Simultaneous determination of Cd(II) and Cu(II) using stripping voltammetry in groundwater, soil and *Alhagi maurorum* plants in industrial and urban areas in Northern Border, Saudi Arabia with luminol as a chelating agent

Ahmed Farouk Al-Hossainy

**ABSTRACT**

The cathodic stripping voltammetry of Cu(II) and Cd(II) speciation was re-optimized by using luminol (Lu) in groundwater, soil and *Alhagi maurorum* plants, finding differences with the pre-existing method and a different interpretation for the electroactive species. The main findings are that optimum sensitivity is obtained at 0.3–142.5 ng/mL and 0.065–60.0 ng/mL for copper and cadmium, respectively, that the complexes responsible for adsorption on the electrode are CuLu and CdLu, and that the sensitivity of the method is much improved in the absence of dissolved oxygen. The limit of detection of the method was 0.011 ± 0.001 ng/mL for Cu(II) and 0.013 ± 0.001 ng/mL for Cd(II). The interference of some common ions: Cr(III), Fe(III), Zn(II), Ni(II), Co(II) and Mo(II) was studied. It was concluded that application of this method for the determination of Cu(II) and Cd(II) in groundwater, soil and *Alhagi maurorum* plants led to satisfactory results.

**Key words** | adsorptive stripping voltammetry, copper and cadmium, groundwater, luminol ligand, soil and *Alhagi maurorum* plants

**INTRODUCTION**

In the last few decades, analytical methods based on different concepts and experimental procedures in biological and human samples have become very important (Rashed et al. 2010). The ideal speciation technique should be able to quantify the amount of free and bound metal in the species, which seems to play an important role in bioavailability and consequently on the toxicity of metals (Beltagi & Ghoneim 2009; Iwona & Mieczyslaw 2014). Spectroscopic techniques, such as graphite furnace atomic absorption spectrometry (GF-AAS) and inductively coupled plasma mass spectroscopy (ICP-MS), but also neutron activation analysis have very low detection limits and have been widely used for trace metal analysis. However, these methods can be used for speciation analysis only by coupling them to separation procedures (Sergio et al. 2015).

Copper is essential for a variety of biochemical processes (Ivo et al. 2015) and is needed for certain critical enzymes to function in the body (Guanghan et al. 2004). Copper is also involved in the functioning of the nervous system, in maintaining the balance of other useful metals in the body such as zinc and molybdenum, and other bodily functions. Copper deficiency causes ischaemic heart disease, anaemia; bone disorders (Karen et al. 2015). The maximum tolerable daily intake for copper is 0.5 mg/kg body weight (Sudkate et al. 2015).

Cadmium is considered to be one of the most toxic elements for living organisms. The environmental persistence of the metal in concert with their intensive use by modern society has, over the years, created a concentration in the biosphere. Continuous exposure to low levels of cadmium may result in bioaccumulation and health effects, by both occupational and environmental exposition (Eliza et al. 2014). The determination of copper and cadmium in biological materials using the flame atomic absorption spectrometry method poses several problems, mainly due to the low concentrations of these metals, in which a great variation of the matrix from sample to sample and contamination has been obtained (Nail & Ramazan 2015).

Adsorptive stripping voltammetry (AdSV) is an extremely sensitive electrochemical technique for measuring trace metal concentrations in biological samples (Mariola et al. 2015; Paulo et al. 2015). The method is based on the
formation and interfacial accumulation of a metal complex on the working mercury electrode and subsequent measurement of the reduction peak of the accumulated complex. This technique has been used to measure the trace element concentrations in different matrices, such as water (Hong et al. 2015), soil (Danielle et al. 2014) and plants (Abbas et al. 2012).

Luminol \((C_8H_7N_3O_2)\) is a versatile chemical that exhibits chemiluminescence, with a striking blue glow, when mixed with an appropriate oxidizing agent. Luminol is a white-to-pale-yellow crystalline solid that is soluble in most polar organic solvents, but insoluble in water (Ernest et al. 1934). Luminol behavior in aqueous NaOH and \(H_2SO_4\) solutions has been studied by steady-state fluorescence and time-resolved single-photon counting techniques (Jian et al. 2010). In addition, a selective method is presented for the simultaneous determination of copper and cadmium in food samples by AdSV. In preliminary studies, it has been proven that copper and cadmium react with luminol, giving rise to the formation of Cu–Lu and Cd–Lu complexes. These complexes have adsorptive characteristics on the hanging mercury drop electrode (HMDE) and can be reduced in a reduction step (Shahryar et al. 2011).

In this work, a sensitive adsorptive stripping voltammetric method was used for the simultaneous determination of copper and cadmium in biological samples. At the same time, the results were compared with those obtained by both AAS and inductively coupled plasma-atomic emission spectrometry (ICP-AES). A new sensitive and rapid adsorptive voltammetric method for the determination of ultra-trace levels of copper and cadmium in groundwater, soil and Alhagi maurorum plants was developed based on AdSV of copper and cadmium complexes with luminol. In addition, the effect of environmental areas on samples metal levels was studied. The procedure is based on the reduction of the complexes of those metal ions with luminol after accumulation at the surface of an HMDE.

1. Polluted areas include the following:
   - Arar is located in the north of Saudi Arabia, at 30°54′21″N, 41°8′20″E, in the heart of a vast rocky limestone plain. It lies about 1,100 km northwest of Riyadh, and about 60 km from the Iraqi border.
   - Turaiif is a town in Northern Borders Province, Saudi Arabia, close to the border with Jordan. It is located at a bend in Highway 85 as it turns west to Jordan. It is located at around 31°40′39″N, 38°39′11″E.
   - Sakakah is an oasis town in northwestern Saudi Arabia and is the capital of Al Jawf Province. It is located just to the north of the An Nafud desert. It is located at around 29°58′11″N, 40°13′32″E.
2. Unpolluted areas include Aluwayqilah, Zalom and Hazem Algalamid:
   - Aluwayqilah is located in the north of Saudi Arabia, at 30°22′5.09″N, 42°13′55.05″E (bend in highway 85). It lies about 950 km northwest of Riyadh.
   - Zalom is a town in Northern Borders, Saudi Arabia (bend in highway 80). It is located at around 30°10′1.69″N, 40°17′46.94″E.
   - Hazem Algalamid is a town and crossroads in Saudi Arabia near the Iraqi border. It is located at around 31°16′54.40″N, 40°6′13.46″E.

**Reagents**

All the chemicals were analytical grade and were used without further purification. The standard \(\text{Cu(II)}\) and \(\text{Cd(II)}\) solutions were obtained from reference material (National Institute of Standards & Technology 2008) at a concentration of 1 mmol L\(-1\). A 1.0 × 10\(^{-3}\) M solution of luminol (Sigma Aldrich, St Louis, MO, USA) was prepared by dissolving the appropriate amount of luminol in NaOH (Merck, Kenilworth, NJ, USA) 1.0 M. Borate buffer solutions were used for fixing the pH in the range of 5.5–10.0.

The NaBO\(_3\) (3.5 g of Aldrich product number 24,412-0) was added to 500 mL distilled water and thoroughly dissolved. Then, NaCO\(_3\) (25 g) and luminol (9.5 g) were added and dissolved. The solution was then decanted into a plastic spray bottle, ready to use. It was applied as a fine mist on the surface to be tested.

**Samples**

One gram of each soil sample from S\(_2\) (soil without digestion at the surface) and S\(_3\) (soil without digestion at 50 cm depth from the ground surface) was stirred in
bidistilled water for 1 hour and then filtered. The filtrate of S2 and S3 were transferred into a 100 mL measuring flask and diluted to the required volume (100 mL) by bidistilled water. The second filtrate sample S4 (soil with digestion at the surface) and S5 (soil with digestion at 50 cm depth from the ground surface) were mixed with 5 mL concentrated nitric acid and evaporated to dryness (Benjamin et al. 2015). The residue was dissolved in the bidistilled water and transferred into a 100 mL measuring flask and diluted to the required volume with bidistilled water.

In addition, 1 g of each *Alhagi maurorum* plant (polluted and unpolluted areas) samples S6 (leaves), S7 (stems) and S8 (roots) after drying and grinding were dissolved in 5 mL concentrated nitric acid and evaporated to dryness. All glassware and polyethylene bottles were soaked in 2 M nitric acid for at least 1 week, washed 3 times with bidistilled water and finally soaked in 0.1 M hydrochloric acid until ready for use.

**Instrumentation**

For the voltammetric measurements, a fully computerized Electrochemical Trace Analyzer Model 273 APAR (EG&G) was used, interfaced with an electrode assembly model 303A (EG&G) of a static mercury drop electrode in the HMDE mode \((A = 2.6 \times 10^{-2} \text{ cm}^2)\). Stirring was performed with a Teflon coated bar at about 400 rpm using a magnetic stirrer (IKA Labortechnik, Staufen, Germany), Ag/AgCl (in saturated KCl), reference electrode and an auxiliary electrode of a platinum wire. At the beginning of the experiment, the solutions were deoxygenated with high purity nitrogen for 10 min, whereas before each analysis step, the solution was deoxygenated for 10 min.

**Experimental procedure**

Standard solutions of luminol (2 mL, \(1.0 \times 10^{-4} \text{ M}\)), borate buffer (pH 7.5), (5 mL, 0.10 M) and different concentrations of Cu(II) and Cd(II) were added to an electrochemical cell, and the volume was made up to 10 mL with ethanol (Shahryar et al. 2011). A nitrogen flow was applied for 10 min to remove the electroactive molecular oxygen. The accumulation potential \((-0.01 \text{ V})\) was applied to a fresh mercury drop for 60 s while the solution was stirred. Following the accumulation period, the stirring was stopped, and after 10 s the voltammograms were recorded by applying a negative going potential. Each scan was repeated four times with a new drop for each analyzed solution and the mean of the voltammograms was obtained. The currents of Cu(II) and Cd(II) were used as a measure of ion concentrations. All data were obtained at ambient temperature. Experimental conditions for the simultaneous determination of Cu(II) and Cd(II) in underground water by differential pulse adsorptive stripping voltammetry (DPASV) and differential pulse anodic stripping voltammetry (DPASV) are presented in Table 1.

**Quality assurance**

The reliability of the procedure for determining ultra-trace Cu(II) and Cd(II) in underground water by DPASV and DPASV has been checked by analyzing standard reference materials. The standard reference material (National Institute of Standards & Technology 2008) was analyzed for copper and cadmium. The results agree with certified values, Table 1. The validity of the method was further determined by cross-method check, spike recovery and replicate analysis.

### Table 1 | Experimental conditions for the simultaneous determination of Cu(II) and Cd(II) in underground water and reference material (WMP8435) by differential pulse AdSV and differential pulse anodic stripping voltammetry

<table>
<thead>
<tr>
<th>Metals</th>
<th>Method</th>
<th>(D_p \text{ (mV)})</th>
<th>(F_p \text{ (mV)})</th>
<th>(D_t \text{ (s)})</th>
<th>(S_c \text{ (mV/s)})</th>
<th>Certified (ppm)</th>
<th>Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td>DPASV</td>
<td>-152</td>
<td>0.00</td>
<td>90</td>
<td>10</td>
<td>0.4600</td>
<td>0.45875</td>
</tr>
<tr>
<td></td>
<td>DPASV</td>
<td>-555</td>
<td>0.00</td>
<td>60</td>
<td>100</td>
<td>0.4600</td>
<td>0.46008</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>DPASV</td>
<td>-600</td>
<td>+0.05</td>
<td>150</td>
<td>10</td>
<td>0.0002</td>
<td>0.00019</td>
</tr>
<tr>
<td></td>
<td>DPASV</td>
<td>-729</td>
<td>-0.90</td>
<td>60</td>
<td>100</td>
<td>0.0002</td>
<td>0.00021</td>
</tr>
</tbody>
</table>

Supporting electrolyte, HMDE: hanging mercury drop electrode; \(D_p\): deposition potential; \(F_p\): final potential; \(D_t\): deposition time; \(S_c\): potential scan rate.
RESULTS AND DISCUSSION

Luminol (Lu) can act as a bidentate chelating agent, coordinating with both a metal ion at the amino group and one of the hydrazide carbonyls to form a six-membered ring. The divalent metals of Cu(II) and Cd(II) can form stable 1:2 complexes with Schiff bases such as luminol, Scheme 1.

Adsorptive and voltammetric characteristics of the Cu(II) and Cd(II)-luminol complexes

Adsorptive stripping voltammograms of the mixture of metal ions Cu(II) and Cd(II) and luminol system between \(-0.3\) and \(-0.9\) V in a solution containing different concentrations of these metals under optimal conditions are shown in Figure 1(a). Two separated peaks for the reduction of cadmium and copper complexes with luminol were observed, which were due to the difference in the thermodynamic stability of their complexes. Comparison of the voltammograms revealed that the height of the copper and cadmium peaks depends not only on the duration of the pre-concentration step, but also on the stripping initial potential, which revealed the adsorptive nature of the response (Meijiao et al. 2015).

The influence of pH using (BO₃)⁻ media on the cathodic stripping peak currents of Cu(II) and Cd(II) was studied in the pH range of 5.5–10.0. It was found that at pH 7.5 the...
peak currents of cadmium and copper were at their maximum values. Thus, pH 7.5 was adopted for further studies. An optimum luminol concentration of $1 \times 10^{-4}$ M was selected for further experiments.

The limit of detection (LOD), expressed as the concentration ($c_1$), or the quantity ($q_1$), is derived from the smallest measure ($x_1$), that can be detected with reasonable certainty for a given analytical procedure. The value of $x_1$ is given by the equation:

$$x_1 = \bar{x}_{bi} + k s_{bi}$$

where $x_{bi}$ is the mean of the blank measures, $s_{bi}$ is the standard deviation of the blank measures and $k$ is a numerical factor chosen according to the confidence level desired, which have been recently reported by the International Union of Pure and Applied Chemistry (IUPAC 1997). Conversely, according to Currie (1995, 1997, 1999), the IUPAC and International Organization for Standardization (ISO), detection limits (minimum detectable amounts) are based on the theory of hypothesis testing and the probabilities of false positives $\alpha$, and false negatives $\beta$. However, quantification limits are defined in terms of a specified value for the relative standard deviation.

A comparison with other previous research is summarized in Table 2, in which it explains LOD, linear in the concentration range (LR) and interferences for real samples using different techniques. The given detection limits of the elements under investigation revealed that the proposed scheme of analysis under optimal conditions is very sensitive and useful for ultra-trace determination of copper and cadmium elements. The detection limits LOD (µg/L) of the investigated metals, defined as the metal concentration yielding an analytical peak equal to the minimum detectable one, can be calculated as: $LOD = 5(SD/m)$, where, SD is the standard deviation of the blank and $m$ is the slope of the calibration line (Jonatas et al. 2012).

The pre-concentration time is short, 60 s, and the limit of detection and linear dynamic range of method are very good. The majority of Zn$^{2+}$, Ba$^{2+}$, Mn$^{2+}$, Ca$^{2+}$ cations and CN$^-$ anions less than 100-fold excess do not interfere with the determination of copper and cadmium. It was found that there was no intermetallic effect between copper and cadmium ions in this system.

### Determination of Cu(II) and Cd(II) concentrations in groundwater, soil and Alhagi maurorum plant

The results of Cu(II) and Cd(II) concentrations in groundwater, soil and Alhagi maurorum plants as determined by AdSV are presented in Figures 2 and 3. The levels of the mean cadmium concentrations in groundwater, soil and Alhagi maurorum plants all sampled in different

<table>
<thead>
<tr>
<th>Method</th>
<th>Real samples</th>
<th>LOD (µg/L)</th>
<th>LR (µg/L)</th>
<th>Interferences</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cu</td>
<td>Cd</td>
<td>Cu</td>
<td>Cd</td>
</tr>
<tr>
<td>DPA$_2$SV</td>
<td>Biological samples</td>
<td>0.011</td>
<td>0.014</td>
<td>0.3–142.5</td>
<td>0.065–60</td>
</tr>
<tr>
<td>AdSV</td>
<td>Natural water</td>
<td>0.16</td>
<td>0.34</td>
<td>5.3–99.8</td>
<td>11.5–72.4</td>
</tr>
<tr>
<td>SWC</td>
<td>Real water sample</td>
<td>–</td>
<td>0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DPASV</td>
<td>Perch fish</td>
<td>0.017</td>
<td>–</td>
<td>0.4–50</td>
<td>–</td>
</tr>
<tr>
<td>AdCSV</td>
<td>Water samples</td>
<td>–</td>
<td>0.01</td>
<td>1.0–13.0</td>
<td>–</td>
</tr>
<tr>
<td>SWC</td>
<td>Water</td>
<td>0.012</td>
<td>0.028</td>
<td>0.05–10</td>
<td>0.12–40</td>
</tr>
<tr>
<td>ASV</td>
<td>Glazed ceramic</td>
<td>2.7</td>
<td>0.25</td>
<td>0–200</td>
<td>0–200</td>
</tr>
<tr>
<td>AdCSV</td>
<td>Food</td>
<td>–</td>
<td>1.180</td>
<td>1–50</td>
<td>10–200</td>
</tr>
</tbody>
</table>

SWC: square wave cathodic stripping voltammetry; DCA$_2$SV: differential pulse cathodic adsorptive stripping voltammetry; DPASV: differential pulse anodic stripping voltammetry; A$_2$SV: adsorptive stripping voltammetry; ASV: anodic stripping voltammetry; A$_2$CSV: adsorptive cathodic stripping voltammetry.

---

**Table 2 | Comparison between advantages of present work and previous studies**

Downloaded from https://iwaponline.com/wst/article-pdf/72/7/1127/467238/wst072071127.pdf by guest on 07 November 2018
locations, are 0.696 ± 0.0124 ppm, 0.516 ± 0.0175 ppm, 0.596 ± 0.009 ppm, 0.709 ± 0.005 ppm, 0.684 ± 0.005 ppm and 0.787 ± 0.007 ppm, respectively, as shown in Figure 2(a)–2(c). The results of the studied copper mean in groundwater, soil and *Alhagi maurorum* plants are 10.443 ± 0.01 ppm, 9.296 ± 0.005 ppm, 11.146 ± 0.012 ppm, 16.558 ± 0.0465 ppm, 13.752 ± 0.0216 ppm and 16.339 ± 0.021 ppm, respectively, Figure 3(a)–3(c).

Sample bottles were transported to the field and back to the laboratory individually in double bags (colorless PE zip-type bags) inside double large PE bags and carried and stored in a plastic trunk. Any manipulation of the samples in the laboratory was done in a particulate-free environment. Several analytical techniques, such as AAS, ICP-AES or voltammetry, have proved to be powerful analytical tools for the determination of trace metals in biological samples (groundwater, soil and *Alhagi maurorum* plants).

**Cadmium**

The voltammograms obtained in the course of simultaneous determination of Cu(II) and Cd(II) are presented in Figure 1(a). The relative standard deviations for Cd(II) determination at a concentration of $2 \times 10^{-9}$ mol L$^{-1}$ was 5.17% ($n = 6$). In addition, the measurements for the calibration graph for Cd(II) in the presence of $1 \times 10^{-8}$ mol L$^{-1}$ Cu(II) was estimated. The parameters of the calibration curve and limit of detection obtained for Cd(II) in the presence of constant concentration of Cu(II) are also given in Table 1.

The mean Cd(II) concentration of underground water is 0.4085 ± 0.0053 ppm. Significantly, by using analysis of variance one-way statistics ($p < 0.01$), a high concentration (0.563 ± 0.0082 ppm) of cadmium was observed in Sakakah city samples, while Zalom city (0.315 ± 0.0027 ppm) reveals ($P < 0.01$) the lowest Cd(II) concentration (Figure 4(a)). The increase of Cd(II) concentration in groundwater from Turaif and Sakakah city may be related to excess Cd(II) in the environment surrounding the cement and phosphate factory. The presence of Cd(II) at the highest level in the underground of Sakakah city may be related to air and water pollution input from industrial activities in this area.

The concentrations of the cadmium Cd(II) dissolved in distilled water (after acidic digestion) can be determined simultaneously by the method of standard addition under the optimal conditions described above. The content of cadmium in *Alhagi maurorum* plant samples is shown in Figure 2.
The higher content of cadmium was observed at Sakakah city in root samples (0.791 ± 0.003 ppm), but the lower content of cadmium was observed at Zalom city in leaves of *Alhagi maurorum* plant samples (0.124 ± 0.0016 ppm). As to the depth distribution of the cadmium metal related to areas, as seen in Figure 4(b) the content of cadmium in the *Alhagi maurorum* plant samples tends to increase with industrial areas, but no regular trend was observed in the distribution of cadmium in urban areas. The mean cadmium level of industrial *Alhagi maurorum* plant samples (0.3617 ± 0.0029 ppm) and urban areas (0.2484 ± 0.0078 ppm) are significant (*P* < 0.01).

For the determination of cadmium in soil samples from industrial and urban areas in Northern Border, Figure 4(c), all samples had to be pre-treated before acidic digestion, so that speciation analysis was not possible. The results compared favorably with the AdSV technique and the values are summarized in Figure 3. An attempt was made to extend the method to simultaneous determination of cadmium in soil samples. With 0.7046 ± 0.01535, 0.8885 ± 0.00656, 1.108 ± 0.008 and 0.549 ± 0.0141 ppm values of means of urban, industrial areas, the highest content of cadmium was found in Sakakah city (at surface) and the smallest content of cadmium was found in Zalom city (at surface).

**Copper**

The method was applied to the determination of the copper in biological samples by dissolving the samples directly before application of the procedure. Environmental systems may affect the copper content in groundwater, *Alhagi maurorum* plant and soil samples (Figure 5(a)–5(c)). Location is one of the environmental system components that affect copper content in biological samples. The following results represented the relationship between copper content and different study areas (Aluwayqilah, Zalom, Hazem Algalamid, Arar, Turaiif and Sakakah city).

Understanding copper content in biological samples was established primarily by Horria *et al.* (2002), Othman (2003, 2004) and Othman & Mahmoud (2003), who showed transition metal concentrations in biological samples, such as
soil, underground water and plants, collected from Egypt. The industrial and urban areas from the Northern Border area have the following ranges: Cu(II), 4.816 ± 0.0027–20.837 ± 0.0637 ppm and 0.81 ± 0.0081–17.983 ± 0.0078 ppm, respectively. The highest Cu(II) concentration was also found at S_{2} (soil without digestion at surface) in Arar city, and the mean value was (11.5344 ± 0.01926) higher than that in urban areas.

Overall, the studied concentration of copper elements in biological samples generally showed different distribution patterns. Higher concentrations were more frequently observed in industrial areas and the mean values were all higher than in urban areas. In general, sampling sites of lower metal contents were located in Zalom city, while the higher content sites were distributed in the Arar area. A possible explanation for this is that these sampling sites were located near the cement factory, phosphate factory and Aramco Company, with frequent anthropogenic activities along the Northern Border areas and domestic wastewater being discharged.

In conclusion, overall results, biological samples from Sakakah and Arar cities exhibited significantly ($P < 0.01$) higher values of Cu(II) and Cd(II) in their biological samples than those from the other areas. Biological control samples from Zalom area exhibited the lowest values of studied heavy metals in their biological samples, which reveals that the Zalom environment is free from pollution by heavy metal Cu(II) and Cd(II) contaminations. Biological heavy metal concentrations differ according to several factors; one is the location of the subject, which differs from one area to another and from one city to another in the same country.

**Analytical results**

For the analysis of copper and cadmium heavy metals, most of the analytical multi-element techniques used are ICP-AES, AAS, and SV. For Cu(II) and Cd(II) heavy metal analysis, ppb and sub-ppb concentration AdSVs were applied, which make use of electrolytic accumulation of the metal at the stationary electrode surface, mainly HMDEs on solid electrodes, e.g., glassy carbon is based on adsorptive accumulation of the species on the electrode. This technique has been developed for various cations, anions and organic molecules.
Copper and cadmium concentrations were determined by DPAdSV after complexing with luminol concentration of $1 \times 10^{-4}$ M. Figure 1 shows the DPAdSV voltammograms for determination of Cu(II) and Cd(II) simultaneously by addition of standard copper and cadmium nitrate solutions to the sample in the same electrolysis cell using micropipettes.

**Linearity test of calibration plots**

Under optimized conditions, a linear relationship between the reduction peak current of Cu$^{2+}$ and Cd$^{2+}$ complexes and the concentrations of Cu$^{2+}$ and Cd$^{2+}$ was obtained. To verify the linear relationship between peak currents and metal concentrations, four calibration graphs were constructed under optimum conditions; after 60 s accumulation time is illustrated in Figure 6. The calibration graphs were performed for copper and cadmium separately and in the presence of 30.0 ng/mL of cadmium and 30.0 ng/mL of copper, respectively. The result of this study indicated that in all cases the current concentration relationships were linear in the concentration range of 0.3–142.5 ng/mL and 0.065–60.0 ng/mL for copper and cadmium, respectively.

The current of the metal peak increased linearly with standard additions, with a slope similar to that of the supporting electrolyte. Linearity is valid for the studied metals through long periods within the concentration levels normally found in biological samples. The formation of intermetallic compounds between the investigated metal ions may cause an error in their determination. The standard addition method was chosen here because the matrix effects were less influenced by this procedure.

**Comparison of the analytical methods**

A comparative study was carried out between the results of Cu(II) and Cd(II) concentrations in biological samples obtained using AAS, ICP-AES and DPAdSV (Table 3).
It was proved that the results obtained using AAS and ICP-AES for Cd(II) (0.729 ± 0.0079, and 0.714 ± 0.0042 ppm) and Cu(II) (9.502 ± 0.0217, and 9.431 ± 0.0204, respectively) in roots of Alhagi maurorum plant samples are nearly in agreement with those obtained using DPA dSV for the same elements (0.722 ± 0.004 ppm) for Cd(II) and (9.473 ± 0.0193 ppm, for Cu(II), respectively).

Generally, the data obtained by ICP-AES are in close agreement with those obtained by SV for some metals and slight differences for the others. However, the slight differences that may be found sometimes between both techniques are mainly due to the manipulation of the analyst and metal interferences in cases of ICP-AES while the standard addition method is used to perform the SV technique. The standard addition method is more accurate than the calibration curves, since additions of the standard

### Interferences

The interference of foreign ions was studied for a solution containing Cu(II) and Cd(II) at a concentration of $2 \times 10^{-9}$ mol L$^{-1}$. The influence of the added foreign ions on the Cu(II) and Cd(II) peak currents is presented in Table 4. To validate the selectivity of the proposed stripping voltammetric method for simultaneous determination of Cu(II) and Cd(II) ions, various cationic and anionic ions, as potential interferents, were tested. Interference was taken as the level causing an error in excess of 3%. The results show that less than 1,000-fold excess of Al$^{3+}$, Pb$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, Na$^+$, K$^+$, Li$^+$, F$^-$, Br$^-$, Cl$^-$, I$^-$, S$_2$O$_3^{2-}$, and CO$_3^{2-}$; 100-fold excess of Cr$^{3+}$, Zn$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Fe$^{3+}$ and Mo$^{2+}$ did not interfere with the determination of $2 \times 10^{-9}$ mol L$^{-1}$ each of copper and cadmium ions. In the presence of Ni(II), Co(II) and Mo(VI) a decrease of Cd(II) signal was observed.

In conclusion, the method of copper and cadmium determination by differential pulse cathodic AdSV proposed in this paper is a very low-cost method because the reagents and techniques are not expensive. Another advantage is that the time required to carry out the analysis is shorter than other polarographic methods in which determination of copper and cadmium in biological samples is made. It should be pointed out that with this method, it is possible to determine very low quantities of copper and cadmium because the detection limits are 0.011 and 0.014 ng mL$^{-1}$.

<table>
<thead>
<tr>
<th>Item metal</th>
<th>Cd(II)</th>
<th>Cu(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAS (mean ± SD)</td>
<td>ICP-AES (mean ± SD)</td>
</tr>
<tr>
<td>Aluwayqilah ($n = 5$)</td>
<td>0.970 ± 0.0175</td>
<td>0.958 ± 0.0051</td>
</tr>
<tr>
<td>Zalom ($n = 5$)</td>
<td>0.507 ± 0.0203</td>
<td>0.547 ± 0.0244</td>
</tr>
<tr>
<td>Hazem Algalamid ($n = 5$)</td>
<td>0.661 ± 0.0111</td>
<td>0.694 ± 0.0100</td>
</tr>
<tr>
<td>Sakakah ($n = 5$)</td>
<td>0.787 ± 0.0042</td>
<td>0.759 ± 0.0025</td>
</tr>
<tr>
<td>Arar ($n = 5$)</td>
<td>0.739 ± 0.0047</td>
<td>0.735 ± 0.0032</td>
</tr>
<tr>
<td>Turaif ($n = 5$)</td>
<td>0.718 ± 0.0023</td>
<td>0.693 ± 0.0031</td>
</tr>
<tr>
<td>Mean</td>
<td>0.729 ± 0.0079</td>
<td>0.714 ± 0.0042</td>
</tr>
</tbody>
</table>

$n$: number of samples; SD: standard deviation.
Application

The proposed method was successfully applied for determination of trace concentrations of copper and cadmium in several biological samples, Figure 7. The Cu(II) and Cd(II) concentrations of biological samples were determined using the recommended procedure under optimum conditions, using the standard addition method. The results presented in Table 2 show the high sensitivity of the proposed method.

CONCLUSION

A simple, fast, highly sensitive and specific DPA_{dSV} method was developed for simultaneous determination of Cu(II) and Cd(II) as luminol-complexes in biological samples (underwater, soil and Alhagi maurorum plant samples) without significant interference from foreign cations, anions and organics. The method was successfully applied for simultaneous determination of Cu(II) and Cd(II) in biological samples. The limits of detection using the described DPA_{dSV} voltammetry method (0.011 μg/L Cu(II) and 0.014 μg/L Cd(II)) indicate its high sensitivity compared with most previously reported methods for determination of Cu(II) (0.017–0.012 μg/L) and Cd(II) (0.02–0.028 μg/L). Copper and cadmium presented at higher levels in industrial areas than in urban areas. The results obtained for the biological samples by DPA_{dSV} and ICP-AES are in very good agreement, and show that both are suitable for the determination of Cu(II), and Cd(II) in biological samples at micro-levels.

REFERENCES


Currie, L. A. 1997 Detection: international update, and some emerging dilemmas involving calibration, the blank and
multiple detection decisions. Chemometrics and Intelligent Laboratory Systems 37, 151–181.


Hong, L., Meixian, L. & Dragan, M. 2015 Simultaneous determination of copper, lead, and cadmium ions at a Mo6S9-xLx nanowires modified glassy carbon electrode using differential pulse anodic stripping voltammetry. Electrochimica Acta 154, 184–118.


Iwona, R. & Mieczyslaw, K. 2014 Determination of lead and cadmium by anodic stripping voltammetry at bismuth film electrodes following double deposition and stripping steps. Sensors & Actuators B 204, 136–141.


Nail, A. & Ramazan, G. 2015 A new cloud point extraction procedure for determination of inorganic antimony species in beverages and biological samples by flame atomic absorption spectrometry. Food Chemistry 175, 507–515.


Othman, A. F. & Mahmoud, A. G. 2005 Square-wave stripping voltammetry for direct determination of eight heavy metals in...


First received 3 February 2015; accepted in revised form 3 June 2015. Available online 19 June 2015