-4} M buffer concentration.

In summary, Cd, Cr, Cu and Mn emission intensities can be maximized using the experimental conditions of experiment 8, i.e. 6 min irradiation time, pH of 9.0 and a 22×10^{-4} M buffer concentration. Under these conditions the Pb intensity is close to attaining a maximum value. If Ni determination were a priority this irradiation time could be increased to 10 min, the pH lowered to 6.0 and the buffer concentration maintained constant

Table 3
Analytical features of CPE ICP OES procedure obtained using optimized values

Parameter	Cd		Cr		Cu		Mn		Ni		Pb	
	WPC	CPE	WPC	CPE	WPC	CPE	WPC	CPE	WPC	CPE	WPC	CPE
A	33685	745.15	15746	567.85	15640	718.95	57902	1447.6	5118.2	332.02	4139.3	161.3
B	3014.5	1260.5	2181.7	5003.5	2707.9	2105.8	4800.4	14738	2127.5	3207.4	2754.9	1339.5
R^2	0.9997	0.9983	0.9993	0.9989	0.9998	0.9986	0.9986	0.9983	0.9990	0.9971	0.9988	0.9974
LOD (μg L^{-1})	5.9	0.081	21.2	0.79	17.4	0.38	20.8	0.83	22.6	0.28	37.1	0.69
LOQ (μg L^{-1})	19.2	0.27	70.7	2.64	57.8	1.27	69.4	2.77	75.3	0.93	123.7	2.32
%R.S.D. (20 μg L^{-1})	–	1.5	–	2.2	–	3.5	–	2.6	–	2.5	–	2.5
IF	–	22	–	36	–	46	–	25	–	65	–	39

WPC, determination without preconcentration; CPE, determination after preconcentration using the cloud point extraction procedure; A, slope of analytical curve; B, intercept of analytical curve; LOD, limit of detection; LOQ, limit of quantification; %R.S.D., relative standard deviation (repeatability to 5.0 μg L^{-1}, $N = 8$); IF, improvement factor.

Table 4
Determination of Cu, Pb, Cd, Cr, Ni and Mn for the certified reference material (Orchard leaves, NIST 1571) using the developed procedure

Sample	Metal	Certified values (μg g^{-1})	Determined values (μg g^{-1})[a]
	Cu	12 ± 1	12.3 ± 0.3
	Pb	45 ± 3	50 ± 8
Orchard leaves	Cd	0.11 ± 0.01	0.10 ± 0.01
(NIST 1571)	Cr	2.6 ± 0.3	2.7 ± 0.2
	Ni	1.3 ± 0.12	1.26 ± 0.05
	Mn	91 ± 4	92 ± 1

[a] Mean ± standard deviation ($n = 3$).

at 22×10^{-4} M, which are the conditions for experiment 1 given in Table 1.

3.1. Analytical features

Table 3 presents some analytical characteristics of the developed procedure and allows a comparison with the procedure without the preconcentration stage of the cloud point. The concentrations of the standards for construction of the analytical curves for the CPE ICP OES procedure were in the 5.0–30.0 μg L^{-1} range ($N = 6$). Without the preconcentration stage, the concentrations of the calibration standards were in the 0.1–5.0 μg mL^{-1} ($N = 5$) range. Improvement factors, defined as the ratio of the slopes of the analytical curves before and after the preconcentration, were in the 22–65 range.

The limits of detection (LOD) and quantification (LOQ) of the developed procedure were calculated taking eight consecutive measurements of the blank signal and using the background equivalent concentration (BEC) [25], according to the expressions presented below:

$$BEC = \frac{\text{average blank intensity}}{\text{slope of the analytical curve}},$$

$$LOD = \frac{3 \times BEC \times R.S.D.}{100}, \quad LOQ = \frac{10 \times BEC \times R.S.D.}{100}$$

3.2. Accuracy and application

To evaluate the accuracy of the developed procedure, the studied metals were determined for a certified reference material (Orchard leaves, NIST 1571) supplied by the National Institute of Standards and Technology. The results are presented in Table 4.

The method proposed was applied for analysis of three oil-refinery saline effluent samples. The concentrations achieved varied between 2.6 and 5.4 μg L^{-1} for copper, 2.5 and 3.5 μg L^{-1} for lead, 0.27 and 0.41 μg L^{-1} for cadmium, 2.6 and 3.2 μg L^{-1} for chromium, 1.2 and 2.1 μg L^{-1} for nickel and 26.2 and 32.0 μg L^{-1} for manganese. Spike tests were also executed to evaluate the accuracy in these samples. Results show that recoveries between 90 and 109% were found using the proposed procedure. It can be seen in Table 5. These results show that this procedure can be satisfactorily used for these kinds of matrices.

Table 5
Spike test and determination of Cu, Pb, Cd, Cr, Ni and Mn from oil-refinery saline effluents using the developed procedure ($N = 3$)

Sample	Metal	Added ($\mu g\, L^{-1}$)	Found[a] ($\mu g\, L^{-1}$)	Recovery (%)
1	Cu	–	2.6 ± 0.2	–
		5.0	7.1 ± 0.3	90
	Pb	–	<LOQ	–
		10.0	10.9 ± 1.0	109
	Cd	–	<LOQ	–
		10.0	10.8 ± 1.0	108
	Cr	–	<LOQ	–
		10.0	9.7 ± 0.5	97
	Ni	–	1.2 ± 0.2	–
		5.0	6.4 ± 1.1	104
	Mn	–	26.2 ± 2.1	–
		10.0	36.8 ± 1.2	106

[a] Mean $\pm$ standard deviation ($n = 3$).

4. Conclusions

The application of a Doehlert design and principal component analysis (PCA) led to finding optimized conditions for the simultaneous extraction of Cu, Pb, Cd, Ni, Cr and Mn using cloud point extraction.

The behavior of the Cu, Pb, Cd, Cr and Mn ion intensities as a function of irradiating time, pH and buffer concentration are essentially the same as described by the first principal component. The Ni ion intensity behaves differently and is described by the second principal component. Both components describe 95% of the total data variance.

Highest responses for the simultaneous extraction of Cu, Cd, Cr and Mn are expected when this is done at pH 9.0 with a buffer TRIS 0.0022 mol L^{-1} concentration and a 6 min microwave irradiation time of the sample. These conditions should result in a near maximum intensity of Pb and an intermediate intensity value for Ni. Maximum Ni intensity values are expected by increasing the irradiation time to 10 min lowering the pH to 6.0 and maintaining the buffer concentration constant at 22×10^{-4} M, which are the conditions for experiment 1 given in Table 1.

The applicability of the proposed procedure to samples of digested vegetable leaves was demonstrated by the determination of Pb, Cd, Ni, Cr and Mn in a certified reference material (Orchard leaves, NIST 1571). The values found by the proposed procedure in the determination of these metals were in agreement with the certified values of the reference material. The applicability of this procedure to metal determination in saline effluents was verified by spike tests. The recoveries of the metals added to the samples were between 90 and 109%.

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