



# Simultaneous determination of Cu, Pb, Cd, Ni, Co and Zn in bioethanol fuel by adsorptive stripping voltammetry and multivariate linear regression



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## HIGHLIGHTS

- Simultaneous determination of six trace metals in bioethanol fuel samples.
- Multivariate determination of six analytes with a voltammetric method.
- MLR aided by variable selection provided excellent quantitative predictions.
- Peak alignment and baseline correction are crucial for DPAdSV signal processing.

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## ABSTRACT

In this work it is proposed the one-voltammogram simultaneous determination of Cu(II), Pb(II), Cd(II), Ni(II), Co(II), and Zn(II) at ppb and sub-ppb levels in bioethanol fuel samples by adsorptive stripping voltammetry. Mixed ligands were employed providing stripping peaks for all analytes at the same voltammetric scan under optimized conditions. To perform the determination, multivariate linear regression aided by successive projections algorithm (SPA-MLR) was evaluated. The voltammograms were preprocessed with the asymmetric least squares (AsLS) baseline correction algorithm and the *icoshift* peak alignment tool. An excellent analytical performance was obtained, despite the inherent complexity of the simultaneous determination, with average apparent recovery =  $96\% \pm 16\%$ , and better prediction results in comparison with the benchmark method of partial least squares (PLS).

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## 1. Introduction

The relevance of biofuels in the global energetic matrix is already established and its quality control becomes crucial. To ensure the quality of biofuels is necessary to establish quality standards, aiming to set limits on levels of contaminants that will not affect the emissions, the engine performance and integrity, as well as the safety in transportation and handling.

Ethanol produced on a large scale from sugarcane industries is the biofuel most widely used in Brazil. As automotive fuel, bioethanol can be used in its hydrated form, or added in its anhydrous form to type A gasoline to formulate type C gasoline in the proportion of 25% [1]. An important control performed for bioethanol is

the control of trace metals. The presence of metals in biofuels may occur due to the absorption of metals from the soil by the plant used as raw material, as well as they can be incorporated during the processes of production, storage and transportation [2]. Trace metals in bioethanol can be an indicative of metallic corrosion during storage and their presence can accelerate oxidation processes resulting in the formation of gums [3]. Therefore, it is necessary to determine and control the concentration of these impurities in order to reduce the undesirable effects of these contaminants.

In general, the analysis of trace metals in ethanol fuel has traditionally been carried out by means of spectroanalytical techniques: flame atomic absorption spectrometry (FAAS) [4,5], electrothermal atomic absorption spectrometry (ETAAS) [6,7] and inductively coupled plasma mass spectrometry (ICP-MS) [8]. Electroanalytical techniques such as stripping voltammetry can also be used in the determination of trace metals due to their low detection limits and possibility of simultaneous determinations. In this sense, a

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few works can be found in the literature, always covering the determination of Cu(II), Cd(II), Pb(II), and Zn(II) by anodic stripping voltammetry (ASV), either in ethanol fuel or gasoline [3,9–11]. However, the electroanalytical simultaneous determination of more metals in fuels is not reported, mainly due to the increasing difficulties directly related to the increasing number of complex mutual interference processes such as, for example, the formation of intermetallic compounds, which could lead to considerable errors of the estimated concentrations [12,13].

In this sense, the use of chemometrics presents itself as a powerful analytical alternative to circumvent the described drawbacks, generate accurate calibration models, expanding the number of analytes determined simultaneously, and also providing the one-voltammogram simultaneous determination of multiple analytes in the presence of unknown interferences, even if the selectivity is poor. A collection of representative works regarding the application of chemometrics to the simultaneous determination of metals by voltammetric techniques [14–32] reveals that stripping voltammetry and partial least squares regression (PLS) is the preferred combination of techniques. However, despite the several papers published, this is not an established methodology. The papers point out to a limit on the number of analytes determined simultaneously, with only one work describing the determination of up to five analytes, and at ppm level [29]. The expected natural evolution of this analytical approach to allow the determination of more analytes at ppb and sub-ppb levels has not been reported.

In this paper, we present a method for the simultaneous determination of six metals – Cu(II), Pb(II), Cd(II), Ni(II), Co(II) and Zn(II) – at ppb and sub-ppb levels by differential pulse adsorptive stripping voltammetry (DPAdSV) and multivariate calibration. The determination of more elements was accomplished by using a suitable combination of ligands – nonspecific and or selective – in a mixed-ligand operation [33–35], providing the formation of surface-active redox chelates with all metals, not necessarily at sufficient separation of the peak potentials, which were properly resolved by chemometrics. Multivariate calibration models were built based on multiple linear regression (MLR), which generally requires the use of a tool for selecting non-redundant variables in the independent variables matrix in order to minimize multicollinearity problems [13]. The variable selection tool used in this work was the successive projections algorithm (SPA) [36]. For comparison, partial least squares (PLS) regression models were obtained from full voltammograms. The method was applied to the determination of trace metals in commercial bioethanol fuel samples. At the best of our knowledge, only a few works can be found regarding the analysis of fuels with chemometrics methods, but none comprising multivariate calibration [37,38]. In addition, this is the first application of SPA-MLR to adsorptive stripping voltammetric data.

## 2. Material and methods

### 2.1. Reagents, solutions and samples

High-purity deionized water ( $R \geq 18 \text{ M}\Omega \text{ cm}$ ) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all aqueous solutions. In addition, all reagents were analytical grade and used without further purification. Stock solutions ( $1000 \text{ mg L}^{-1}$  cadmium, copper, lead, nickel, cobalt, or zinc in 2% (v/v)  $\text{HNO}_3$ ) were purchased from Sigma–Aldrich and used to prepare secondary  $10 \text{ mg L}^{-1}$  stock solutions. The ligands dimethylglyoxime (DMG, Merck) and 8-hydroxyquinoline (oxine, Sigma–Aldrich) were employed in order to form complexes with all analytes. DMG is known to form complexes with Ni(II), Co(II), and Zn(II) [39,40], while oxine chelates Cu(II), Cd(II), and Pb(II)

[33,35]. DMG was prepared as a  $0.10 \text{ mol L}^{-1}$  solution in ethanol (Sigma–Aldrich) and oxine was prepared as a  $0.10 \text{ mol L}^{-1}$  solution in  $0.15 \text{ mol L}^{-1}$  HCl. HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid sodium salt) buffer (Sigma–Aldrich) was prepared at pH 9 and  $0.01 \text{ mol L}^{-1}$ .

Hydrated ethyl alcohol fuel samples were purchased at local fuel stations. Samples were pretreated by heating 100 mL of ethanol fuel at  $110 \text{ }^\circ\text{C}$  until complete evaporation, and the residue recovered with 0.5% (v/v)  $\text{HNO}_3$  in a 25 mL volumetric flask [5].

### 2.2. Instrumentation and analytical procedure

Electrochemical recordings were conducted using a potentiostat Autolab PGSTAT 101 (Metrohm Autolab, The Netherlands) coupled to a polarographic module 663 VA Stand (Metrohm Autolab, The Netherlands), and controlled by Nova 1.7 software in a conventional three-electrode electrochemical cell. The reference and auxiliary electrodes were an Ag/AgCl (saturated KCl) and a Pt wire, respectively. A hanging mercury drop electrode (HMDE) was used as working electrode. All measurements were carried out at room temperature ( $25 \pm 1 \text{ }^\circ\text{C}$ ). Mercury is practically innocuous at conditions employed in this work. Moreover, the amount of Hg used for one determination is dramatically reduced with HMDE. However, it is advisable careful use of Hg given its known toxicity.

To perform the voltammetric analysis, a  $100 \mu\text{L}$  aliquot of the pretreated ethanol fuel sample was placed in the voltammetric cell with  $10 \text{ mL}$  of  $0.01 \text{ mol L}^{-1}$  HEPES buffer at pH 9,  $0.50 \text{ mmol L}^{-1}$  DMG, and  $0.01 \text{ mmol L}^{-1}$  oxine. This mixture was purged with ultrapure  $\text{N}_2$  for 60 s. The optimized DPAdSV parameters were

**Table 1**  
Experimental design. Concentration values in  $\mu\text{g L}^{-1}$ .

Exp.	Cu	Pb	Cd	Ni	Co	Zn
<i>Calibration set</i>						
1	1.80	6.20	3.40	1.80	0.30	3.60
2	1.80	1.04	0.56	2.90	0.20	6.50
3	0.33	1.04	5.60	1.20	0.60	3.60
4	0.33	10.4	2.30	2.90	0.30	2.10
5	3.30	4.10	5.60	1.80	0.20	2.10
6	1.30	10.4	3.40	1.20	0.20	5.10
7	3.30	6.20	2.30	1.20	0.50	6.50
8	1.80	4.10	2.30	2.40	0.60	5.10
9	1.30	4.10	4.50	2.90	0.50	3.60
10	1.30	8.30	5.60	2.40	0.30	6.50
11	2.60	10.4	4.50	1.80	0.60	6.50
12	3.30	8.30	3.40	2.90	0.60	0.65
13	2.60	6.20	5.60	2.90	0.05	5.10
14	1.80	10.4	5.60	0.29	0.50	0.65
15	3.30	10.4	0.56	2.40	0.05	3.60
16	3.30	1.04	4.50	0.29	0.30	5.10
17	0.33	8.30	0.56	1.80	0.50	5.10
18	2.60	1.04	3.40	2.40	0.50	2.10
19	0.33	6.20	4.50	2.40	0.20	0.65
20	1.80	8.30	4.50	1.20	0.05	2.10
21	2.60	8.30	2.30	0.29	0.20	3.60
22	2.60	4.10	0.56	1.20	0.30	0.65
23	1.30	1.04	2.30	1.80	0.05	0.65
24	0.33	4.10	3.40	0.29	0.05	6.50
25	1.30	6.20	0.56	0.29	0.60	2.10
<i>Validation set</i>						
1	0.80	9.00	1.40	2.00	0.30	4.20
2	2.80	7.10	1.40	1.40	0.30	5.60
3	2.20	2.50	4.90	0.70	0.40	2.80
4	1.60	5.10	3.80	2.60	0.50	1.40
5	2.20	9.00	2.80	0.70	0.10	5.60
6	2.80	5.10	3.80	1.40	0.50	2.80
7	0.80	7.10	4.90	2.00	0.30	1.40
8	2.20	9.00	2.80	0.70	0.30	1.40
9	0.80	7.10	1.40	2.00	0.50	2.80
10	2.80	5.10	4.90	2.60	0.10	4.20

**Table 2**

Figures of merit of the univariate analytical curves obtained for each analyte.

Analyte	Linear range ( $\mu\text{g L}^{-1}$ )	R	Sensitivity ( $\text{nA } \mu\text{g}^{-1} \text{L}$ )	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )
Cu(II)	0.03–6.40	0.99	20.8	0.0086	0.026
Pb(II)	1.00–10.4	0.98	6.07	0.19	0.66
Cd(II)	0.11–5.60	0.99	20.0	0.033	0.11
Ni(II)	0.03–5.90	0.99	37.9	0.0087	0.029
Co(II)	0.05–2.90	0.99	67.3	0.014	0.044
Zn(II)	0.33–6.50	0.98	4.49	0.098	0.33

potential step ( $\Delta E_s$ ) = 2 mV; deposition potential ( $E_d$ ) =  $-0.8$  V; deposition time ( $T_d$ ) = 30 s; pulse amplitude ( $\Delta E_p$ ) = 75 mV; modulation time ( $T_p$ ) = 40 ms; scan rate ( $\nu$ ) = 20  $\text{mV s}^{-1}$ . Solutions were stirred during deposition step. After 5 s of equilibration period, voltammograms were recorded by applying a negative-going potential scan from  $-0.3$  V to  $-1.3$  V. The analysis was performed three times using a new drop and the average voltammogram was used in the chemometric analysis. Since certified reference materials were unavailable for trace elements in bioethanol, results were evaluated by apparent recovery tests.

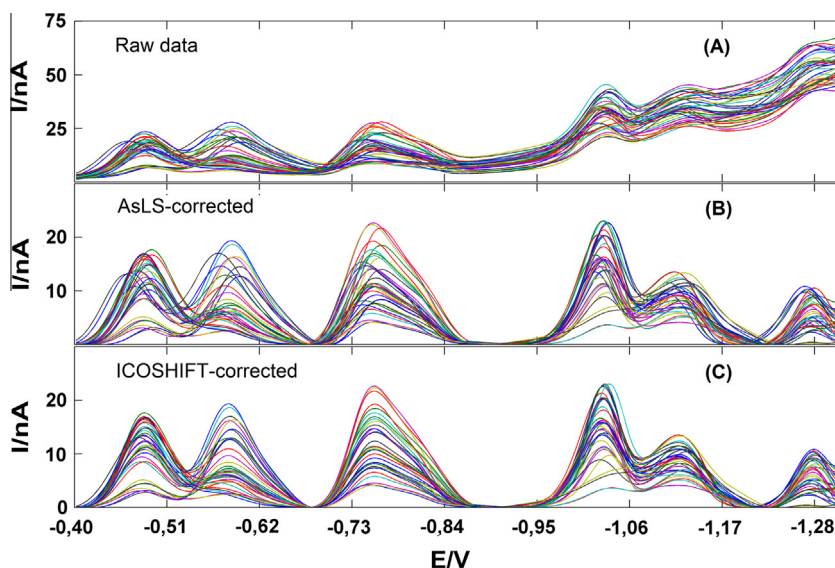
### 2.3. Experimental design and data analysis

Calibration set was obtained following a Brereton design [41] and comprised twenty-five samples (aqueous solutions) containing Cu(II), Pb(II), Cd(II), Ni(II), Co(II), and Zn(II) at five different concentration levels for each metal. The use of such a design allows

the construction of the calibration set with a smaller number of experiments containing the same representative distribution of a set built with a large number of samples, making it possible to routine laboratory analysis and determination of multiple analytes [41]. Validation set comprised ten samples prepared with random concentrations of the analytes at four levels. Each sample was analyzed in triplicate (independent replicates) and the resulting average voltammogram was used in the calculations. Table 1 shows the concentration levels employed for each metal in the calibration and validation sets.

To perform baseline correction we used the asymmetric least squares (AsLS) algorithm [42] that combines smoothing and asymmetric weighting of deviations related to the smoothed signal to estimate a polynomial function, which is subtracted from the original voltammogram. To work properly, multivariate calibration algorithms require the same underlying processes must be associated to the same variables in all the samples. Therefore, if the stripping peaks in the voltammogram show non-systematic shifts, this could impair the predictive power of multivariate models [13]. However, peak shifting could be minimized with an alignment algorithm. This tool adds an offset in the axis of potentials such that the position of the peaks shall correspond with those in a reference voltammogram. In this work, peak alignment was carried out by using the *icoshift* algorithm [43].

AsLS and *icoshift* algorithms, as well as SPA-MLR calibration, were performed in MATLAB<sup>®</sup> R2008a environment (The MathWorks<sup>™</sup> Inc., USA). For the sake of comparison, PLS regression was performed on the full voltammograms with the software Pirouette<sup>®</sup> 4.0 (Infometrix Inc., USA). The choice of the number of



**Fig. 1.** (A) Voltammograms obtained for calibration and validation sets. (B) Voltammograms after baseline correction by AsLS algorithm. (C) Baseline-corrected voltammograms after peak alignment with the *icoshift* tool.

**Table 3**

Figures of merit obtained for multivariate calibration models applied to the simultaneous voltammetric determination of Cu, Pb, Cd, Ni, Co, and Zn.

Analyte	PLS					SPA-MLR				
	RMSEP ( $\mu\text{g L}^{-1}$ )	N	$R_{\text{pred}}$	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )	RMSEP ( $\mu\text{g L}^{-1}$ )	N	$R_{\text{pred}}$	LOD ( $\mu\text{g L}^{-1}$ )	LOQ ( $\mu\text{g L}^{-1}$ )
Cu(II)	0.22	2	0.96	0.020	0.060	0.16	4	0.98	0.093	0.36
Pb(II)	0.63	6	0.95	0.61	1.8	0.86	2	0.95	0.53	1.6
Cd(II)	0.31	2	0.93	0.036	0.11	0.34	2	0.93	0.074	0.22
Ni(II)	0.15	2	0.99	0.0086	0.026	0.16	3	0.98	0.036	0.12
Co(II)	0.050	4	0.94	0.0087	0.026	0.030	2	0.98	0.0053	0.016
Zn(II)	0.37	7	0.95	0.47	1.4	0.38	6	0.94	0.63	1.9

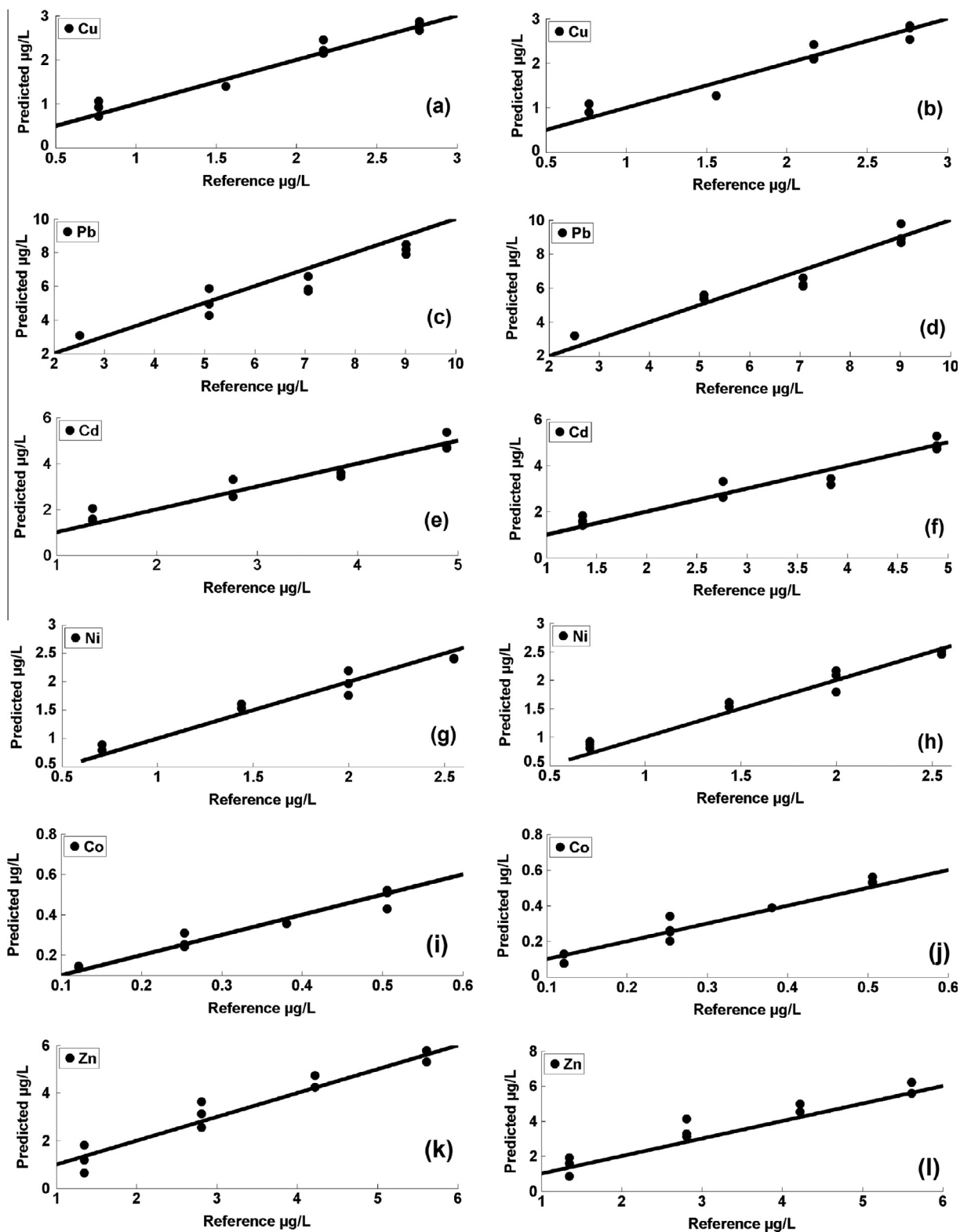


Fig. 2. Prediction versus reference values of validation samples. SPA-MLR: (a), (c), (e), (g), (i), and (k). PLS: (b), (d), (f), (h), (j), and (l).

latent variables and real variables for PLS and SPA-MLR models, respectively, was performed using test series and a validation set. All calculations were performed on the blank-subtracted mean-centered voltammograms. The figures of merit correlation coefficient ( $R$ ), root mean square error (RMSE), and number of latent variables or pure variables selected ( $N$ ) were used to evaluate the quality of the models.

The limits of detection (LOD) and quantification (LOQ) were calculated for the univariate and multivariate calibration models. For univariate models LOD and LOQ were calculated using the equation  $\text{LOD (or LOQ)} = ks/b$ , where  $k = 3.3$  for LOD, and  $k = 10$  for LOQ,  $s$  is the standard deviation of 10 measured blank samples, and  $b$  is the slope of the univariate analytical curve. For multivariate models, LOD and LOQ were calculated using a similar

**Table 4**  
Apparent recovery obtained from the analysis of ethanol fuel samples by proposed method.

Sample	Metal	Added ( $\mu\text{g L}^{-1}$ )	PLS		SPA-MLR	
			Recovery ( $\mu\text{g L}^{-1}$ )	Recovery (%)	Recovery ( $\mu\text{g L}^{-1}$ )	Recovery (%)
Sample A	Cu	0.30	0.10	33	0.27	90
	Pb	3.00	3.23	108	2.80	93
	Cd	2.00	2.50	125	1.69	85
	Ni	1.00	1.21	121	1.09	109
	Co	0.50	0.48	96	0.51	102
	Zn	2.00	1.73	87	2.48	124
Sample B	Cu	0.30	0.19	63	0.30	100
	Pb	3.00	1.49	50	4.00	133
	Cd	2.00	2.45	123	1.68	84
	Ni	1.00	1.47	147	1.09	109
	Co	0.50	0.51	102	0.50	100
	Zn	2.00	0.84	42	1.90	95
Sample C	Cu	0.30	0.16	53	0.23	78
	Pb	3.00	1.33	44	2.91	97
	Cd	2.00	2.07	104	1.92	96
	Ni	1.00	0.68	68	0.76	76
	Co	0.50	0.33	66	0.40	80
	Zn	2.00	0.48	24	1.42	71
Average recovery				81 $\pm$ 36	96 $\pm$ 16	

equation:  $\text{LOD (or LOQ)} = k \sigma \|b_k\|$ , where  $k = 3.3$  for LOD, and  $k = 10$  for LOQ,  $\sigma$  is the standard deviation of the net analytical signal (NAS), and  $\|b_k\|$  is the Euclidean norm of the vector of regression coefficients estimated from the multivariate calibration model for analyte  $k$  [44]. The idea of using NAS is extracting the part of the signal that is directly related to the concentration of the analyte of interest [45,46]. The NAS is obtained using an approach based on the PLS regression vector [47] to calculate the NAS vector  $r^*$ :

$$r^* = b(b^T b)^{-1} b^T r \quad (1)$$

In the Eq. (1),  $r$  ( $j \times 1$ ) is the voltammogram vector ( $j$  is the number of variables) and  $b$  ( $j \times 1$ ) is the regression vector of the PLS regression. In this work, were used the norms of 15 NAS vectors ( $r$ ) corresponding to 15 voltammograms ( $r$ ) of the reference signal.

### 3. Results and discussion

Initially, after optimization of DPAdSV parameters, we investigated the individual electroanalytical behavior of the six metallic cations in the range  $1 \times 10^{-10}$  mol L $^{-1}$  up to  $1 \times 10^{-7}$  mol L $^{-1}$ . This study is indispensable once the chemometric tools used in the simultaneous determination are based on linear relationships between independent and dependent variables. The stripping voltammograms were characterized by a peak current centered at  $-0.48 \text{ V} \pm 0.04 \text{ V}$ ,  $-0.59 \text{ V} \pm 0.02 \text{ V}$ ,  $-0.73 \text{ V} \pm 0.02 \text{ V}$ ,  $-1.00 \text{ V} \pm 0.01 \text{ V}$ ,  $-1.10 \text{ V} \pm 0.03 \text{ V}$ , and  $-1.26 \text{ V} \pm 0.02 \text{ V}$  for Cu(II), Pb(II), Cd(II), Ni(II), Co(II), and Zn(II), respectively. Linear relationships between peak current and concentration were observed with correlation coefficients higher than 0.980 (Table 2).

The univariate models were subjected to an ANOVA test and it was observed no evidence of lack of fit for the linear models at 95% confidence level. Table 2 shows the figures of merit of the univariate analytical curves obtained for each analyte. The linear ranges determined in this step were used to establish the minimum and maximum concentrations employed in the experimental design presented in Table 1. It was established that the values should cover at least one decade of concentration. Therefore, the simultaneous determination of Cu(II), Pb(II), Cd(II), Ni(II), Co(II), and Zn(II) was performed at the ranges  $0.33\text{--}3.30 \mu\text{g L}^{-1}$ ,  $1.00\text{--}10.4 \mu\text{g L}^{-1}$ ,

$0.56\text{--}5.60 \mu\text{g L}^{-1}$ ,  $0.29\text{--}2.90 \mu\text{g L}^{-1}$ ,  $0.05\text{--}0.60 \mu\text{g L}^{-1}$ , and  $0.65\text{--}6.50 \mu\text{g L}^{-1}$ , respectively.

Fig. 1 presents the voltammograms obtained for calibration and validation sets. The voltammograms exhibit six stripping peaks at potentials similar to those found in the individual analysis. However, important features are also observed such as poor peak resolution, peak shifting, peak overlapping, and baseline changes, resulting in a complex signal. In this sense, a multiple standard addition simultaneous analysis could be a hard task. In fact, the univariate analytical curves obtained after simultaneous standard addition show that, for all analytes, the linearity is obeyed only on a very narrow concentration range (between  $5 \times 10^{-10}$  mol L $^{-1}$  and  $1 \times 10^{-9}$  mol L $^{-1}$ , data not shown). Increasing the concentration, the erratic behavior of peak currents reveals the complex relationship of competition between the complexes formed.

SPA-MLR and PLS models were built independently for each metal. Cross-validation was not appropriate because led to models with overfitting. For this reason, we used an external validation set with 10 samples. The latent variables in PLS models and the real variables selected for SPA-MLR models were chosen based on the lowest root mean square error of prediction (RMSEP), which was estimated from sample concentrations on validation set.

Table 3 presents the figures of merit RMSEP,  $R_{pred}$ , number of latent variables and real variables ( $N$ ) used in SPA-MLR and PLS models, as well as LOD and LOQ. It is observed that both multivariate calibration approaches have similar analytical performances. Importantly, the use of raw or partially corrected data (voltammograms uncorrected for baseline and peak alignment, Fig. 1) did not provide satisfactory results. Fig. 2 shows the plots of predicted versus reference concentrations for validation set samples obtained by SPA-MLR and PLS models. The observation of these plots confirms the satisfactory values of  $R_{pred}$  and small RMSEP shown in Table 3. For each analyte, the lowest RMSEP values correspond to relative errors of prediction of 10–13%, sufficient to accept the method as accurate.

The developed method was applied to bioethanol fuel samples. Three samples were collected and analyzed in triplicate with the obtained models and the concentrations found were below or close to the lower limit of linear response range. Therefore, the fortification of the samples with the six metals was carried out and the apparent recoveries obtained show a good agreement between



the added and found concentrations. Table 4 shows the average results of apparent recovery using SPA–MLR and PLS models.

As one can observe, SPA–MLR outperformed PLS once the average recovery for prediction with SPA–MLR was  $96\% \pm 16\%$ , and for PLS was  $81\% \pm 36\%$ . PLS shows as a less precise choice and led to an underestimation of metal concentrations in the analysis of ethanol fuel samples. Thus, although both calibration approaches provided similar performances in the analysis of aqueous solutions, SPA–MLR was more robust to matrix effects when analyzing bioethanol fuel. These superior results can be understood in terms of the fewest variables selected by SPA – mainly at the reduction peak of the metal – avoiding the use of regions of the voltammogram in which the interference could be more intense [48] or with little information about the analyte concentration.

#### 4. Conclusions

In this work, it is demonstrated the application of multivariate calibration to the simultaneous determination of trace levels of six metals by differential pulse adsorptive stripping voltammetry at a hanging drop mercury electrode. The proposed method performs the determination of Cu(II), Pb(II), Cd(II), Ni(II), Co(II) and Zn(II) at ppb and sub-ppb levels by exploring the versatility of mixed ligands and the convenience of chemometric procedures in the analysis of aqueous solutions and bioethanol fuel samples. This method can be expanded to assess the content of more metals by applying the right combination of ligands.

Peak alignment and baseline correction tools were applied in the pretreatment of the voltammograms and proved fundamental to provide adequate prediction results in multivariate calibration. SPA–MLR was evaluated as regression algorithm and presented predictive power similar to PLS in the analysis of aqueous solutions. However, for the analysis of bioethanol fuel samples, SPA–MLR was superior to PLS, presenting correct values of apparent recovery, and showing to be more robust to matrix effects. As one can see, the proposed method is accurate (as confirmed by relative error prediction values and recovery tests), sensitive (LOD for simultaneous determination  $<1 \mu\text{g L}^{-1}$  for all analytes), precise, and can be used as a routine tool for trace metal ions determination in bioethanol samples.

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#### References

- [1] Ambrozini ARP, Kuri SE, Monteiro MR. Corrosão metálica associada ao uso de combustíveis minerais e biocombustíveis. *Quim Nova* 2009;32:1910–6.
- [2] Chaves ES, Lepri FG, Silva JSA, Quadros DPC, Saint-Pierre TD, Curtius AJ. Determination of Co, Cu, Fe, Mn, Ni and V in diesel and biodiesel samples by ETV–ICP–MS. *J Environ Monit* 2008;10:1211–6.
- [3] Tormin TF, Narciso LCD, Richter EM, Muñoz RAA. Batch-injection stripping voltammetry of metals in fuel bioethanol. *Fuel* 2014;30:952–6.
- [4] Korn MGA, Santos DSS, Welz B, Vale MGR, Texeira AP, Lima DC, et al. Atomic spectrometric methods for the determination of metals and metalloids in automotive fuels – a review. *Talanta* 2007;73:1–11.
- [5] De Oliveira MF, Balbo VR, Saczk AA, Okumura LL, Fernandes AP, De Moraes M, et al. Quantitative assay of copper, iron, nickel, and zinc in fuel ethanol samples by flame atomic absorption spectrometry. *Chem Tech Fuels Oil* 2008;44:430–4.
- [6] Saint-pierre TD, Aucélio RQ, Curtius AJ. Trace elemental determination in alcohol automotive fuel by electrothermal atomic absorption spectrometry. *Microchem J* 2003;75:59–67.
- [7] Saint-pierre TD, Maranhão TA, Frescura VI, Curtius AJ. Determination of Cd and Pb in fuel ethanol by filter furnace electrothermal atomic absorption spectrometry. *Quim Nova* 2008;31:1626–30.
- [8] Saint-pierre TD, Maranhão TA, Frescura VI, Curtius AJ. The development of a method for the determination of trace elements in fuel alcohol by electrothermal vaporization–inductively coupled plasma mass spectrometry using external calibration. *Spectrochim Acta B* 2005;60:605–13.
- [9] Munoz RAA, Angnes L. Simultaneous determination of copper and lead in ethanol fuel by anodic stripping voltammetry. *Microchem J* 2004;77:157–62.
- [10] De Oliveira MF, Saczk AA, Okumura LL, Fernandes AP, De Moraes M, Stradiotto NR. Simultaneous determination of zinc, copper, lead, and cadmium in fuel ethanol by anodic stripping voltammetry using a glassy carbon–mercury-film electrode. *Anal Bioanal Chem* 2004;380:135–40.
- [11] Trindade JM, Martiniano LC, Gonçalves VRA, Souza AG, Marques ALB, Baugis GL, et al. Anodic stripping voltammetry coupled with design of experiments for simultaneous determination of  $\text{Zn}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{Pb}^{+2}$ , and  $\text{Cd}^{+2}$  in gasoline. *Fuel* 2012;91:26–32.
- [12] Pinto L, Lemos SG. Comparison of different PLS algorithms for simultaneous determination of Cd(II), Cu(II), Pb(II), and Zn(II) by anodic stripping voltammetry at bismuth film electrode. *Electroanal* 2014;26:299–305.
- [13] Marreto PD, Zimer AM, Faria RC, Mascaro LH, Pereira EC, Frago WD, et al. Multivariate linear regression with variable selection by a successive projections algorithm applied to the analysis of anodic stripping voltammetry data. *Electrochim Acta* 2014;127:68–78.
- [14] Gonzáles MJG, Renedo OD, Martínez MJA. Speciation of antimony by adsorptive stripping voltammetry using pyrogallol. *Talanta* 2007;71:691–8.
- [15] Domínguez O, Arcos MJ. Simultaneous determination of chromium (VI) and chromium (III) at trace levels by adsorptive stripping voltammetry. *Anal Chim Acta* 2002;470:241–52.
- [16] Lastres E, De Armas G, Catusas M, Alpizar J, García L, Cerda V. Use of neural networks in solving interferences caused by formation of intermetallic compounds in anodic stripping voltammetry. *Electroanal* 1997;9:251–4.
- [17] Ensafi AA, Khayamian T, Atabati M. Simultaneous voltammetry determination of molybdenum and copper by adsorption cathodic differential pulse stripping method using a principal component artificial neural network. *Talanta* 2002;57:785–93.
- [18] Henrion A, Henrion R, Henrion G, Scholz F. Application of partial least-squares regression for signal resolution in differential pulse anodic stripping voltammetry of thallium and lead. *Electroanal* 1990;2:309–12.
- [19] Alves GMS, Magalhães JMCS, Soares HMVM. Simultaneous determination of copper(II), lead(II) and zinc(II) at bismuth film electrode by multivariate calibration. *Electroanal* 2001;23:1410–7.
- [20] Cauchi M, Bessant C, Setford S. Simultaneous quantitative determination of cadmium, lead, and copper on carbon-ink screen-printed electrodes by differential pulse anodic stripping voltammetry and partial least squares regression. *Electroanal* 2008;20:2571–7.
- [21] Ensafi AA, Khayamian T, Benvidi A, Mirmomtaz E. Simultaneous determination of copper, lead and cadmium by cathodic adsorptive stripping voltammetry using artificial neural network. *Anal Chim Acta* 2006;561:225–32.
- [22] Garcia JM, Martin E, Jiménez AI, Jiménez F, Arias JJ. Application of partial least squares regression to highly overlapped differential-pulse polarographic peaks. *J Electroanal Chem* 1994;373:235–9.
- [23] Herrero A, Ortiz MC. Piecewise direct standardization method applied to the simultaneous determination of Pb(II), Sn(IV) and Cd(II) by differential pulse polarography. *Electroanal* 1998;10:717–21.
- [24] Antunes MC, Simão JE, Duarte AC. Resolution of voltammetric peaks using chemometric multivariate calibration methods. *Electroanal* 2001;13:1041–5.
- [25] Antunes MC, Simão JE, Duarte AC, Tauler R. Multivariate curve resolution of overlapping voltammetric peaks: quantitative analysis of binary and quaternary metal mixtures. *Analyst* 2002;127:809–17.
- [26] Herrero A, Ortiz MC. Multivariate calibration transfer applied to the routine polarographic determination of copper, lead, cadmium and zinc. *Anal Chim Acta* 1997;348:51–9.
- [27] Chan H, Butler A, Falck DM, Freund MS. Artificial neural network processing of stripping analysis responses for identifying and quantifying heavy metals in the presence of intermetallic compound formation. *Anal Chem* 1997;69:2373–8.
- [28] Sanz MB, Sarabia LA, Herrero A, Ortiz MC. Multivariate analytical sensitivity in the determination of selenium, copper, lead and cadmium by stripping voltammetry when using soft calibration. *Anal Chim Acta* 2003;489:85–94.
- [29] Ni Y, Jin L. Simultaneous polarographic chemometric determination of lead, copper, vanadium, cadmium and nickel. *Chemometr Intell Lab* 1999;45:105–11.
- [30] Ni Y, Kokot S. Does chemometrics enhance the performance of electroanalysis? *Anal Chim Acta* 2008;626:130–46.
- [31] Ni Y. Simultaneous determination of copper, cadmium and nickel by ratio derivative polarography. *Talanta* 1998;47:137–42.
- [32] Ni Y, Wang L. Simultaneous polarographic determination of lead (II) and tin (II) by multivariate calibration. *Anal Lett* 1999;32:2081–9.
- [33] Colombo C, van den Berg CMG. Simultaneous determination of several trace-metals in seawater using cathodic stripping voltammetry with mixed ligands. *Anal Chim Acta* 1997;337:29–40.
- [34] Wang J, Lu J, Wang J, Luo D, Tian B. Simultaneous measurements of trace chromium and uranium using mixed ligand adsorptive stripping analysis. *Anal Chim Acta* 1997;354:275–81.

- [35] Santos-Echeandía J. Direct simultaneous determination of Co, Cu, Fe, Ni and V in pore waters by means of adsorptive cathodic stripping voltammetry with mixed ligands. *Talanta* 2011;85:506–12.
- [36] Soares SFC, Gomes AA, Galvão Filho AR, Araújo MCU, Galvão RKH. The successive projections algorithm. *TRAC-Trend Anal Chem* 2013;42:84–98.
- [37] Pinto L, Lemos SG. Multivariate optimization of the voltammetric determination of Cd, Cu, Pb and Zn at bismuth film. Application to analysis of biodiesel. *Microchem J* 2013;110:417–24.
- [38] Silva AC, Paz JEM, Pontes LFBL, Lemos SG, Pontes MJC. An electroanalytical method to detect adulteration of ethanol fuel by using multivariate analysis. *Electrochim Acta* 2013;111:160–4.
- [39] Moforbos M, Economou A, Voulgaropoulos A. Simultaneous determination of nickel(II) and cobalt(II) by square wave adsorptive stripping voltammetry on a rotating-disc bismuth-film electrode. *Anal Chim Acta* 2004;519:57–64.
- [40] Rezaei B, Rezaei E. Simultaneous determination of trace amounts of nickel, cobalt, and zinc in the wastewater of a galvanic workshop by using adsorptive cathodic stripping voltammetry. *J Anal Chem* 2006;61:262–5.
- [41] Brereton RG. *Chemometrics, data analysis for the laboratory and chemical plant*. 1st ed. USA: John Wiley & Sons; 2003.
- [42] Peng J, Peng S, Jiang A, Wei J, Li C, Tan J. Asymmetric least squares for multiple spectra baseline correction. *Anal Chim Acta* 2010;683:63–8.
- [43] Tomasi G, Savorani F, Engelsen SB. *Icoshift*: an effective tool for the alignment of chromatographic data. *J Chromatogr A* 2011;43:7832–40.
- [44] Oliveira AC, Goicoechea HC, Iñón FA. MVC1: an integrated MatLab toolbox for first-order multivariate calibration. *Chemometr Intell Lab* 2004;73:189–97.
- [45] Lorber A, Faber K, Kowalski BR. Net analyte signal calculation in multivariate calibration. *Anal Chem* 1997;69:1620–6.
- [46] Ferré J, Faber KM. Net analyte signal calculation for multivariate calibration. *Chem Int Lab Syst* 2003;69:123–36.
- [47] Faber KM. Efficient computation of net analyte signal vector in inverse multivariate calibration models. *Anal Chem* 1998;70:5108–10.
- [48] Soares SFC, Galvão RKH, Araújo MCU, Silva EC, Pereira CF, Andrade SIE, et al. A modification of the successive projections algorithm for spectral variable selection in the presence of unknown interferents. *Anal Chim Acta* 2011;689:22–8.