Determination of zinc by square-wave adsorptive stripping voltammetry using alizarin as a chelating agent

Ahmad H. Alghamdi *

Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

Abstract   The adsorptive collection of zinc(II) complex with alizarin ligand, coupled with the square-wave voltammetric technique at the hanging mercury drop electrode, yields a very sensitive electroanalytical procedure for the determination of zinc. The optimized experimental conditions include: supporting electrolyte (carbonate buffer), pH (11), alizarin concentration (1×10^{-6}! $mol\,l^{-1}$), accumulation time (60 s), accumulation potential (−0.1 V), scan rate (700 mV s^{-1}), pulse amplitude (0.06 V) and SW frequency (80 Hz). The monitored stripping voltammetric current was linear over the range of $5 \times 10^{-8} – 4 \times 10^{-7}\,mol\,l^{-1}$ and the detection limit was $1 \times 10^{-8}\,mol\,l^{-1}$. The relative standard deviation was calculated as 1.3% ($n = 10$) for $1 \times 10^{-8}\,mol\,l^{-1}$ Zn(II) and the obtained electrochemical signal was stabile for up to 60 min. Possible interferences by either co-existing metal ions or other chelating agents were also investigated. The applicability of the proposed SW-AdSV method to the analysis of foodstuff was assessed by the determination of zinc content in instant coffee samples. The accuracy of the obtained voltammetric analytical results was validated by comparing with that obtained by atomic absorption spectrometric method and conducting the necessary statistical evaluation.

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1. Introduction

Square-wave adsorptive stripping voltammetry (SW-AdSV) has received increasing attention as a stripping voltammetric technique for ultra-trace quantitative analysis because it can

* Tel.: +966 14676001; fax: +966 14675992.
E-mail address: ahalgamdy@gmail.com

be used to perform an electroanalytical measurement faster than conventional differential pulse voltammetry and with higher sensitivity. The unique features and problems of this outstanding electrochemical method are reported in several monographs (Brainina and Neyman, 1993; Wang, 1994; Dewald, 1996). SW-AdSV procedure is well suited for the determination of ultra-trace amounts of inorganic ions through the adsorbed complexes. The applications of this advanced electrochemical method to the determination of various metal ions have been reviewed elsewhere (Brainina et al., 2000; Abu Zuhri and Wolfgang, 1998; Zaitsev et al., 1999).

In adsorptive stripping voltammetric approach, the metal ion of interest is absorbed on the working electrode by means of a non-electrolytic process prior to the voltammetric scan. By this electroanalytical technique, the analytes are determined as

complexes with different organic ligands adsorbed on the electrode surface. Over the years a large number of chelating agents of various classes were used for the determination of more than sixty elements in almost all groups of periodic system of elements (Zaitsev et al., 1999). Dimethylglyoxime (DMG), 8-hydroxyquinoline (Oxine), nioxime, catechol, cupferron, tropolone and solochrome violet RS are some selected examples of such very popular chelating agents. Alizarin is a natural dye and chelating agent, which has been used successfully in the stripping voltammetric determination of some metals including Mo (Tyszczuk and Korolczuk, 2008), Al(III) (Wang et al., 1987), In(III) (Zhao et al., 1996).

The determination of trace concentration levels of zinc is of interest in different fields such as environmental surveillance, food control and occupational hazards (Venugopal and Luckey, 1978; Underwoodm, 1977). Because of the industrial significant of zinc and as well as its environmental concern, a sensitive and yet simple analytical method for its trace determination is required. Accordingly, adsorptive stripping voltammetry has been effectively employed for the determination of zinc ions in various real samples after its prior complexation with some organic surface-active substances such as Morin (Shams et al., 2004), 2-quinolinethiol (Paneli and Voulgaropoulos, 1994, tetramethylene dithiocarbamate (TMDC) Hernandez-Brito et al., 1993, ammonium pyrrolidine dithiocarbamate (APDC) Ornella et al., 1995; Van, 1985, propyl gallate (Young et al., 2003), carbidopa (Gholivand et al., 2007) and xylenol orange (Colston and Robinson, 1997).

The present work examines the reliability and validity of SW-AdSV electrochemical method for the determination of trace amounts of zinc in food samples via the application of alizarin as a chelating agent.

2. Experimental

2.1. Apparatus

All square-wave adsorptive stripping measurements were carried out with 797 AV computrace (Metrohn, Herisau, Switzerland) in connection with Dell computer and controlled by (VA computrace 2.0) control software. Stripping voltammograms were obtained via a Hewlett–Packard laser jet printer. A conventional three electrode system was used in the hanging mercury drop electrode (HMDE) mode. This three electrode system was completed by means of a platinum auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode. Atomic absorption spectrometric determination of zinc metal was achieved by Perkin–Elmer Flame Atomizer (Model 2280). The pH was measured with Metrohn 744 pH meter. Oxford adjustable micropipette (Ireland) was used to pipette microliter volumes standard solutions.

2.2. Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Stock solution of Zn(II) of 1×10^{-2} mol^{-1} was prepared by dissolving the appropriate amount of nitrate salts (BDH, UK) in distilled water in 100-ml volumetric flasks. A 1×10^{-2} mol^{-1} stock solution of alizarin (BDH, UK) was prepared by dissolving an accurately weighed amount of pure substances. Standard solutions of zinc

metal ions or ligands with lower concentration were prepared daily by diluting the stock solutions with distilled water. Britton–Robinson supporting buffer (pH $\approx$ 2, 0.04 mol l^{-1} in each constituent) was prepared by dissolving 2.47 g of boric acid (Winlab, UK) in 500 ml distilled water containing 2.3 ml of glacial acetic acid (BDH, UK) and then adding 2.7 ml of ortho-phosphoric acid (Riedal-deHaen, Germany) and diluting to 1 l with distilled water. The carbonate buffer was 0.1 mol l^{-1} in both sodium hydrogen carbonate (Winlab, UK) and disodium carbonate (BDH, UK), while phosphate buffer was prepared from 0.1 mol l^{-1} in both phosphoric acid (Riedal-deHaen, Germany) and sodium dihydrogen phosphate (Winlab, UK). The acetate buffer was prepared from 0.02 M in both sodium acetate (Winlab, UK) and acetic acid (BDH, UK). Five different brands of popular instant coffee samples were purchased from city of Riyadh markets.

2.3. Procedure

The general procedure adopted for obtaining square-wave adsorptive stripping voltammograms was as follows: a 20 ml aliquot of an appropriate buffer at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of the alizarin chelating agent and zinc metal ion were added. The test solutions were purged with nitrogen for 5 min initially, while the solution was stirred. The accumulation potential of -0.1 V versus Ag/AgCl was applied to a new mercury drop while the solution was stirred for 60 s (unless otherwise stated). Following the preconcentration period, the stripping was stopped and after 10 s had elapsed, cathodic scans were carried out over the range from 0.0 to -1.3 V. For the analysed foodstuff samples (instant coffee) a pretreatment conventional wet digestion method utilizing acid mixture was applied. All measurements were obtained at room temperature.

3. Results and discussion

3.1. Preliminary observations

For test solution (acetate buffer pH 3) containing 5×10^{-6} mol l^{-1} Zn(II), preliminary stripping voltammetric measurements should that zinc ions exhibited a very weak and nearly flatted electrochemical peak at $E_p = -958$ mV (versus Ag/AgCl reference electrode) as can be seen from Fig. 1 (line A). However, the addition of 3×10^{-7} mol l^{-1} alizarin to the previous acetate supporting electrolyte solution provided a well-defined (line B) cathodic peak at -952 mV. In fact, the zinc ions exhibited a good affinity towards alizarin molecules forming a very stable alizarin–Zn(II) complex which is strongly adsorbed onto the HMDE surface. This obtained well-developed stripping voltammetric peak was found to response perfectly to the extra addition of either 5×10^{-7} mol l^{-1} alizarin or 5×10^{-7} mol l^{-1} Zn(II) (lines C and D, respectively), which probably reflect the formation and adsorption of the suggested complex. The observed SW-AdSV peak is most probably due to the cathodic reduction of Zn(II) in the adsorbed complex with alizarin and the electrochemical mechanism of the reduction process of alizarin–Zn(II) complex is illustrated in Fig. 2.

Clearly, this proposed electrochemical reduction mechanism suggested an irreversible reductive process for zinc ions
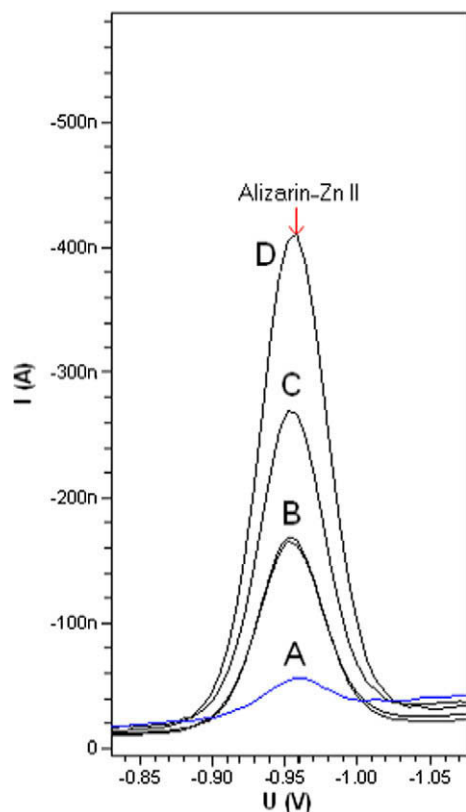
Figure 1 Square-wave adsorptive stripping voltammetric scan of Zn(II) in the presence of alizarin: A = acetate buffer pH 3 + 5×10^{-7} mol l^{-1} Zn(II), B = A + 3×10^{-7} mol l^{-1} alizarin, C = A + B + 5×10^{-7} mol l^{-1} alizarin and D = A + B + C + 5×10^{-7} mol l^{-1} Zn(II).

Figure 3 Cyclic voltammogram for the formed complex between 1×10^{-7} mol l^{-1} Zn(II) and 5×10^{-7} mol l^{-1} alizarin in pH 3 acetate buffer at 50 mV s^{-1} scan rate.

succeeding cathodic scans exhibit a gradual decrease in the voltammetric peak intensity, that seemed to indicate the adsorptive characteristic of this complex at the surface of the employed working electrode. Anyhow, the interfacial accumulation of this ligand–metal ion complex onto the HMDE surface can be used as an effective accumulation step in order to enhance the electroanalytical determination of zinc ions.

3.2. Optimum parameters and experimental conditions

3.2.1. Effect of pH

The sensitivity of the SW-AdSV approach for the electrochemical analysis of Zn(II) metal ion via their formed complex with alizarin, is strongly dependent upon the pH and nature of supporting buffer which are the main factors influencing the formation of the observed complex, its stability, its adsorption process and its electrochemical reduction process. Among the investigated supporting electrolytes (acetate, phosphate, carbonate and Britton–Robinson buffer) at different pH values, the best voltammetric peak resolution and sensitivity have been observed when utilizing 0.1 mol l^{-1} carbonate buffer, which will be selected for all subsequent experiments.

The influence of pH value of the used buffer solution on the monitored stripping voltammetric peak current and potential was also investigated. When the SW-AdSV peak current of 5×10^{-7} mol l^{-1} Zn(II) in the presence of 1×10^{-6} mol l^{-1} alizarin was measured as a function of pH over the range 7.5–11.5, the zinc voltammetric signal was found to increase steadily over the examined pH range as can be seen from Fig. 4. How-

in the adsorbed complex, an assumption which was confirmed by cyclic voltammetric measurement of 1×10^{-7} mol l^{-1} Zn(II) ions and 5×10^{-7} mol l^{-1} alizarin chelating agent in pH 3 acetate buffer at 50 mV s^{-1} scan rate. As can be noticed from Fig. 3, which exhibits the cyclic voltammogram of alizarin–Zn(II) complex, the absence of the anodic peak at the reverse scan for the stripping voltammetric peak at −831 mV confirmed the irreversible nature of the evaluated reduction process. The first electrochemical peak observed at 655 mV in the given cyclic voltammogram is more probably resulted from the cathodic reduction of the electroactive anthraquinone moiety in the alizarin free molecules. Furthermore, when repetitive cyclic voltammetric measurements for alizarin complex with Zn(II) were carried out, a well-developed SW-AdSV peak was observed at −841 mV for the first cathodic scan, however,
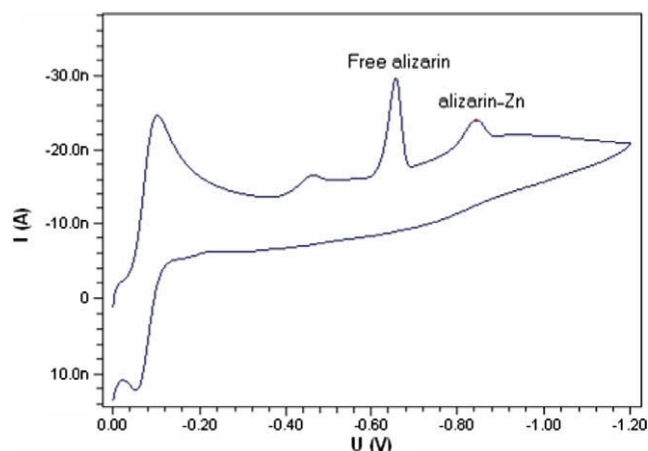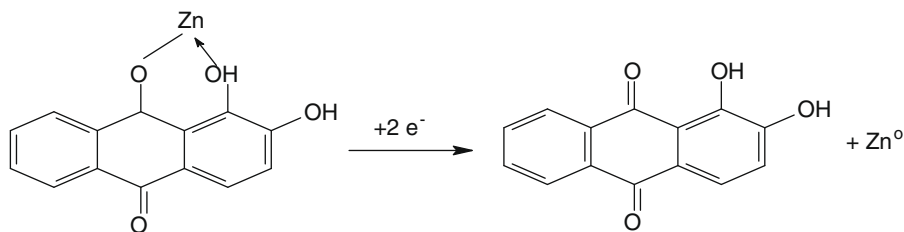

Figure 2 Mechanism of the electrochemical reduction process for alizarin–Zn(II) complex.

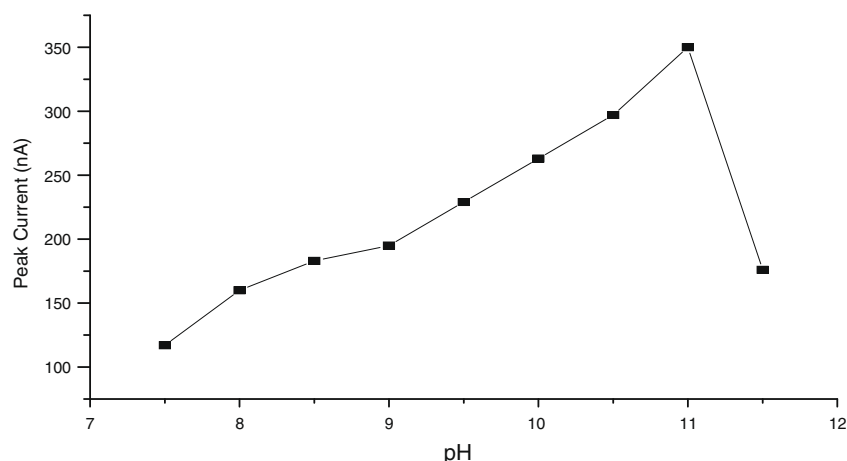**Figure 4** Effect of pH on SW-AdSV signal of 5×10^{-7} mol l^{-1} Zn(II) with 1×10^{-6} mol l^{-1} alizarin in acetate buffer after 90 s accumulation time at 0.0 V accumulation potential and 500 mV s^{-1} scan rate.

ever, a sharp peak current decline was observed at pH 11.5, thus carbonate buffer solution with pH 11 seems to be the appropriate choice to ensure the highest voltammetric peak current. In addition, it was observed that the peak potentials for Zn/alizarin complex exhibited a shift in the negative direction by 149 mV, when pH value was varied over the 7.5–11.5 pH range.

3.2.2. Effect of alizarin ligand concentration
Varying the concentration of alizarin chelating agent also plays an important role in the investigated stripping voltammetric procedure. Fig. 5 indicates that an increase of alizarin concentration from 1×10^{-7} to 1×10^{-6} mol l^{-1} at a constant Zn(II) concentration of 1×10^{-7} mol l^{-1} initiate a steady enhancement for the current intensity of the monitored SW-AdSV signal. However, addition of higher alizarin concentration level (2×10^{-6} mol l^{-1} – 4×10^{-6} mol l^{-1}) caused leveling off of the voltammetric peak height. Accordingly, it seems that alizarin concentration of 1×10^{-6} mol l^{-1} might be adequate to ensure maximum stripping voltammetric, hence, for further work this chelating agent concentration was selected.

3.2.3. Effect of accumulation time and potential
Fig. 6 illustrates the relationship of the stripping voltammetric peak current versus accumulation time for 1×10^{-7} mol l^{-1} Zn(II) with 1×10^{-6} mol l^{-1} alizarin over various adsorption times. Variation of the accumulation time over the range 0–150 s at a preconcentration potential of 0.0 V showed a gradual enhancement for Zn(II) SW-AdSV peak current up to 60 s and decreased with longer preconcentration times. As a consequence, accumulation time of 60 s was selected as an optimum for further studies.

When the adsorption of formed alizarin–Zn(II) complex was measured as a function of accumulation potential at 60 s collection time over the range from $+0.2$ V to -0.8 V, it was observed that monitored peak current increased slightly when applying -0.1 V as the optimal accumulation potential.

3.2.4. Effect of potential sweep conditions
The scan rate parameter of the voltammetric technique was also varied in order to establish the optimum scan rate for the developed SW-AdSV procedure. It was found that the aliz-
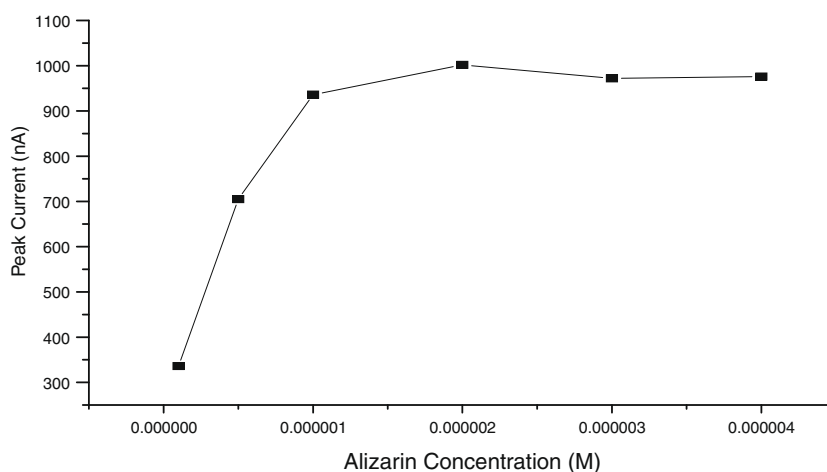

Figure 5 Effect of alizarin concentration on the stripping voltammetric peak current of 5×10^{-7} mol l^{-1} Zn(II) in pH 11 acetate buffer after 90 s accumulation time at 0.0 V accumulation potential and 500 mV s^{-1} scan rate.
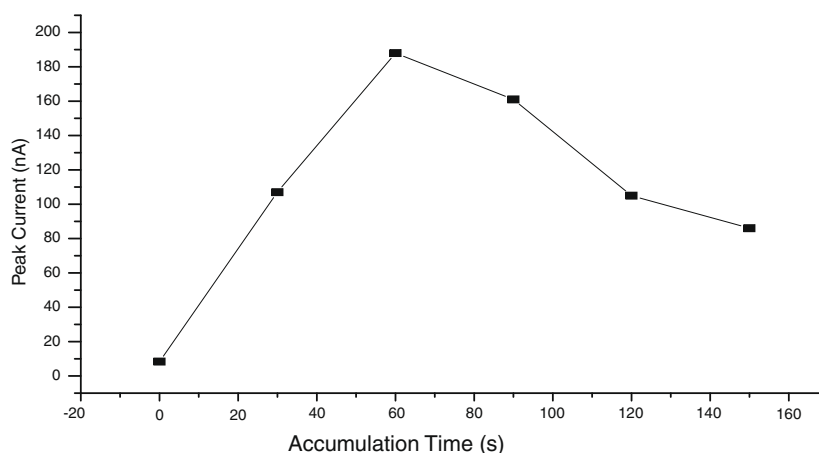
Figure 6 Effect of accumulation time on the SW-AdSV peak current of 1×10^{-7} mol l^{-1} Zn(II) with 1×10^{-6} mol l^{-1} alizarin in pH 11 acetate buffer. Accumulation potential: 0.0 V and scan rate: 500 mV s^{-1}.
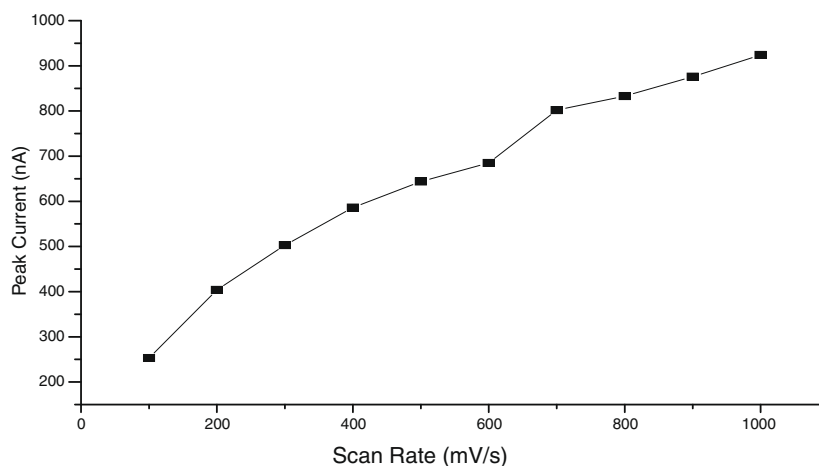

Figure 7 Effect of scan rate on the stripping voltammetric peak height of 1×10^{-7} mol l^{-1} Zn(II) with 1×10^{-6} mol l^{-1} alizarin in pH 11 acetate buffer. Accumulation time: 60 s and accumulation potential: -0.1 V.

arin–Zn(II) SW-AdSV peak height increased gradually, over 100–1000 mV s^{-1} scan rate range. However, the width and the shape of the examined stripping voltammetric peak slightly distorted when utilizing scan rate higher than 700 mV s^{-1}. Consequently, 700 mV s^{-1} scan rate is recommended as an optimal for subsequent electroanalytical studies. The dependence of SW-AdSV peak currents on scan rate is presented in Fig. 7.

Moreover, the impact of varying the square-wave frequency on the SW-AdSV current intensity was also evaluated. The effect of this operating variable was studied over the range 20–90 Hz and it was concluded that in order to assure maximum peak current, 80 Hz square-wave frequency was the ideal choice for this operational parameter. Additionally, varying the value of excitation wave pulse amplitude also plays an important role for the measured stripping voltammetric current intensity. Increasing this parameter over the range 0.01–0.1 V, resulted in a substantial enhancement of the voltammetric peak current up to 0.06 V value and gradually decreased thereafter. Hence, for further electrochemical investigation, 0.06 V excitation wave pulse amplitude value was adopted.

3.2.5. Effect of instrumental parameters
The monitored SW-AdSV peak current can be further maximized by optimizing other experimental factors that can affect the adsorption accumulation process for the formed alizarin–Zn(II) complex. The influence of the surface size of the mercury drop working electrode on the observed stripping voltammetric response was evaluated. The relationship between the measured peak current and the surface area of the drop was found to be linear for the surface areas of 0.15–0.60 mm^2. For optimal sensitivity, 0.60 mm^2 drop surface area (drop size 8) will be used for all next electrochemical measurements. In addition, the effect of convection rate was also studied by ascending the stirring rate from 100 to 3000 rpm. Although 3000 rpm stirring rate provided the maximum peak height, yet, 2000 rpm stirring speed was chosen as optimum value, which provided adequate preconcentration effect without affecting the drop stability.

3.3. Analytical performance

Once the most ideal and suitable chemical conditions and instrumental parameters for the stripping voltammetric deter-

Table 1 Optimal conditions for voltammetric SW-AdSV measurement of Zn(II).

Parameter	Studied range	Optimal
Supporting buffer	Phosphate, B–R buffer, carbonate, acetate	Carbonate buffer
pH	pH 7.5–11.5	pH 11
Alizarin concentration	1×10^{-7}–4×10^{-6} mol l^{-1}	1×10^{-6} mol l^{-1}
Accumulation time	0–150 s	60 s
Accumulation potential	$+0.3$ V–(-0.8) V	-0.1 V
Scan rate	100–1000 mV s^{-1}	700 mV s^{-1}
SW frequency	20–90 Hz	80 Hz
Pulse amplitude	0.01–0.1 V	0.06 V
Drop size	2–8	Size 8
Stirrer speed	100–3000 rpm	2000 rpm

mination of zinc metal ions were established (Table 1), calibration plot for this metal ion was recorded to estimate the analytical characteristics of the developed SW-AdSV method.

3.3.1. Calibration graph and detection limit

The dependence of alizarin–Zn(II) voltammetric peak current on the concentration of Zn(II) was obtained when successive additions of the standard Zn(II) solution were added to the voltammetric cell. As represented in Fig. 8, a proportional and linear relationship between Zn(II) and the monitored SW-AdSV signal was observed. Least squares analysis of the studied concentration range (5×10^{-8}–4×10^{-7} mol l^{-1}) yielded the following regression equation:

$$i_p \ (nA) = 911 + 5.94 \times 10^9 \ C \ (mol \ L^{-1}) \quad r = 0.996 \quad n = 5$$

where i_p is the stripping voltammetric peak current in amperes, C is the analysed zinc metal ion concentration and r is the correlation coefficient.

The effective preconcentration during the adsorption process of the formed complex makes it possible to achieve a very low detection limits. For this metal ion analysed via the optimized SW-AdSV procedure, the detection limit of 1×10^{-8} mol l^{-1} was calculated based on the signal-to-noise (S/N = 3) characteristic.

3.3.2. Reproducibility and stability

The high sensitivity of SW-AdSV approach is also accompanied by good precision of the obtained results. This can be attributed to the reproducible HMDE surface area and adsorption process for the formed alizarin–Zn(II) complex. Under the previously experimental optimized conditions, the reproducibility of the measured analytical signals was checked from 10 successive measurements of 1×10^{-7} mol l^{-1} Zn(II) in the presence of 1×10^{-6} mol l^{-1} alizarin. The precision of the method in terms of relative standard deviation was 1.3 RSD%. The stability of 1×10^{-7} mol l^{-1} Zn(II) solution was also investigated and monitored electrochemical signal was fond to give nearly a constant voltammetric current intensity up to 60 min.

3.3.3. Interference studies

The major sources of interferences are likely to be from co-exciting metal ions capable of forming complexes with the used chelating agent, hence, affecting SW-AdSV response via an overlapping peak or competing for adsorptive sites or available ligand. The effect of the following ions, which are of great significance in industrial or environmental matrices, was tested at the optimal experimental conditions. As a matter of fact, the interfering metal ions were added at different concentrations
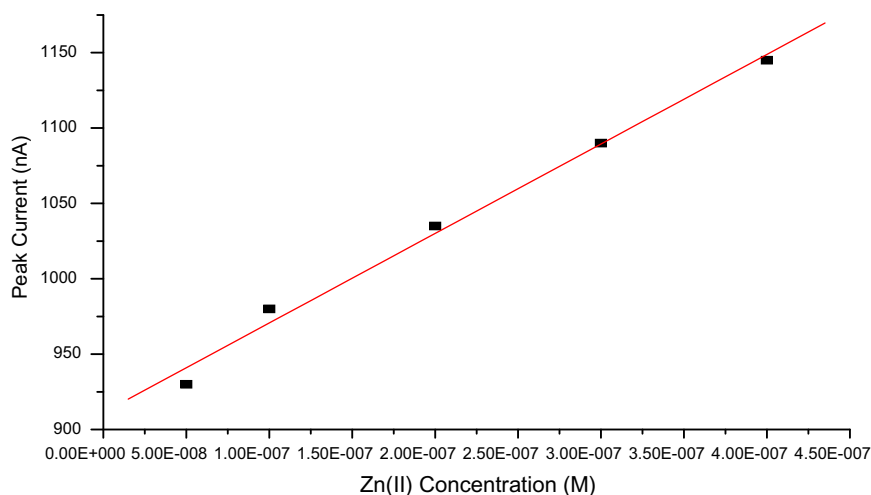

Figure 8 Calibration graph for Zn(II) in the presence of 1×10^{-6} mol l^{-1} alizarin in pH 11 acetate buffer after 60 s accumulation time at -0.1 V accumulation potential and 700 mV s^{-1} scan rate.

Table 2 Practical application of SW-AdSV for the determination of zinc content in instant coffee samples and comparison with the results obtained by AAS technique.

Instant coffee brand	SW-AdSV (ppm)	AAS (ppm)	Paired t-test value
Nescafe Red Mug 1	507 ± 4	501 ± 30	t = 0.4
Nescafe Red Mug 2	707 ± 21	703 ± 40	t = 0.2
Nescafe Kanjara	719 ± 19	717 ± 43	t = 0.1
Bon café Classic	652 ± 3	663 ± 26	t = 0.7
Zidnee Classic	709 ± 34	714 ± 6	t = 0.2

(1-fold, 5-fold and 20-fold) higher than the concentration of Zn(II) (1×10^{-7} mol l^{-1}). Actually, very mild or no significant interference was observed upon adding 1-fold or 5-fold concentration of Pb(II), Fe(III), Fe(II), Al(III) and Cd(II). However, the presence of Cd(II) at higher concentration (20-fold) caused 19.8% decrease in zinc voltammetric peak current. On the other hand, the noticeable interference influence was observed when Cu(II) and Ni(II) metal ions were present in the test solution especially at higher concentration levels (20-fold). In fact, the monitored Zn(II) voltammetric peak height increased by 34% and 38% of its original height after the addition of 20-fold of Cu(II) and Ni(II), respectively.

Furthermore, the competitive effect of complexing agents on the Zn(II) SW-AdSV peak current was modeled by the additions of 5×10^{-7} mol l^{-1} and 1×10^{-6} mol l^{-1} of some other chelating agent such as EDTA, DMG and cupferone to the test solution. There was totally no effect for the addition of EDTA and DMG chelating agents at these concentration level on the stripping voltammetric peak current of Zn(II) metal ion. However, a very mild increase in the SW-AdSV peak height by 5.7% after the addition of 1×10^{-6} mol l^{-1} cupferone ligand.

3.4. Analytical applications and accuracy

In order to validate the practical reliability of the optimized SW-AdSV method, five different foodstuff samples (namely instant coffee) were analysed for their zinc contents by the recommended voltammetric procedure (Table 1) after conventional wet digestion. In fact, zinc concentrations were calculated via the standard addition approach in order to minimize the sample matrix interference. Typical analytical results correspond to the mean values of four replicate determinations are summarized in Table 2. The accuracy of the developed electroanalytical method was verified by comparing the analytical results obtained by SW-AdSV method with that obtained by atomic absorption spectrometric (AAS) method for the same instant coffee samples. As can be seen from Table 2, the results obtained by the developed voltammetric method compared favorably with those obtained by the spectroscopic reference method. The agreement of the compared result was tested by the paired t-test statistical approach (Miller and Miller,

1994). The means of both analytical methods were found to be not differ significantly, since all the calculated t-test value were less than the critical value (2.45) at the 95% confidence level ($P = 0.05$). There is no statistical evidence that the means of the proposed voltammetric method differ significantly from the means of the reference methods (null hypotheses is obtained).

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