Application of experimental design on determination of aluminum content in saline samples by adsorptive cathodic stripping voltammetry

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Abstract: Adsorptive cathodic stripping voltammetric determination of aluminum at ng mL^{-1} levels in salt samples based on the metal complexation with Calcon (1-(2-hydroxynaphthylazo)-2 naphthol-4-sulfonic acid) and the subsequent adsorptive deposition onto a hanging mercury drop electrode was studied. Central composite design was used as a design method. Several chemical and instrumental parameters (pH, ligand concentration, deposition time, deposition potential, and complexing time) were involved in the experimental design. Analytical parameters such as repeatability, linearity, and accuracy were also investigated and the detection limit was found as 0.32 ng mL^{-1}.

Key words: Aluminum, Calcon, adsorptive cathodic stripping voltammetry, central composite design

1. Introduction

Aluminum is the third most abundant element and its compounds are used as coagulants in drinking water treatment.[1] Aluminum has been implicated in the pathogenesis of some disorders observed in patients with chronic renal failure while undergoing hemodialysis.[2] Therefore, monitoring the aluminum level of dialysis fluids and serum samples is essential in preventing toxic effects in uremic patients.

Several methods have been developed for the determination of aluminum in various samples such as milk, wine, serum, and hemodialysis concentrates.[3−6] Among the atomic techniques, electrothermal atomic absorption spectrometry has shown satisfactory detection limits for the determination of the aluminum in various samples.[7,8] However, the hindrance of the salt content for the direct determination of aluminum in samples with saline matrices has reduced the application of atomic techniques.

Stripping voltammetry, on the other hand, provides an inexpensive way for sensitive and selective analysis in saline matrices.[9] However, direct electrochemical detection of aluminum is difficult since it is reduced at very negative potentials. Therefore, indirect determination of aluminum is carried out with adsorptive cathodic stripping voltammetry (AdCSV) based on the adsorptive accumulation of aluminum complexes with reducible ligands. Early studies included solochrome violet RS (SVRS) and 1,2-dihydroxyanthraquinone-3-sulfonic acid (DASA).[10,11] The SVRS complex gives an adsorptive cathodic peak at –0.61 V whose intensity increases linearly as a function of aluminum concentration. DASA was reported to display improved sensitivity (limit of detection:

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0.027 ng mL^{-1}). However, these methods require a preheating step due to the slow reaction between trivalent aluminum and the complexing reagent.[12]

The efficiency of a variety of ligands was evaluated by comparing their voltammetric response through the applying of a linear scan mode after preconcentration onto the mercury film electrode as Al(III) complexes.[13] Although it was stated that cupferron is the best ligand for Al(III) determination, the cathodic peak locates at very negative potentials close to hydrogen evolution and, therefore, in terms of signal-to-background characteristics, search for another reagent is required.

Calcon (1-(2-hydroxynaphthylazo)-2 naphthol-4-sulfonic acid, or solochrome dark blue BS or Blue Black Eriochrome), on the other hand, was firstly proposed as a metallochromic indicator.[14] As shown in Figure 1, it is an oxygen–nitrogen donor hydroxy monoazo chelating reagent and complexes with Al(III) via its –OH groups. It has been employed as a complexing reagent for polarographic and AdCSV determination of aluminum in tap water and hemodialysis solutions.[15]
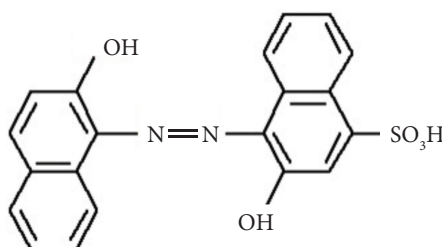

Figure 1. Structure of Calcon.

Calcon is reduced at a hanging mercury drop electrode (HMDE) around –0.3 V and its aluminum complex gives a well-separated peak around –0.5 V, which is less affected by calcium and zinc interferences than SVRS and DASA complexes. However, early studies indicated that the deposition conditions are strongly affected by ligand concentration, and self-adsorption of the free ligand hinders the precise determination of aluminum as it competes with the complex for active sites of the electrode surface.[16] Therefore, the influence of variables on the peak current and their interaction should be optimized carefully.

In this study, the AdCSV method as based on aluminum complexation with Calcon and optimization of the instrumental and chemical parameters by using the central composite design (CCD) approach was investigated to prove that Calcon is actually more eligible than other complexing reagents that suffer from the interferences of ionic content of saline samples. Hemodialysis solutions were then analyzed by AdCSV under optimal conditions.

2. Experimental

2.1. Instrumentation

All measurements were carried out using a Metrohm 757 VA Computrace voltammetric analyzer (Herisau, Switzerland) equipped with a multimode electrode in the HMDE mode. The 3-electrode system was completed by means of a platinum auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode. Differential pulse (DP) mode was used throughout the study with 50 mV amplitude and a scan rate of 15 mV s^{-1}. The pH measurements were made with a Thermo Orion 4-Star ion analyzer (Waltham, MA, USA). An Agilent 7500ce ICP–MS (Tokyo, Japan) was used as a complementary method to compare the amount of aluminum in the solutions.

2.2. Reagents

Standard aluminum solutions were prepared daily by appropriate dilution of a stock aluminum solution (1000 mg L^{-1}) prepared by dissolving aluminum metal (Merck, Darmstadt, Germany) in hydrochloric acid. HEPES [N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid), 0.1 M] (Sigma, Taufkirchen, Germany) was prepared in ultrapure water. The pH of the HEPES solution was adjusted to 7.0 by dropwise addition of a concentrated NaOH solution. Acetic acid/acetate buffer was prepared from 1.0 M acetic acid and the pH was adjusted to 4.5 by the addition of NaOH. Fresh Calcon (Merck) solutions were prepared immediately before analysis by dissolving 58 mg of pure substance in 10 mL of ultrapure water. All solutions were prepared with Milli-Q water (18.2 MΩ).

2.3. Data analysis

CCD analysis was carried out using MATLAB 5.3 (MathWorks Inc., Natick, MA, USA) programming language. The statistical tests included in the Microsoft Excel Solver Add-In and Student's t-test were done with the help of the Microsoft Office Excel program (Microsoft Office 2000, Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

For AdCSV determination of aluminum via its reducible Calcon complex, competitive adsorption of the free ligand and the complex on the electrode surface necessitated the careful optimization of deposition parameters. CCD was employed for this comprehensive optimization. The most significant instrumental and chemical variables were identified with preliminary studies and several variables were kept fixed in the determination of aluminum in saline samples by AdCSV.[16] The fixed variables were as specified in the experimental section and the concentration of Al(III) was kept at a constant of 10 ng mL^{-1} unless otherwise stated. For this purpose, of the known variables that potentially influence the peak enhancement, 5 were chosen for the CCD optimization.

CCD with 4 design variables [$(2^4 + 2 \times 4 + 1) = $ n] was modified by the addition of the fifth variable (2n). Four experiments were carried out at an intermediate (level 0) that can be attributed to experimental error. Variables with their intervals are shown in Table 1. The final design with a total of 58 experiments was used to establish the peak enhancement tendencies and to improve the experimental conditions for the aluminum measurements. In this case the intervals of the first 4 variables were taken at 5 levels according to the CCD rules. The fifth variable, namely pH factor, was only taken at 2 levels, being 4.5 at the low level and 7.0 at the high level considering the pH dependency for formation of the complex. The matrix and results of the CCD are shown in Table 2.

Table 1. Levels for the variables of the CCD.

Variables		−2	−1	0	+1	+2
Calcon concentration (M)	C	0.5×10^{-5}	1.0×10^{-5}	1.5×10^{-5}	2.0×10^{-5}	2.5×10^{-5}
Complexation time (s)	t_R	150	200	250	300	350
Deposition voltage (V)	E	−0.45	−0.40	−0.35	−0.30	−0.25
Deposition time (s)	t_d	10	20	30	40	50
Medium pH	pH		4.5		7.0	

The response component used to build the CCD model was the current intensity of the first measurement of the Al(III)-Calcon complex peak current ($-i_p$, nA) for each experiment. Therefore, achieving the best sensitivity signal for the determination of aluminum was evaluated according to these intensities.

Table 2. Design matrix with coded variables and values for the response.

Exp.	C	tR	E	t_d	pH	Response	Exp.	C	tR	E	t_d	pH	Response
1	1	1	1	1	1	**6.18**	**30**	1	1	1	1	−1	**4.43**
2	1	1	1	−1	1	**19.82**	**31**	1	1	1	−1	−1	**3.56**
3	1	1	−1	1	1	**5.62**	**32**	1	1	−1	1	−1	**21.46**
4	1	1	−1	−1	1	**7.33**	**33**	1	1	−1	−1	−1	**18.53**
5	1	−1	1	1	1	**6.70**	**34**	1	−1	1	1	−1	**2.84**
6	1	−1	1	−1	1	**17.79**	**35**	1	−1	1	−1	−1	**4.98**
7	1	−1	−1	1	1	**4.91**	**36**	1	−1	−1	1	−1	**14.38**
8	1	−1	−1	−1	1	**5.50**	**37**	1	−1	−1	−1	−1	**13.62**
9	−1	1	1	1	1	**4.91**	**38**	−1	1	1	1	−1	**4.61**
10	−1	1	1	−1	1	**34.30**	**39**	−1	1	1	−1	−1	**4.77**
11	−1	1	−1	1	1	**7.38**	**40**	−1	1	−1	1	−1	**27.91**
12	−1	1	−1	−1	1	**5.71**	**41**	−1	1	−1	−1	−1	**21.41**
13	−1	−1	1	1	1	**20.31**	**42**	−1	−1	1	1	−1	**4.33**
14	−1	−1	1	−1	1	**25.99**	**43**	−1	−1	1	−1	−1	**3.05**
15	−1	−1	−1	1	1	**5.89**	**44**	−1	−1	−1	1	−1	**26.28**
16	−1	−1	−1	−1	1	**5.93**	**45**	−1	−1	−1	−1	−1	**21.27**
17	2	0	0	0	1	**9.02**	**46**	2	0	0	0	−1	**6.37**
18	−2	0	0	0	1	**24.82**	**47**	−2	0	0	0	−1	**22.20**
19	0	2	0	0	1	**18.78**	**48**	0	2	0	0	−1	**16.22**
20	0	−2	0	0	1	**14.45**	**49**	0	−2	0	0	−1	**15.73**
21	0	0	2	0	1	**16.04**	**50**	0	0	2	0	−1	**3.67**
22	0	0	−2	0	1	**2.66**	**51**	0	0	−2	0	−1	**15.97**
23	0	0	0	2	1	**6.05**	**52**	0	0	0	2	−1	**10.71**
24	0	0	0	−2	1	**21.79**	**53**	0	0	0	−2	−1	**11.70**
25	0	0	0	0	1	**22.47**	**54**	0	0	0	0	−1	**21.58**
26	0	0	0	0	1	**22.14**	**55**	0	0	0	0	−1	**25.68**
27	0	0	0	0	1	**21.77**	**56**	0	0	0	0	−1	**22.02**
28	0	0	0	0	1	**20.84**	**57**	0	0	0	0	−1	**23.41**
29	0	0	0	0	1	**22.45**	**58**	0	0	0	0	−1	**24.04**

The design matrix of the CCD employed coded values for variables and voltammeter response according to a quadratic model. This model was a second-order polynomial equation as shown below:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k-1} \sum_{j=2(i<j)}^{k} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2. \tag{1}$$

The coefficients of the polynomial were represented by β_0 (constant terms), β_1, β_2, β_3...(linear effects), β_{12}, β_{13}, β_{34}...(interaction effects), and β_{11}, β_{22}, β_{33}...(quadratic effects). The calculated regression coefficients of the second-order polynomial equation and their linear, interaction, and quadratic terms are presented in Table 3. The most influential of the terms in the statistical model for peak enhancement was related not only to instrumental but also classical chemical parameters. The positive and negative signs of the regression coefficients of linear, quadratic, and interaction terms identified the effect of the variables on the Al(III)–Calcon complex peak height. A positive coefficient indicated that the variable directly affects the response whereas a negative coefficient meant that increasing the value of this factor diversely affects the response. There are certain ways to assess the significance of each parameter based on the size of the coefficients using Student's

t-test, an F-test, and normal probability distribution. In this study, Student's t-test was used to decide the most significant variables for peak enhancement.

Table 3. Regression coefficients of the second-order polynomial equation.

Regression coefficients	Value	Regression coefficients	Value
β_0	22.6400		
β_1	–2.7009	$\beta_3\beta_5$	–5.6542
β_2	0.4962	$\beta_4\beta_5$	2.1877
β_3	–0.8832	$\beta_1\beta_2\beta_3$	–0.1481
β_4	–1.6432	$\beta_1\beta_2\beta_4$	0.7334
β_5	0.1584	$\beta_1\beta_2\beta_5$	–0.0468
β_{11}	–2.0230	$\beta_1\beta_3\beta_4$	0.6153
β_{22}	–1.8501	$\beta_1\beta_3\beta_5$	1.9192
β_{33}	–3.5281	$\beta_1\beta_4\beta_5$	–0.5196
β_{44}	–2.7832	$\beta_2\beta_3\beta_4$	–0.9043
$\beta_1\beta_2$	0.5705	$\beta_2\beta_3\beta_5$	–0.0683
$\beta_1\beta_3$	–0.1739	$\beta_2\beta_4\beta_5$	0.9656
$\beta_1\beta_4$	–0.1190	$\beta_3\beta_4\beta_5$	1.3683
$\beta_1\beta_5$	0.1389	$\beta_1\beta_2\beta_3\beta_4$	0.8682
$\beta_2\beta_3$	–0.6556	$\beta_1\beta_2\beta_3\beta_5$	–0.6057
$\beta_2\beta_4$	–0.6386	$\beta_1\beta_3\beta_4\beta_5$	–0.2765
$\beta_2\beta_5$	0.2087	$\beta_2\beta_3\beta_4\beta_5$	0.7738
$\beta_3\beta_4$	–2.3278	$\beta_1\beta_2\beta_3\beta_4\beta_5$	–0.6315

The statistical significance obtained by Student's t-test is the percentage probability, and the calculated results are shown in Figure 2. A large confidence means that the factor is significant.[17]
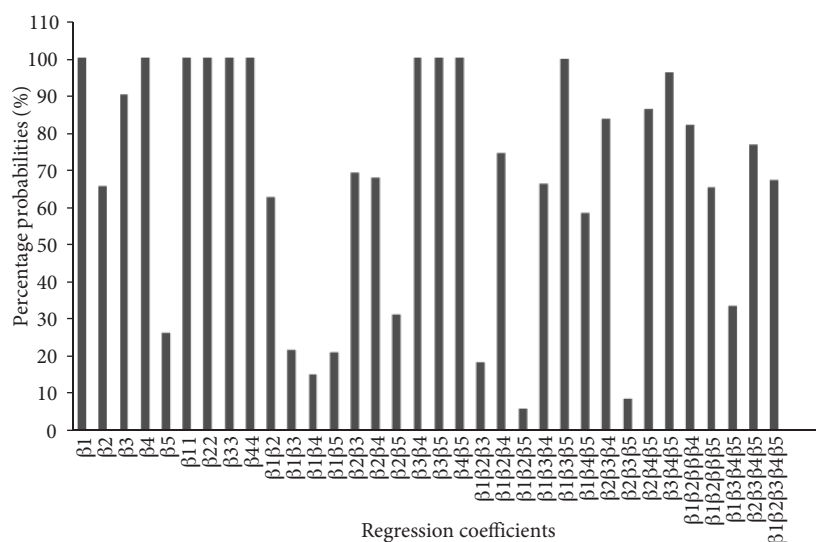

Figure 2. Percentage probability results obtained from the t-test.

Normally, high probabilities are expected if a factor is significant in excess of 95%. Based on this idea, Calcon concentration and deposition time seem to be the most significant factors in linear interactions. On the

other hand, the interactions of the factors show that Calcon concentration, medium pH, deposition potential, and time affected the peak current of the Al(III)–Calcon complex. To interpret these results visually, the response surface methodology was used for the significant factors that were obtained from Student's t-test.

Considering the enhanced stability of the Al (III)–Calcon complex in neutral pH, a better response is expected around pH 7.0. In light of the regression coefficients it can be concluded that, even if the effect of pH alone has minor effects on the results, its interactions with deposition potential and time are important contributors to the complex peak. These interactions are illustrated in Figures 3 and 4. The importance of pH arises from the hydrogen ion involvement in the reduction mechanism of the complex on the HMDE. Therefore, the pH of the solution influenced the deposition potential of the complex. Furthermore, due to the stability of the complex at neutral pH, the deposition of complex on the electrode surface was deposition time-dependent.
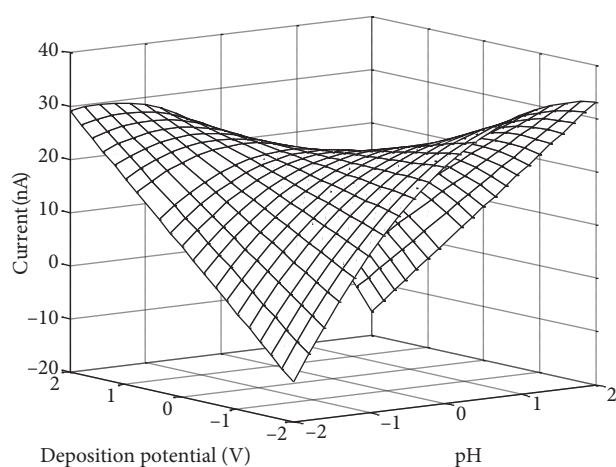
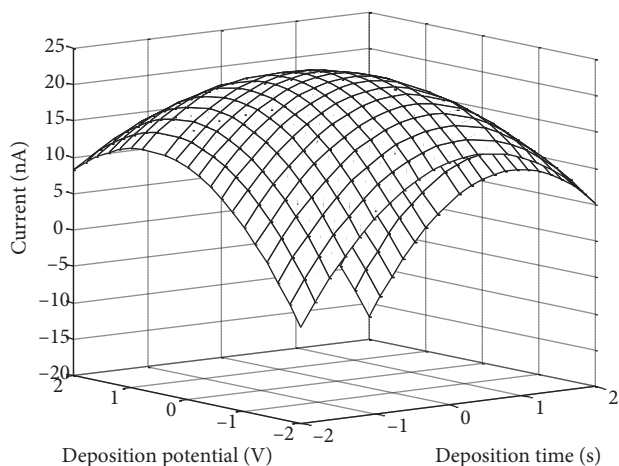**Figure 3.** Response surface for the CCD for optimization of the deposition potential and pH.
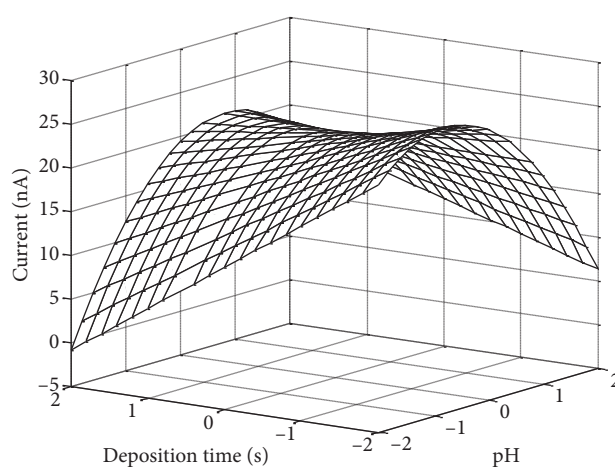
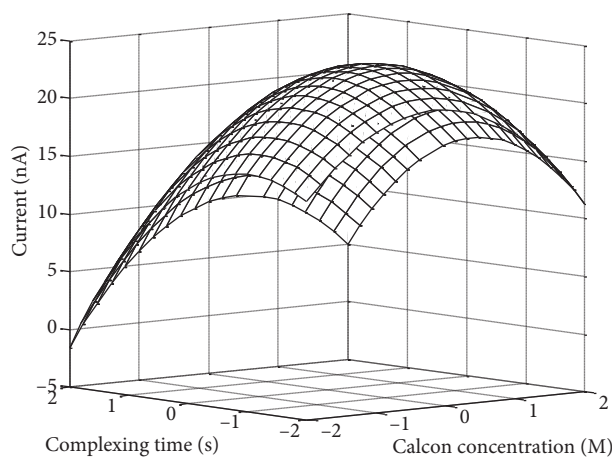**Figure 4.** Response surface for the CCD for optimization of the deposition time and pH.

The negative effect of deposition time can be attributed to the self-adsorption of the free ligand on the HMDE for longer deposition times. Due to the competitive adsorption between the free ligand and the complex, the extent of complex adsorption on HMDE was found to be critically dependent on deposition time. The interaction term of deposition potential and time also has a negative sign, which means that reducing the deposition time requires a decrease in the deposition potential as shown in Figure 5. Consequently, optimum deposition potential was found to be more negative than the peak potential of free ligand. To prevent the adsorption of free ligand on HMDE, appropriate deposition time should be determined.

Figure 6 shows the surface plot of the Calcon concentration and complexing time effects on the instrumental response. The linear effects of these variables prove that smaller Calcon concentrations and higher complexing times yield maximum peak current, as can be predicted from the kinetic aspects of the reactions. The quadratic term as related to complexing time clearly showed this interaction.

Another assessment of the results was the influence of Calcon concentration and deposition time on instrumental response. As reported above, the competitive adsorption between the free ligand and complex affected the peak enhancement of the complex. Therefore, the deposition step should be performed after sufficient time has been allowed for the complexation reaction.

Figure 5. Response surface for the CCD for optimization of the deposition time and deposition potential.

Figure 6. Response surface for the CCD for optimization of the Calcon concentration and complexing time.

The predicted responses along with the residuals when using the full model by taking all the coefficients were calculated and the actual response as a function of predicted response was plotted. Figure 7 shows the actual response as a function of predicted response with a correlation coefficient of 0.9286, which indicates a reasonable fit for this model for the given system.
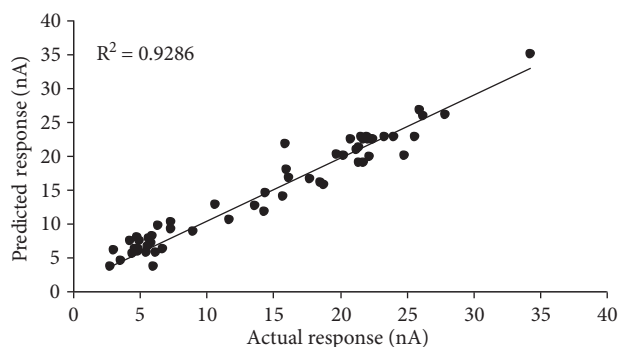

Figure 7. The plot of the actual response vs. the predicted response.

By means of the response surface designs, further statistical approaches have also been applied to find the set of experimental conditions that lead to maximize responses. In this study the Solver Add-In utility from Microsoft Excel was used in order to find the optimal conditions of aluminum determinations by AdCSV. As such, a set of suitable experimental conditions for all the variables is proposed in Table 4, not only with regard to the enhancement of the Al(III)–Calcon complex peak, but also for the improvement of resolution between peaks. The analytical performance characteristics were evaluated using the proposed set of experimental conditions that were obtained via CCD.

As shown in Figure 8, the linearity was studied for Al(III) concentrations up to 8.0 ng mL^{-1} with a correlation coefficient of 0.9970. The detection limit (DL) as calculated according to DL $= 3\ \sigma/m$, where σ is the standard deviation of the blank and m is the slope of the calibration line, was found to be 0.32 ng mL^{-1}. The limit of quantification (QL $= 10\ \sigma/m$) was determined to be 1.06 ng mL^{-1}.

Table 4. Operational conditions for response optimization.

Variables	Coded level	Real value
Calcon concentration (M)	−1.41	1.1×10^{-5}
Complexing time (s)	0.13	257
Deposition potential (mV)	−1.56	−0.43
Deposition time (s)	0.75	38
pH	1	7
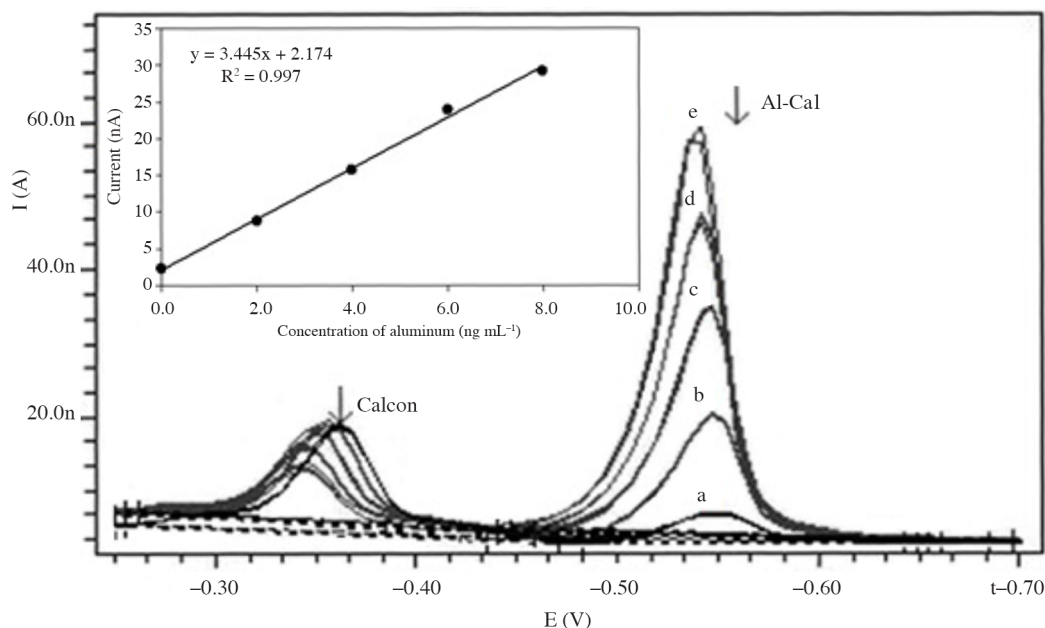
Figure 8. DPAd CSV voltammograms of the Al(III)–Calcon complex peak with the subsequent addition of Al(III) to be a) 2.0, b) 4.0, c) 6.0, d) 8.0, and e) 10.0 ng mL^{-1} in the cell containing 1.1×10^{-5} M Calcon at pH 7.0; the calibration graph is given in the inset.

The accuracy of the method for aluminum was determined by replicate analyses of reference certified water (NIST – SRM 1640), using both ICP–MS and AdCSV. The certified aluminum level was given as 52.00 ± 1.50 ng mL^{-1}. The replicate measurements were done 4 times with ICP–MS and the aluminum level was found to be 53.01 ± 0.99 ng mL^{-1}. The aluminum level of the SRM with AdCSV was determined as 51.80 ± 1.23 ng mL^{-1}.

4. Conclusion

A chemometric approach to adsorptive cathodic stripping voltammetric determination of aluminum at ng mL^{-1} levels in salt samples based on metal complexation with Calcon has been presented. Calcon (1-(2-hydroxynaphthylazo)-2 naphthol-4-sulfonic acid) is less affected by calcium and zinc interferences than other complexes, but self-adsorption of the free ligand hinders the precise determination of aluminum as it competes with the complex for active sites of the electrode surface. Therefore, CCD has been followed to show that this reagent is more eligible than other complexes. Both chemical and instrumental variables were studied simultaneously to reveal their main trends and to find a set of suitable experimental conditions for the trace

determination of aluminum in dialysis and saline samples. The method has been validated with certified reference samples of fresh water with satisfactory results.

Acknowledgment

References

1. WHO. *Guidelines for Drinking Water Quality*, Vol. 1, Geneva, 1984.

2. Massey, R.; Taylor, D. *Aluminum in Food and the Environment*, Vol. 73, Royal Society of Chemistry, Cambridge, 1989.

3. Gayon, J. M. M.; Parajon, J. P.; Sanz-Medel, A.; Fellows, C. S. *J. Anal. Atom. Spectrom.* **1992**, *7*, 743–747.

4. Almeida, A. A.; Cardoso, M. I.; Lima, J. L. F. C. *J. Anal. Atom. Spectrom.* **1997**, *12*, 837–841.

5. Lopez, F. F.; Cabrera, C.; Lorenzo, M. L.; Lopez, M. C. *Sci. Total Environ.* **2000**, *257*, 191–198.

6. Tang, S.; Parsons, P. J.; Slavin, W. *Analyst* **1996**, *121*, 195–198.

7. de Amorim, F. R.; Bof, C.; Franco, M. B.; de Silva, J. B. B.; Nascentes, C. C. *Microchem. J.* **2006**, *82*, 168–173.

8. Barroso, M. F.; Ramos, S.; Oliva-Teles, M. T.; Delerue-Matos, C.; Sales, M. G. F.; Oliveira, M. B. P. P. *Food Addit. Contam. B* **2009**, *2*, 121–130.

9. Wang, J. *Analytical Electrochemistry*, 3rd ed., John Wiley & Sons, New York, 2006.

10. Wang, J.; Farias, F. A. M.; Mahmoud, J. S. *Anal. Chim. Acta* **1985**, *172*, 57–61.

11. Van den Berg, C. M. G.; Murphy, K.; Riley, J. P. *Anal. Chim. Acta* **1986**, *188*, 177–182.

12. Dawnar, A. J.; Powell, K. J.; Money, S. D. *Anal. Chim. Acta* **1997**, *349*, 111–116.

13. Lo Balbo, A.; Dall'Orto, V. C.; Sobral, S.; Rezzano, I. *Anal. Lett.* **1998**, *31*, 2717–2728.

14. West, T. S. *Complexometry with EDTA and Related Reagents*, BDH Chemicals Ltd., East Yorkshire, UK, 1969.

15. Metrohm, Application Bulletin No. 131/1 e, Metrohm AG, Herisau, Switzerland.

16. Yılmaz, S., *Voltammetric Determination of Aluminum in Different Samples*, MSc, Ege University Graduate School of Natural and Applied Sciences, İzmir, 2000.

17. Brereton, R. G. *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*, John Wiley & Sons, West Sussex, UK, 2004.