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Experimental designs in the development of a new method for the sensitive determination of cadmium in seawater by adsorptive cathodic stripping voltammetry

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Abstract

A new differential pulse adsorptive cathodic stripping voltammetric (DPAdCSV) method for the direct determination of cadmium at subnanomolar levels in saline waters based on metal complexation with 2-acetylpyridine salicyloylhydrazone (APSH) and subsequent adsorptive deposition onto a hanging mercury drop electrode (HMDE) is presented. A study strategy based on experimental designs has been followed. Operating conditions were improved with exploratory (Plackett–Burman) and surface response (central composite) experimental designs, involving several chemical and instrumental parameters (pH, ligand concentration, pulse amplitude, time interval for voltage step, voltage step, deposition potential and deposition time). Analytical parameters as repeatability, linearity and accuracy were also investigated and a detection limit (DL) of 0.06 nM was achieved which could be lowered by extending the adsorption time. The interference of other metals and major salts present in seawater was also studied. The method was validated with reference water samples: NIST-SRM 1643d and BCR-CRM 505, showing good concordance with the certified values.

Keywords: Cadmium; 2-Acetylpyridine salicyloylhydrazone; Adsorptive cathodic stripping voltammetry; Seawater

1. Introduction

Cadmium is known to be a hazardous environmental pollutant with toxic effects for the living organisms in aquatic ecosystems [1]. Adverse effects of cadmium are produced not only because of its high toxicity even at trace concentrations, but also due to bioaccumulation processes along the food chain. As water plays an important role in nature as carrier, among others, the anthropogenic inputs of cadmium can be easily widespread over the aquatic medium. Therefore, cadmium can stay accessible to living organisms, including microorganisms and microalgae which are at the first steps of the food chain and involved in important biological processes [2].

Several methods have been already developed for the determination of cadmium in water samples. Undoubtedly, analytical techniques like neutron activation analysis (NAA), X-ray fluorescence analysis, but mainly those methods based on atomic absorption and emission spectroscopy are the most widely used for this purpose. Particularly, ETAAS has shown satisfactory detection limits (DL) for the determination of cadmium at nanomolar levels in water samples.

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Unfortunately, the influence of the salt content for the direct determination of metals in samples with saline matrices, as well as the lack of multielemental analysis and the incapacity for speciation studies has reduced their application to saline water samples.

Thus, in recent years, stripping voltammetry techniques, particularly anodic (ASV) and adsorptive cathodic (AdCSV) stripping voltammetry, have shown numerous advantages, including speed of analysis, good selectivity and sensitivity, low costs of instrumentation compared with other techniques, good performance with saline matrices like seawater and estuarine water samples and the possibility of simultaneous analysis of mixtures [3]. Thus, stripping voltammetry has become a valuable tool not only for the determination of trace levels of heavy metals in water samples, but also for speciation studies concerning these metals [4,5].

Because of the great sensitivity enhancement obtained with AdCSV methods, several complexing agents have been studied for the adsorptive collection of complexes with cadmium on the hanging mercury drop electrode (HMDE). It has been previously described the use of oxine [6,7], 1-(2-pyridylazo)-2,7-dihydroxynaphthalene [8], calcein blue [9], glyoxylic acid thiosemicarbazide [10], ω-mercaptocarboxylic acids [11], 2,5-dimercapto-1,3,4-thiadiazole [12] and 2-mercapto-5-phenil-ammino-1,3,4-thiadiazole [13] as complexing agents for the voltammetric determination of cadmium.

In addition, the high sensitivity and selectivity of stripping voltammetric techniques make them very suitable for trace metal speciation studies. Thus, ASV and AdCSV are widely used for dissolved organic/inorganic speciation measurements in seawater. Although ASV methods provide speciation information about the equilibrium concentration principally of free metal ions, AdCSV is used by ligand competition for the determination of labile trace metal fractions and also natural metal complexing ligands [4]. Therefore, the study of other ligands with different complexation capacities, like proposed in this paper, would allow its application in further speciation studies in seawater at different detection windows.

In this field, hydrazones have shown interesting properties for medical [14,15] and analytical [16,17] purposes. Several analytical spectrophotometric methods for the determination of metal ions have been previously developed using aroylhydrazones as analytical reagents [18–26] as well as in liquid–liquid extraction methods as extracting agents of metal ions [27]. In addition, the electrochemical behaviour of certain aroylhydrazones has been also studied, showing interesting characteristics as complexing agents for the determination of metal ions by voltammetry [28].

In this work, we present a new AdCSV method based on a new ligand, 2-acetylpyridine salicyloylhydrazone (APSH), which belongs to the group of aroylhydrazones. Previous works about this ligand, regarding to spectral and metallochromic properties of APSH [29] as well as electrochemical properties by direct current polarography (DCP), differential pulse polarography (DPP) and alternating current polarography (ACP) [30] showed its interesting properties as complexing agent of metal ions. However, nothing has been previously reported about the complexation capacity of APSH in presence of metal ions for the potential use in metal determination by AdCSV. For this purpose, a strategy based on factorial experimental designs has been used not only to achieve a systematic study but also to prove the interesting possibilities of this methodology in voltammetric studies.

2. Experimental

2.1. Apparatus

A Metrohm model 746 VA Trace Analyzer processor with a Metrohm 747 VA Electrode Stand with automated hanging mercury drop electrode were used for the voltammetric measurements. The reference electrode was Ag/AgCl, saturated AgCl, 3 M KCl and the auxiliary electrode was a platinum wire. Solutions were stirred during the purging and deposition steps by a rotating PTFE rod. Obtained scans were evaluated with the Metrohm 746 VA processor. The pH was measured with a Crison model 2001 pH meter and pH measurements were calibrated against pH 4 and 7 standards on the NBS pH scale.

Organic matter was removed from water samples by UV irradiation with a Metrohm model 705 UV Digester in quartz tubes.

Although fortunately, cadmium contamination of samples is unusual in lab work, a strict protocol has been followed in order to avoid contamination of solutions and samples. The 747 VA Electrode Stand was placed inside a clean fume hood, whilst sample handling and preparation of solutions were performed using polyethylene gloves and under a laminar flow hood cabinet Crusair model 9005-FL. Reagent solutions were stored in plastic containers, mainly low density polyethylene (LDPE) and polystyrene flasks. All containers and voltammetric cells were carefully cleaned sequentially, first with detergent, then by rinsing with water and by soaking (24 h) with 2 M HNO_3 (Merck, Germany) and finally with thorough rinses with deionised water.

2.2. Reagents and solutions

APSH was synthesised as previously reported [29] by reaction between 2-acetylpyridine (Sigma–Aldrich, Germany) and 2-hydroxybenzohydrazide (Sigma–Aldrich, Germany) in ethanol medium, and subsequently purified. A 10^{-3} M stock solution of APSH solution in absolute ethanol extra pure (Scharlau, Spain) was prepared weekly and kept at 4 °C in darkness when not in use.

Water used for sample preparation, dilution of the reagents and rinsing purposes was purified by reverse osmosis with a Elix 3 system followed by deionisation with a Milli-Q^{50} system (both from Millipore, USA). A 5% (v/v) ethanol was kept in all measuring solutions to assure the solubility of APSH.

Britton–Robinson buffer solutions were used to investigate pH in the range 2–12. These solutions were prepared according to previously reported [31] using *o*-boric acid, *o*-phosphoric acid, acetic acid and sodium hydroxide of Suprapur grade (Merck, Germany). A constant ionic strength of 1.5 M was maintained in the buffers by adding KCl Suprapur grade (Merck, Germany). Once the pH range was shortened, HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (Sigma–Aldrich, Germany) buffer solutions were used in the range pH 7.0–8.0. All salts and acids were analytical or Suprapur Merck grade.

Hydrogen peroxide 30% Suprapur (Merck, Germany) was added to the samples prior to UV digestion giving a final concentration of 0.02 M. Dissolved oxygen was removed from the studied solutions by purging with water-saturated nitrogen (99.99%) (Air Liquide, Spain).

Metal ions standard solutions were prepared by dissolution of AAS standard solutions of $1000\,mg\,l^{-1}$ (Merck, Germany) with deionised water. In order to preserve metals, a 0.05 M HNO_3 concentration in all metal solutions was kept by the addition of HNO_3 of Suprapur Merck grade.

2.3. Study strategy and procedures

Usual strategies used for the development of new AdCSV methods are based on one-variable studies, being focused on the study of pH and accumulation parameters (potential and time). Although these one-variable studies are useful to describe the main behaviour of a certain voltammetric peak enhancement, a further step in methods development can be done by the assistance of statistical tools, like the experimental designs, because they also provide information about relationships between several variables and about multivariate optimization of selected responses. Thus, the combination of exploratory and optimization designs may lead to achieve a better comprehension not only about the effects on voltammetric responses and trends to optimization of chemical and/or instrumental variables, but also to solve other problems which may affect the adsorptive cathodic stripping voltammetric determination of metals.

Thus, a three-stage strategy has been followed in this study. Firstly, a set of preliminary experiments was performed in order to help to establish the ranges of critical variables. After this, a Plackett–Burman exploratory design was used to select the most significant variables, which were subsequently analysed by using a surface response design. Perhaps, the most interesting point of this procedure is not only to find which are the most important variables and their main trends in order to improve a voltammetric response, but also the versatility which provide all the information. Thus, this procedure is focused on provide not an unique optimum set of experimental conditions for the variables, but a better understanding of the processes, which allows the researcher to modify the method whenever sample conditions change, which is quite common in environmental studies and is a key point in speciation studies.

The experiments were performed with solutions prepared as follows. An aliquot of deionised water was transferred into the voltammetric cell and the pH adjusted to the desired value by the addition of 10% (v/v) of Britton–Robinson buffer for the preliminary experiments or 1 M HEPES solution during the exploratory and surface response designs. The amount of the buffer additions was obtained by studying the evolution of the pH with the volume of added buffer in deionised water solutions containing cadmium(II) and APSH. Then, APSH and cadmium(II) solutions of certain concentrations were also added, giving final concentrations according to the studied values. In order to assure the solubility of APSH, 5% (v/v) final concentration ethanol was also added for each experiment.

Once the solution has been deoxygenated by purging with nitrogen, the stirrer was connected and deposition began according to conditions determined for each experiment. Then, the stirring was stopped and the solution was left settle for an equilibration time of 10 s and after that, a cathodic sweep using differential pulse modulation was recorded. Scans of each experiment were repeated twice with a new hanging drop.

Statistical analysis was performed using The Unscrambler 7.01 [32] for the Plackett–Burman design and Statgraphics Plus 5.1 [33] for the central composite design.

3. Results and discussion

3.1. Mechanism of the electrode reaction

APSH is an aroylhydrazone characterized by the presence of the triatomic group C=N–N, which exhibit two tautomeric structures (Fig. 1). They form coordination compounds through the oxygen atom of either the carbonyl or the enol group, being noteworthy for its remarkable versatility as chelating agent.

In the AdCSV determination of cadmium(II), this metal ion forms a complex with APSH, which subsequently absorbs on the mercury drop electrode during the deposition step. Thus, during the voltammetric scan, the cadmium(II) in the complex is reduced to elemental cadmium, which contributes to the reduction current. Depending on the pH, a peak of cadmium(II)–APSH complex appeared, whilst one peak corresponding to a reduction process of APSH also appeared but at more negative potential. Previous electrochemical studies of APSH have shown that this ligand exhibits reduction peaks up to a maximum of four. It has also been shown that the position of the peaks depend on the pH value, thus indicating the involvement of protons in the reduction processes. Thus, the separation of the cadmium(II)–APSH complex and ligand peaks have been also studied and improved in the preliminary experiments.

3.2. Preliminary experiments

Because of the existence of cathodic peaks for the ligand, not only the complex capacity of APSH with cadmium at different pH values needed to be taken into account, but also the possibility of peaks overlapping between the free APSH peak and the cadmium(II)–APSH complex peak. Thus, the most interesting chemical parameters were found to be pH and metal to ligand concentration ratio.

3.2.1. Influence of pH

A wide range of pH was investigated from pH 2.0 to 12.0. A time-stable cadmium(II)–APSH peak can be obtained without peaks overlapping between pH 7 and 9. Under pH 7 it can clearly be observed peaks overlapping interfering the measurement of the cadmium(II)–APSH peak whilst above pH 9, the peaks of the free ligand, as well as the cadmium(II)–APSH complex showed considerable decreases in the voltammetric responses (Fig. 2). Although the separation between the cadmium(II) and

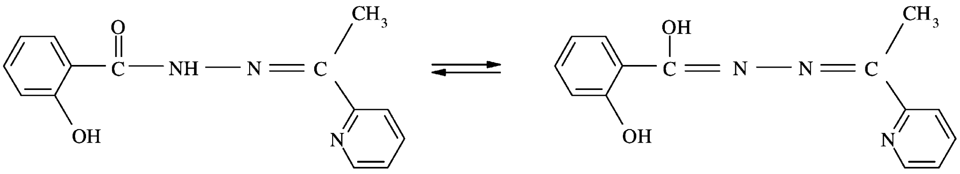

Fig. 1. Tautomeric structures of APSH [29].

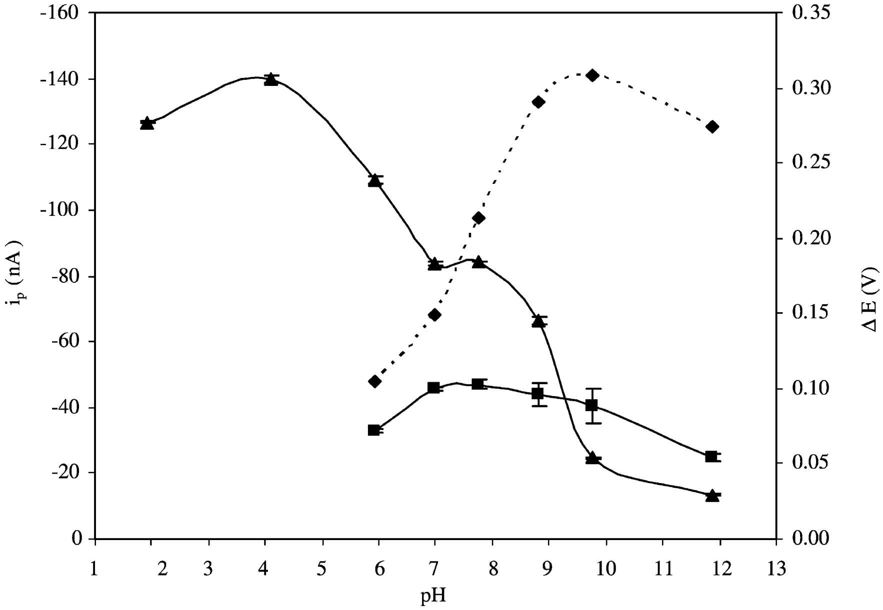

Fig. 2. Influence of pH in peak current of cadmium(II)–APSH complex ((■), solid line) and free APSH ((▲), solid line), and separation between cadmium(II)–APSH complex and free APSH peaks ((◆), dotted line) in Britton–Robinson buffered (10% (v/v)) solutions containing 10^{-5} M APSH and 10^{-6} M cadmium(II). Operating conditions are as follows: DPAdCSV, deposition potential: −0.05 V; deposition time: 60 s; pulse amplitude (*P*): −50 mV; time interval for voltage step (t_s): 0.20 s; voltage step (U_s): 6 mV; sweep rate: 30 mV s^{-1}; rotation speed: 2000 min^{-1}.

free APSH neighbouring peaks was maximum at pH values between 9 and 11, the pH interval 7–9 was chosen as the most convenient for the determination of cadmium(II) because, mainly, at high pH values, hydrolysis reactions of cadmium(II) may cause loss of the analyte. This is the reason why further experiments were performed using 1 M HEPES buffer solution showing good buffer activity from pH 6.8 to 8.2, which is the most common pH range in natural waters.

3.2.2. *Influence of ligand concentration*

In order to establish the complexing behaviour of APSH with cadmium(II) regarding the voltammetric response in CSV of the cadmium(II)–APSH complex, the concentration ratios between cadmium(II) and APSH in solution were also studied at different pH values of the measured Britton–Robinson buffered solutions (pH 7.0, 7.8 and 8.8). In all cases, voltammetric results showed an increase of the cadmium(II)–APSH peak along with an increase of the APSH:Cd concentration ratio up to a metal to ligand ratio of 1:30. Above this M:L ratio, the cadmium(II)–APSH peak did not increase significantly (Fig. 3).

3.2.3. *Effects of varying the deposition potential and time*

The influence of deposition time and potential were also evaluated (Fig. 4). The effect of varying the deposition potential showed a peak enhancement as the potential approached to the characteristic peak potential for the cadmium(II)–APSH complex, being maximum around −0.5 V. Variation of the deposition time showed a linear peak increase until a deposition time of 4 min and non-linear thereafter.

3.3. *Exploratory designs: Plackett–Burman*

A Plackett–Burman design has been used to make a selection of the variables which can be considered significant. For this study, among all the variables that potentially may influence in the development of a method for the determination of cadmium by differential pulse adsorptive cathodic stripping voltammetric

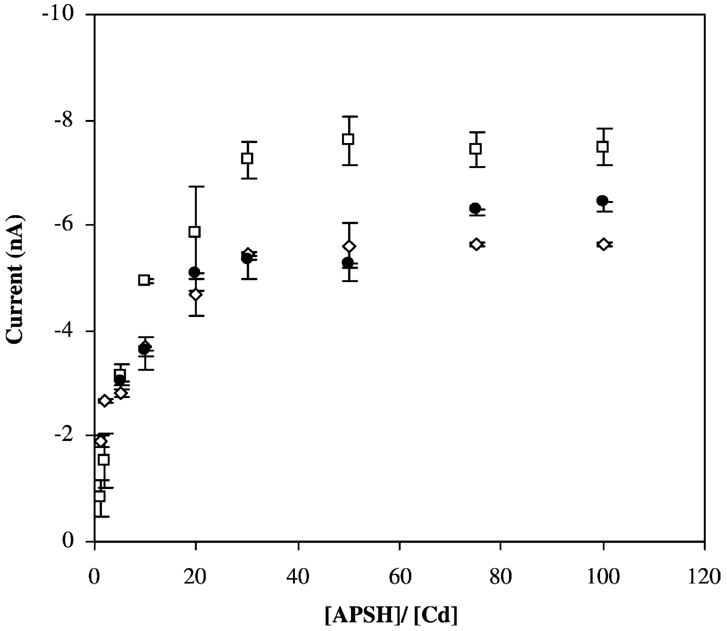

Fig. 3. Effect of varying the APSH concentration on the current of the cadmium(II)–APSH complex peak in Britton–Robinson buffered of pH 7.0 (◇), 7.8 (□) and 8.8 (●) solutions containing 8.9 × 10^{-8} M cadmium(II). Other experimental conditions: differential pulse mode, deposition time and potential: 120 s; −0.05 V; pulse amplitude: −50 mV; potential step: 6 mV.

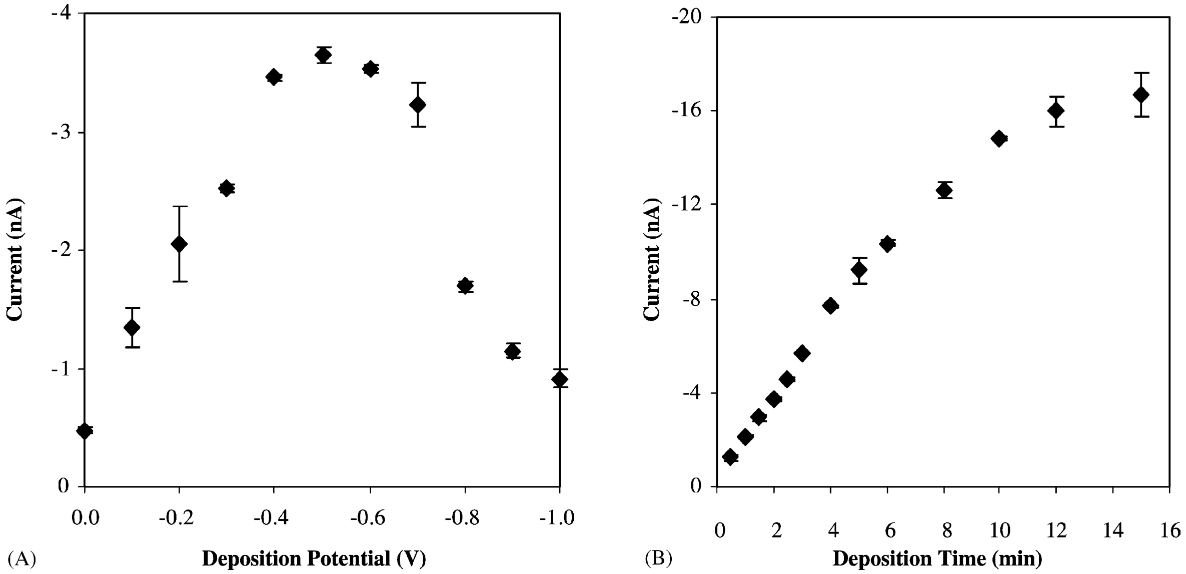

Fig. 4. Effect of varying the deposition potential at a fixed deposition time of 120 s (A), and of varying the deposition time at a fixed absorption potential of −0.5 V (B), on the AdCSV sensitivity for 20 nM cadmium in Britton–Robinson buffered solutions (pH 7.2) containing 1 μM APSH.

Table 1
Levels for the variables of the Plackett–Burman design

Variables		−1	0	+1
pH	pH	7.0	7.5	8.0
Buffer concentration (M)	B	0.02	0.05	0.08
Pulse amplitude (mV)	P	−40	−70	−100
Time interval for voltage step (s)	t_s	0.1	0.5	0.9
Voltage step (mV)	U_s	4	8	12
Deposition time (s)	t_d	60	90	120
Deposition voltage (mV)	E_d	−300	−450	−600

Table 2
Response variables evaluated with the Plackett–Burman design

Analytical performance	Response	Meaning
Sensitivity	i_pCd	Intensity of the first measurement of cadmium–APSH complex peak
Resolution	i_pCd/i_pAPSH	Intensity ratio for cadmium–APSH and free APSH peaks
	ΔE Cd–APSH	Difference of potential between the neighbouring cadmium–APSH and free APSH peaks measured at maximum i_p

Table 4
Analysis of the effects for the variables of the Plackett–Burman design

Variable	i_pCd	i_pCd/i_pAPSH	ΔE Cd–APSH
pH	+	++	+++
B	NS	++	+++
P	++	−−	+++
t_s	−	−−	−−−
U_s	NS	NS	+++
t_d	++	++	−−
E_d	+	++	−

Significance testing method: center. Simbology used for the P-values with negative and positive effects. NS: >0.05; −/+: 0.01–0.05; −−/++: 0.001–0.01; −−−/+++: <0.001.

(DPAdCSV), seven variables including both chemical and instrumental were chosen, according to criteria upon previous experimental basis. Variable intervals are shown in Table 1.

For the Plackett–Burman design, the concentrations of cadmium(II) and APSH were set to 3×10^{-8} M and 2×10^{-6} M, respectively, to ensure a metal to ligand ratio above 1:30 and metal levels around nanomolar concentrations, but high enough to assure valid signals even at the worst operational conditions. The largest drop size of the HMDE was selected and solutions were stirred at 2000 rpm.

Although the main purpose was to achieve the best sensitivity for the determination of cadmium, other aspects as time stability of the peak and possible interferences by peaks overlapping with peaks of APSH were also evaluated. Therefore, three different responses according to the data outputs provided by the Metrohm voltammeter were obtained for each experiment (Table 2). The matrix and results of the Plackett–Burman design and the analysis of the effects are shown in Tables 3 and 4, respectively.

Only two variables did not showed significant influence in the enhancement of the cadmium(II)–APSH peak: buffer concentration (B) and voltage

Table 3
Design matrix with codified variables and values for the response variables evaluated with the Plackett–Burman design

Experiment	pH	B	P	t_s	U_s	t_d	E_d	i_pCd (−nA)	i_pCd/i_pAPSH	ΔE Cd–APSH (mV)
1	+1	+1	+1	−1	+1	−1	−1	20.6	0.22	216
2	−1	+1	+1	+1	−1	+1	−1	24.3	0.22	110
3	−1	−1	+1	+1	+1	−1	+1	16.1	0.11	122
4	+1	−1	−1	+1	+1	+1	−1	19.0	0.48	122
5	−1	+1	−1	−1	+1	+1	+1	23.1	0.75	128
6	+1	−1	+1	−1	−1	+1	+1	30.3	0.57	169
7	+1	+1	−1	+1	−1	−1	+1	13.5	0.61	123
8	−1	−1	−1	−1	−1	−1	−1	11.0	0.27	103
9a	0	0	0	0	0	0	0	26.9	0.42	123
9b	0	0	0	0	0	0	0	25.9	0.40	124
9c	0	0	0	0	0	0	0	26.5	0.40	123

step (U_s). Among the others, pH, pulse amplitude (P), deposition time (t_d) and deposition potential (E_d) showed a positive significant effects in the response. The increase of these variables results in the increase of the cadmium(II)–APSH peak high. Thus, a better response is expected whenever pH gets closer to 8.0, both pulse amplitude and deposition time increase, and the deposition potential approaches to the cadmium reduction potential. All these conclusions seemed to make sense because of the stability of the cadmium(II)–APSH complex at alkaline pH and the well-known behaviour of pulse amplitude as well as the deposition parameters in the voltammetric peaks in AdCSV. Only time interval for voltage step (t_s) exhibit a negative effect. Shorter voltage steps produced higher peaks, which can be related to a higher speed rate during the voltammetric scan.

Regarding peak stability, only three variables showed significant effects. Accumulation time and pH had a negative effect in the peak stability between consecutive measurements. These observations can be explained because of the influence of cadmium hydrolysis reactions at alkaline pH values. Despite the complex stability may be enhanced at alkaline pH, side processes (probably hydrolysis, precipitation and/or adsorption onto electrodes and voltammetric vessel) could be involved in the decrease of free dissolved cadmium. Moreover, this effect seems to be progressive with time, which explains the negative effect in the accumulation time. Nevertheless, this problem may be overcome by using PTFE vessels and neutral or slightly alkaline pH values (i.e. pH 7–8).

Separation efficiency (ΔE Cd–APSH) exhibited positive effects for pH, buffer concentration, pulse amplitude and voltage step, whilst negative effects for time interval for voltage step, deposition time and, in less extent, deposition potential were found. When pH became more alkaline and buffer concentration increased, an improvement of peaks separation was observed. The potential of the cadmium(II)–APSH complex shifted about 40 mV towards negative direction. As a reason for this, the increasing cadmium(II)–APSH complex stability at alkaline pH as well as higher ionic strength can be pointed out. In addition, the increase of the scan rate, in terms of higher U_s values and lower t_s values, also enhanced the peaks separation. Nevertheless, accumulation parameters showed the opposite behaviour.

Peaks height ratio (i_pCd/i_pAPSH) seems to behave similarly to ΔE Cd–APSH, except for pulse amplitude and deposition parameters. In this case, as pH and buffer concentration increased, the cadmium-to-APSH i_p ratio also increased. However, accumulation parameters exhibited the opposite trend observed for ΔE Cd–APSH. This time, higher accumulation produced a higher i_p ratio. As a reason for this, the enhancement of the cadmium–APSH complex adsorption rate on the mercury drop in comparison with the free APSH has been pointed out.

Finally, as buffer concentration (B) and voltage step (U_s) were not significant for i_pCd, whilst deposition potential (E_d) evidenced the well-known behaviour of cadmium(II)–APSH complex peak enhancement when approaching to its characteristic peak voltage, they were fixed in further experiments at those values that compromise the enhancement of i_pCd for a better peak sensitivity, and both i_pCd/i_pAPSH and ΔE Cd–APSH for the improvement of peaks resolution.

3.4. Surface response design: central composite

Once the most significant variables were identified, a cube + star ($2^k + 2k + 1$) central composite design with four design variables was used to establish the peak enhancement tendencies and improved experimental conditions for the cadmium measurements. For this purpose, several variables were kept fixed (Table 5), whilst a new set of 25 ($2^4 + 2 \times 4 + 1$) experiments including two repetitions of the central point were carried out using the variables shown in Table 6. In this case, five levels for each variable were used and the central point was measured three times. The intervals of the instrumental design variables were slightly redefined following the trends obtained

Table 5
Fixed variables for the central composite design

Variable	Value
APSH concentration	2×10^{-6} M
Cadmium concentration	3×10^{-8} M
Drop size	9 a.u.
Stirring speed	2000 rpm
Buffer concentration (HEPES)	0.08 M
Voltage step	8 mV
Deposition potential	−600 mV

Table 6
Design variables and levels for the central composite design

Variable	−2	−1	0	+1	+2
pH	7.00	7.25	7.50	7.75	8.00
P (mV)	−60	−70	−80	−90	−100
t_s (s)	0.05	0.15	0.25	0.35	0.45
t_d (s)	60	80	100	120	140

Table 8
Main trends for the responses in the interval (−2, +2) from the central composite design results

Variables	Interval	i_pCd	i_pCd/i_pAPSH	ΔE Cd–APSH
pH	7.0–8.0	NS	+	+
t_s (s)	0.05–0.45	NS	−	−
P (−mV)	60–100	NS	−	+
t_d (s)	60–140	+	+	NS

in the Plackett–Burman design. Thus, P was set from −60 to −100 mV, t_s from 0.05 to 0.45 s and t_d from 60 to 140 s. The results of the response variables and the general trends observed for the four design variables are shown in Tables 7 and 8, respectively. Main trends of the variables within the experimental intervals exhibited concordance with results obtained in the Plackett–Burman design.

The only observed effect for i_pCd was a positive of t_d, which is easily explainable because of the increase of the cadmium–APSH complex adsorption on the HMDE with the increase of the accumulation time. pH, t_s and P did not exhibit significant effect within the studied intervals for i_pCd. Nevertheless, both responses linked to resolution showed concordance between results of pH and t_s, whilst different results for P. Hence, as pH increases and t_s decreases, the resolution would become improved, as a consequence of an increase of complex stability at pH 8.0 and shorter time interval for voltage steps, which means higher scan speed.

Table 7
Design matrix with codified variables and experimental responses in the central composite design

Experiment	pH	P	t_s	t_d	i_p (Cd) (−nA)	i_pCd/i_pAPSH	ΔE Cd–APSH (mV)
1	−1	−1	−1	−1	23.5	0.65	155
2	+1	−1	−1	−1	21.3	0.61	188
3	−1	+1	−1	−1	22.9	0.51	158
4	+1	+1	−1	−1	21.6	0.46	196
5	−1	−1	+1	−1	23.1	0.37	125
6	+1	−1	+1	−1	23.7	0.57	154
7	−1	+1	+1	−1	22.7	0.35	143
8	+1	+1	+1	−1	24.7	0.41	168
9	−1	−1	−1	+1	27.7	0.73	165
10	+1	−1	−1	+1	28.1	0.76	198
11	−1	+1	−1	+1	28.1	0.60	176
12	+1	+1	−1	+1	27.3	0.71	199
13	−1	−1	+1	+1	30.6	0.59	125
14	+1	−1	+1	+1	28.9	0.78	161
15	−1	+1	+1	+1	31.0	0.50	150
16	+1	+1	+1	+1	28.2	0.56	174
17	−2	0	0	0	24.9	0.44	126
18	+2	0	0	0	27.8	0.58	172
19	0	−2	0	0	26.4	0.72	137
20	0	+2	0	0	25.5	0.40	164
21	0	0	−2	0	26.3	0.76	192
22	0	0	+2	0	23.4	0.37	135
23	0	0	0	−2	17.9	0.41	164
24	0	0	0	+2	31.5	0.70	155
25	0	0	0	0	25.2	0.51	150
26	0	0	0	0	26.8	0.55	151
27	0	0	0	0	25.8	0.53	157

Table 9
Set of predicted variable values for the single response (SRM) and multiple response maximization (MRM) with codified values of the design variables and predicted response values for the SRM and MRM

	Operational conditions for response maximization				Predicted responses	
	pH	P (−mV)	t_s (s)	t_d (s)	SRM	MRM
i_pCd	7.0	60	0.28	140	−33.5 nA	−31.2 nA
i_pCd/i_pAPSH	8.0	60	0.41	140	1.13	1.07
ΔE Cd–APSH	8.0	64	0.05	140	256 mV	199 mV
MRM	8.0	60	0.22	139		

As a consequence of the surface response designs, further statistical approaches in order to find a set of experimental conditions which lead to maximize such responses, have been also done. Therefore, several sets of variable values which maximize individually i_pCd, i_pCd/i_pAPSH and ΔE Cd–APSH, as well as a set of values for the multiple response maximization of the three variables together are shown in Table 9.

Finally, a set of suitable experimental conditions for all the variables during the three stages of the study has been proposed in Table 10, considering not only the enhancement of the cadmium(II)–APSH complex peak but also the improvement of resolution between peaks (Fig. 5). These conditions were performed experimentally in order to check the previously predicted results by multiple response optimization, showing the highest rate of concordance for i_pCd (0.2%), followed by i_pCd/i_pAPSH (9.4%) and ΔE Cd–APSH (13.3%). Although this multiple response optimization was done only with four variables while the others were fixed, the rate of concordance is reasonably good for i_pCd, and in less extent, for the other two response variables.

Table 10
Suitable experimental conditions for the sensitivity and resolution improvement of the cadmium(II)–APSH peak

Variables		Values	Stage
Metal to ligand concentration ratio	Cd:APSH	>1:30	Preliminary experiments
Drop size	d_s	9 a.u.	
Rotation speed	r_s	2000 rpm	
Concentration of HEPES	B	0.08 M	Plackett–Burman Design
Voltage step	U step	8 mV	
Deposition potential	U_d	−0.6 V	
Acidity/alkalinity	pH	8.0	Central composite design
Pulse amplitude	P	−60 mV	
Time interval for voltage step	t step	0.25 s	
Deposition time	t_d	140 s	

3.5. Analytical performance characteristics of the method

The analytical performance characteristics were evaluated using the proposed suitable set of experimental conditions. The linearity was studied for

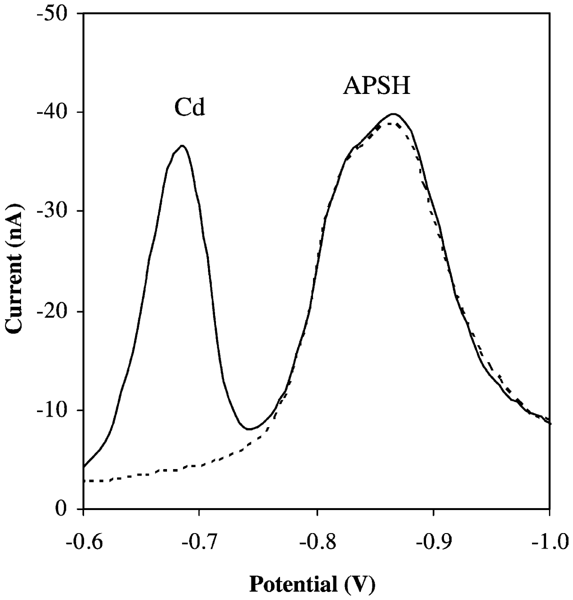

Fig. 5. DPAdCSV voltammograms of 2×10^{-6} M APSH in the absence of cadmium(II) (dotted line) and in the presence of 3×10^{-8} M cadmium(II) (solid line). Operating conditions shown in Table 10.

cadmium(II) concentrations up to 1.2×10^{-7} M and it showed linearity over the range 3×10^{-10}–4×10^{-8} M with a correlation coefficient of 0.9996 and an equation of the linear regression being $i_p(-\text{nA}) = 0.5505 + 0.9683\,C$ (nM). Detection limit, calculated according $\text{DL} = 3\sigma/m$ [34], where σ is the S.D. of the blank and m the slope of the calibration curve, was found to be 0.06 nM. The quantification limit (QL), calculated as $\text{QL} = 10\sigma/m$ [34] was 0.21 nM. By increasing the deposition time these values can be further improved.

The adsorptive accumulation of the cadmium(II)–APSH complex under the optimized conditions results in reproducible peak currents. Measurements of 11 solutions 30 nM cadmium gave a mean peak current of 31 ± 0.8 nA and a relative standard deviation (R.S.D.) of 2.5%. The analytical precision (repeatability) was evaluated by measuring the concentration of eight aliquots of 5 nM cadmium(II) using the standard addition method giving an average concentration of 5.0 ± 0.3 nM.

3.6. Selectivity of the method

Because seawater exhibits higher saline concentrations than fresh waters, the different salts present in seawater have been used to study their influence on the determination of cadmium, being this way the fresh waters also included. Hence, several solutions with saline concentrations have been analysed in order to study their effect on cadmium determination

Table 11
Study of interferences by salts and trace metals for the cadmium determination in solutions containing 5.0 ± 0.3 nM cadmium(II)

Interference	Concentration	Average (nM)	R.S.D. (nM)	Recovery (%)
NaCl	23.94 g l^{-1}	5.3	0.1	106.5
$MgCl_2$	10.83 g l^{-1}	5.1	0.0	103.1
Na_2SO_4	3.99 g l^{-1}	4.9	0.2	99.7
$CaCl_2$	1.49 g l^{-1}	4.9	0.1	98.6
KCl	677 mg l^{-1}	5.0	0.3	100.9
$NaHCO_3$	196 mg l^{-1}	4.9	0.5	99.2
KBr	98 mg l^{-1}	4.8	0.3	96.8
H_3BO_3	27 mg l^{-1}	5.2	0.3	104.0
$SrCl_2$	24 mg l^{-1}	5.1	0.4	102.2
V(V)	100 nM	5.2	0.5	103.8
Fe(III)	100 nM	5.1	0.2	103.0
Pb(II)	30 nM	5.0	0.1	101.4
Bi(III)	3 nM	4.8	0.2	97.4
Ni(II)	100 nM	4.7	0.3	94.7
Co(II)	3 nM	5.2	0.3	104.8
Cu(II)	100 nM	5.2	0.0	105.1
Zn(II)	100 nM	5.3	0.1	106.4

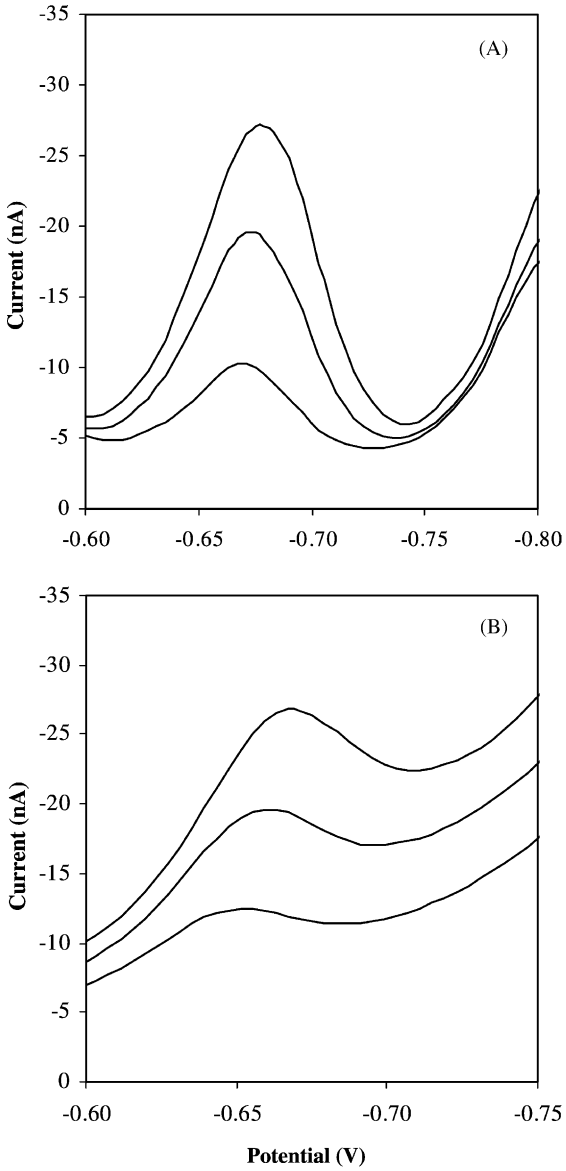

Fig. 6. Successive additions of 1.5×10^{-9} M cadmium(II) to a 1:5 diluted aliquot of sample reference material trace elements in water NIST-SRM 1643d (A) and successive additions of 1 nM to an aliquot of certified reference material trace elements in estuarine water BCR-CRM 505 (B). Operating conditions shown in Table 10.

by the standard addition method. In addition, the influence of other trace metals present in seawater has been also evaluated at higher concentrations that usually found in seawater. For this purpose, cadmium concentration was evaluated in two aliquots for each salt and trace metal in solutions containing 5.0 nM cadmium(II). Results and percentages of recovery are expressed in Table 11.

None of the salts showed to produce interferences for the cadmium determination, as all measured cadmium concentration values were within the 0.3 nM uncertainty given by the standard deviation from the previous precision assessment of the method. Related to trace metals, no interfering effects were found. Zinc produced a peak at −1.09 V, lead at −0.52 V, and nickel at −0.99 V. However, none of these metals apparently interfered in the cadmium determination, even though their concentrations exceeded the usual concentrations in seawater.

3.7. *Accuracy of the method*

The accuracy of the method for cadmium was assessed by replicate analyses of two reference certified water: trace elements in water (NIST-SRM 1643d) and trace elements in estuarine water (BCR-CRM 505). Both samples were certified to contain 6.47 $\mu g\,kg^{-1}$ (57.56 $nmol\,kg^{-1}$) with 6% uncertainty and 0.80 $nmol\,kg^{-1}$ with 0.04 $nmol\,kg^{-1}$ uncertainty cadmium, respectively. Three aliquots of each sample were quantified after UV digestion and neutralization with ammonia, giving mean cadmium concentrations of $6.54 \pm 0.06\,\mu g\,kg^{-1}$ and $0.82 \pm 0.03\,nmol\,kg^{-1}$, respectively (Fig. 6).

4. Conclusions

A new AdCSV method for cadmium analysis based on complexation with 2-acetylpyridine salicyloylhydrazone has been presented. This work has also showed interesting possibilities in further cadmium speciation studies in natural waters based on ligand competition.

A three-stage strategy has been followed to study simultaneously both chemical and instrumental variables in order to find their main trends and to find a set of suitable experimental conditions for the determination of cadmium in water samples, including water samples with saline matrices like seawater and estuarine water. The method has been validated with certified reference samples of fresh and estuarine water with satisfactory results.

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