

Comparative Studies of the Determination of Divalent Cadmium, Lead and Copper in the Boiling Medicinal Herbs by Stripping Voltammetry and by Atomic Absorption Spectrometry

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Abstract: Cadmium, lead and copper were determined in ten Libyan boiling medicinal herbs samples by differential pulse anodic stripping voltammetry as well as by atomic absorption spectrometry. Voltammetric method was done at Hanging Mercury Dropping Electrode (HMDE) in Britton-Robinson buffer solution of pH ~2.1 at $25 \pm 0.1^\circ\text{C}$. The sample preparation was carried out by boiling 2.0g of a finely pulverized plant sample for 10 min, cooled, filtered and completed to 50 mL measuring flask by deionized water. The optimal preconcentration potentials and times for the detection of these metal ions in all sample solutions have been studied. The concentration of each metal ion was determined by the standard addition method. The statistical parameters i.e. slope, standard deviation, correlation coefficient and confidence have been calculated. The levels of Cd(II), Pb(II) and Cu(II) ranged from 0.006-0.103, 0.205-1.751 and 0.198-2.124 $\mu\text{g g}^{-1}$ respectively. Copper was determined by Flame Atomic Absorption Spectrometry (FAAS) and the mean level was ranged from 0.202-2.010 $\mu\text{g g}^{-1}$. On the other hand the mean levels obtained for determination of cadmium and lead by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) ranged from 0.006 to 0.085 and from 0.220-1.850 $\mu\text{g g}^{-1}$ respectively.

Key words: Cadmium, lead, copper, analytical determination, medicinal herbs

INTRODUCTION

Cadmium is one of the few elements that have no constructive purpose in the human body. This element and its compound solution are extremely toxic even in low concentration and will bioaccumulate in organisms and ecosystems. One possible reason for its toxicity is its interference with the action of zinc-containing enzymes. Cadmium may also interfere with biological processes containing magnesium and calcium^[1,2]. Its toxicity threatens the health of the body by weakened immune system, kidney disease and liver damage, effects may include emphysema, cancer and a shortened life span^[1,3-8].

Lead has no biological role in the body. Most lead poisoning symptoms are thought to occur by interfering with an essential enzyme Delta-aminolevulinic acid dehydratase, ALAD, (is a zinc-binding protein which is important in the biosynthesis of heme, the cofactor found in hemoglobin)^[9,10]. It inhibits several enzymes critical to the synthesis of heme, causing a decrease in blood hemoglobin and interferes with a hormonal form of vitamin D, which affects multiple processes in the

body, including cell maturation and skeletal growth. Lead can also cause hypertension, reproductive toxicity and developmental effects. Lead exposure can lead to renal effects such as fanconi-like syndromes, chronic nephropathy and gout^[11-15].

Copper is both vital and toxic for many biological systems, it is critical for energy production in the cells, also involved in nerve conduction, connective tissue, the cardiovascular system and the immune system and excess copper may be absorbed in the intestinal tissues which lead to intestinal disorders, impaired healing and reduced resistance to infections^[16-18].

Recently, several methods of analysis were done for determination of cadmium, lead and copper, e.g. by neutron activation analysis^[19,20], inductively coupled plasma atomic emission spectrometry^[21-24], inductively coupled plasma mass spectrometry^[25,26], spectrophotometry^[27,28], atomic absorption spectroscopy^[19], atomic absorption spectrometry^[24,25,29-31], electrothermal atomic absorption spectroscopy^[32], electrothermal atomic absorption spectrometry^[26,33], atomic absorption spectrophotometry^[34,35], flame atomic absorption spectrometry and flame atomic

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emission spectrometry^[23,33,36,37], a signal volta-mmetric sweep at pH 4.6^[38]. Cadmium and lead were also determined in medicinal plants by differential pulse anodic stripping after preconcentration of the metals in 0.8 M HCl at 0.73V for 180 sec. and the sample preparation was carried out by dry-ashing 1.0 g of plant sample for 2.5 h at 500°C^[39]. This prompted us to study the determination of cadmium, lead and copper by differential pulse anodic stripping voltammetry as well as by flame atomic absorption spectrometry of copper and graphite furnace atomic absorption spectrometry of cadmium and lead in buffer solution of pH ~2.1 as the extension of a series of our investigations^[40-43] for determination of industrial and biological important elements.

MATERIAL AND METHODS

Apparatus: All glassware was soaked in 10% (v/v) HNO₃ for 24h and rinsed three times with distilled water and then in redistilled water before use.

Polarographic analyzer: Differential pulse anodic stripping voltammograms were recorded with an EG and G. Princeton Applied Research Corp. (PAR; Princeton, NJ) model 264 A stripping analyzer, coupled with a PAR 303A Hanging Mercury Drop Electrode (HMDE). The polarographic cell (PAR Model K0060) was fitted with Ag/AgCl saturated KCl and used as a reference electrode with a platinum wire as a counter (auxiliary) electrode. A PAR 305 magnetic stirrer was connected to the 303 A HMDE. A PAR Model RE 0151X-Y recorder was used to collect experimental data. Before measurements the sample solution was deaerated by bubbling for 16 min with nitrogen. During measurements, an inert atmosphere over the solution was maintained by flushing with nitrogen. During the deposition step, the solution was stirred automatically, followed by a quiescent period of 15 sec. before scanning.

- pH was measured with a Fischer Scientific (Pittsburgh, PA, USA) Digital pH Meter Model 810
- GBC 906 atomic absorption spectrophotometer was used for Cu(II) measurement at wavelength 324.7 nm, band-pass 0.7 nm and lamp current 6.0 mA and a AA-6800 Shimadzu (GFA-EX7) Graphite Furnace atomic absorption spectrophotometer was used for Cd(II) and Pb(II) determination at band-pass 0.7 nm, lamp current 8.0 mA and wavelengths 228.9 and 283.2 nm respectively

Solution and reagents: All reagents are of analytical grade. The following solutions were prepared with bidistilled water.

- Solution of each Cd(II), Pb(II) and Cu(II) were prepared respectively by dissolving the required amounts of Cd(NO₃)₂·4H₂O, Pb(NO₃)₂ and Cu(NO₃)₂·2H₂O in bidistilled water. The resulting solutions were then standardized^[44]. Solutions of lower concentrations were prepared by accurate dilution
- Briton-Robinson buffer solution was prepared by dissolving 201 μL glacial acetic acid (AnalaR), 240 μL phosphoric acid (Merck) and 433 mg boric acid (BDH) in 500 mL measuring flask with bidistilled water^[45]
- Ten medicinal herbs samples were collected from Libyan Jamahyria as shown in Table 1. Dried herbal samples were cut into small pieces and then ground into powder. To prepare the boiling of the herbs, 2 g of sample was placed in a beaker containing 40 mL of bidistilled water and boiled for 10 min. After cooling, the extract was filtered through the Whatman No. 541 filter paper and the filtrate was diluted to 50 mL with bidistilled water. Each resulting solution was treated with 10 mL of concentrated nitric acid (AnalaR) and heated nearly dry (repeated three times). This procedure was repeated with 10 mL of a 1:1 (HNO₃/HClO₄) mixture until complete mineralization the cold residue was dissolved in 1 mL of 1M nitric acid and diluted to 10ml with bidistilled water. Nitric acid concentration was adjusted at 0.01 M. A control reagent blank was prepared in the same manner to determine the ultra trace impurities of the metal ions

Analytical procedure: The following parameters were used to perform differential pulse anodic stripping voltammetry (DPASV). Scan rate 10 mVs⁻¹ with duration for 1 sec. and pulse amplitude (ΔE) 25 mV.

For determination of Cd(II), Pb(II) and Cu(II) in medicinal herb samples in the same cell. 5 mL of each sample solution and 1 mL Briton-Robinson buffer solution were transferred to the electrolysis cell and diluted to 10 mL using bidistilled water (pH ~2.1). The solution was deaerated by passing pure nitrogen for 16 min. The deposition potential were controlled at (-0.75, -0.55 and -0.25V vs. Ag/AgCl saturated KCl respectively) and applied to a fresh mercury drop while the solution was stirred. After the deposition step and further 15 sec. (equilibrium time) the voltammogram was recorded.

Table 1: Characteristics of the boiling samples.

Sample No.	Name	Scientific name	Part used
B ₁	Camel's hay sweet rush	<i>Cymbopogon schoenanthus</i> (L) spreng <i>Andropogon schoenanthus</i> L.	The whole herb
B ₂	Juniper, common juniper	<i>Juniperus communis</i> L.	Fresh ripe berries
B ₃	Rosemary, common rosemary	<i>Rosmarinus officinalis</i> L.	leaves and flowering tops
B ₄	Rune, common rue, herb of grace	<i>Ruta graveolens</i> L.	Leaves
B ₅	Stinging nettle, small nettle	<i>Urtica urens</i> L.	The whole plant
B ₆	Sweet marjoram	<i>Origanum majorana</i> L., Majorana <i>hortensis</i> moen	The flowering plant without roots
B ₇	Tuberclad rue	<i>Haplophyllum tuberculatum</i> (forssk) A. Juss	The whole herb without roots
B ₈	White horehound, Horehound	<i>Marrubium Vulgare</i> L.	The herb while flowering without roots
B ₉	Sohbetelhosan	<i>Scorophularia canina</i> L.	The whole herb without roots
B ₁₀	Worm seed	<i>Artemisia herb-alba</i>	Flowering heads

Different concentration from the standard metal ion (individually) were added to the cell using an automatic pipette (Volac 10-100 μL), while keeping the deposition time constant. The solution was stirred and purged with nitrogen for 1 min. after each spike. The concentration of each Cd(II), Pb(II) and Cu(II) in the electrolytic cell were calculated in the sample solutions by using standard addition method, (C_c). Then the concentration in $\mu\text{g g}^{-1}$ of each medicinal herb sample was calculated by the following equation: C in $\mu\text{g g}^{-1} = C_c \times 10^7 \times \text{at wt of the metal ion}$. For cadmium and lead the digested sample solution were treated with concentrated HNO_3 before determination by graphite furnace AAS and the treated sample solution of copper were determined by Flame AAS. The metals were quantified against standard curves prepared at the day of the analysis.

RESULTS AND DISCUSSION

In order to set the optimal condition of the three cations, preliminary measurements were made to obtain the highest peak signal for metal ions Cd(II), Pb(II) and Cu(II) in solution samples. It was noticed that, Britton-Robinson buffer solution (pH ~ 2.1) gave promising results for the determination of Cd, Pb and Cu ions. The effect of deposition potential of each metal ion was studied and it was observed that the highest and best shape peaks for Cd^{2+} , Pb^{2+} and Cu^{2+} were obtained at deposition potentials -0.75 , -0.55 and -0.25 V vs. $\text{Ag}/\text{AgCl}/\text{KCl}_{\text{sat}}$, respectively.

The effect of deposition time on the oxidation peak signals of these metal ions was examined. Figure 1 shown differential pulse anodic stripping voltammograms of Cu(II) in Rosemary sample (B_3) in buffer solution at different deposition times. The optimal deposition times were selected for these metal ions of all sample solutions in a manner that linear relation must be established between deposition times and current signals and listed in Table 2-4.

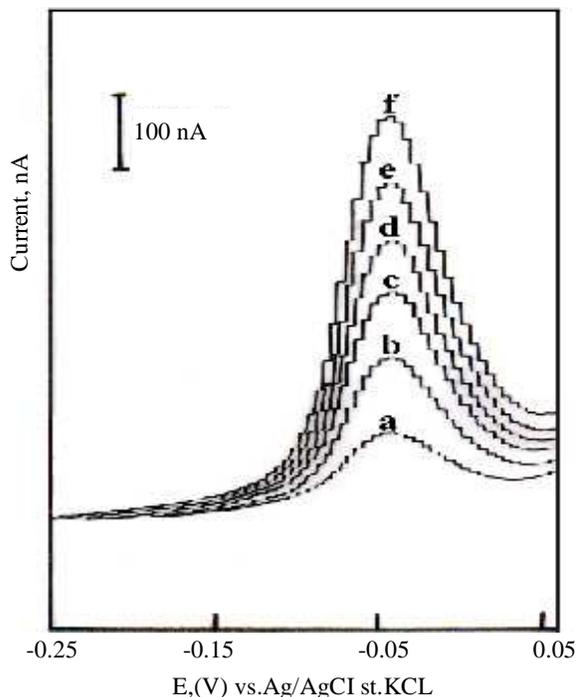


Fig. 1: DPAS Voltammograms of Cu(II) in B_3 sample in presence of 0.028 M Britton-Robinson buffer solution, pH ~ 2.1 at deposition potential -0.25 V and different deposition times. (a): 0 sec, (b): 5 sec, (c): 10 sec, (d): 15 sec, (e): 20 sec, (f): 25 sec

DPAS voltammetric determination of Pb(II):

Figure 2 shows the differential pulse anodic stripping voltammograms of B_3 sample solution in absence and in presence of standard lead nitrate. The plots of peak current against concentration are given in Fig. 3. From the interception of this line with the concentration axis at zero current signal gives the concentration of Pb^{2+} in the voltammetric cell for each sample. After correction for the background current of blank experiments. The

Table 2: Lead content of different boiling samples (a mean value±standard deviation for n = 5 at 95% confidence level)

Sample No.	T _d (sec)	Lead content (mean±SD) (DPSAV) μg g ⁻¹	Regression parameter				Lead content (mean±SD) (GFAAS) μg g ⁻¹	
			Slope	Intercept/ (10 ⁻⁸ mol dm ⁻³)	Corr. Coef.	Confidence Higher Lower		
B ₁	10	0.895±0.03	21.10	92.5	0.9996	0.9320	0.8580	0.920±0.05
B ₂	90	1.360±0.07	2.30	152.3	0.9996	1.4500	1.2730	1.500±0.050
B ₃	30	0.435±0.02	2.40	50.0	0.9994	0.4600	0.4101	0.450±0.010
B ₄	60	0.205±0.01	5.42	55.0	0.9993	0.2174	0.1926	0.231±0.030
B ₅	45	0.228±0.01	4.70	50.0	0.9994	0.2404	0.2156	0.231±0.030
B ₆	20	0.849±0.04	3.08	127.5	0.9996	0.8987	0.7993	0.860±0.060
B ₇	15	1.751±0.10	6.45	55.0	0.9996	1.8750	1.6270	1.850±0.200
B ₈	60	0.742±0.02	1.10	40.0	0.9993	0.7669	0.7171	0.790±0.050
B ₉	10	0.850±0.02	0.96	40.0	0.9994	0.8749	0.8251	0.920±0.040
B ₁₀	45	0.373±0.01	1.82	35.0	0.9992	0.3854	0.3606	0.380±0.020

Table 3: Cadmium content of different boiling samples (a mean value±standard deviation for n=5 at 95% confidence level)

Sample No.	T _d (sec)	Cadmium content (mean±SD) (DPSAV) μg g ⁻¹	Regression parameter				Cadmium content (mean±SD) (GFAAS) μg g ⁻¹	
			Slope	Intercept/ (10 ⁻⁸ mol dm ⁻³)	Corr. Coef.	Confidence Higher Lower		
B ₁	60	0.031±0.0010	8.44	22.0	0.9992	0.0322	0.0298	0.035±0.002
B ₂	90	0.044±0.0020	4.83	20.0	0.9999	0.0465	0.0415	0.045±0.003
B ₃	30	0.103±0.0080	2.18	21.0	0.9995	0.1130	0.0930	0.085±0.009
B ₄	120	0.103±0.0080	5.21	22.0	0.9993	0.0487	0.0413	0.040±0.004
B ₅	90	0.006±0.0005	8.16	7.5	0.9991	0.0066	0.0054	0.006±0.006
B ₆	120	0.042±0.0010	10.09	42.5	0.9995	0.0432	0.0408	0.039±0.002
B ₇	90	0.028±0.0030	6.65	16.0	0.9994	0.0317	0.0243	0.025±0.003
B ₈	60	0.020±0.0020	3.47	7.0	0.9994	0.0225	0.0175	0.021±0.003
B ₉	60	0.006±0.0002	6.35	5.0	0.9993	0.0063	0.0058	0.006±0.001
B ₁₀	60	0.046±0.0030	3.81	16.0	0.9992	0.0497	0.0423	0.045±0.004

Table 4: Copper content of different boiling samples (a mean value±standard deviation for n = 5 at 95% confidence level)

Sample No.	T _d (sec)	Copper content (mean±SD) (DPSAV) μg g ⁻¹	Regression parameter				Copper content (mean±SD) (FAAS) μg g ⁻¹	
			Slope	Intercept/ (10 ⁻⁸ mol dm ⁻³)	Corr. Coef.	Confidence Higher Lower		
B ₁	10	1.135±0.05	18.20	320	0.9994	1.1970	1.073	1.050±0.07
B ₂	45	0.436±0.01	46.90	320	0.9995	0.4480	0.424	0.419±0.02
B ₃	10	1.870±0.20	8.65	250	0.9994	2.1190	1.621	1.950±0.30
B ₄	15	2.124±0.20	11.67	390	1.0000	2.3730	1.875	2.010±0.40
B ₅	30	1.480±0.08	15.95	380	0.9994	1.5795	1.381	1.550±0.06
B ₆	15	0.198±0.02	25.80	80	0.9998	0.2230	0.173	0.210±0.03
B ₇	40	0.219±0.01	90.90	300	0.9995	0.2314	0.207	0.202±0.03
B ₈	10	0.320±0.01	19.34	100	0.9996	0.3324	0.308	0.290±0.03
B ₉	20	0.402±0.01	42.84	260	0.9994	0.4154	0.391	0.380±0.02
B ₁₀	15	0.750±0.02	21.20	260	0.9996	0.7749	0.725	0.760±0.05

limits of detection of the proposed procedure for lead ions under investigation were calculated. The concentration of Pb(II) ions in all samples under consideration using DPASV are shown in Table 2. It was found that, the mean levels of Pb(II) ions are ranged from 0.205-1.751 μg g⁻¹ and the lead content increases in the following order, B₄<B₅<B₁₀<B₃<B₈<B₆<B₉<B₁ <B₂ <B₇. Thus lead content increase in whole herb and flowering heads, while it decreases in the leaves. The average

concentrations of Pb(II) in all samples under investigation within the safe limits set by FAO/WHO.

DPASV voltammetric determination of Cd(II):

Figure 4 shows the differential pulse anodic stripping voltammograms of Cd(II) in B₂ sample spiked with different concentration of cadmium ions in Briton-Robinson Buffer Solution of pH ~2.1. On plotting of i_p vs. Cd(II) concentrations for all medicinal herb samples in the same supporting electrolyte at the same

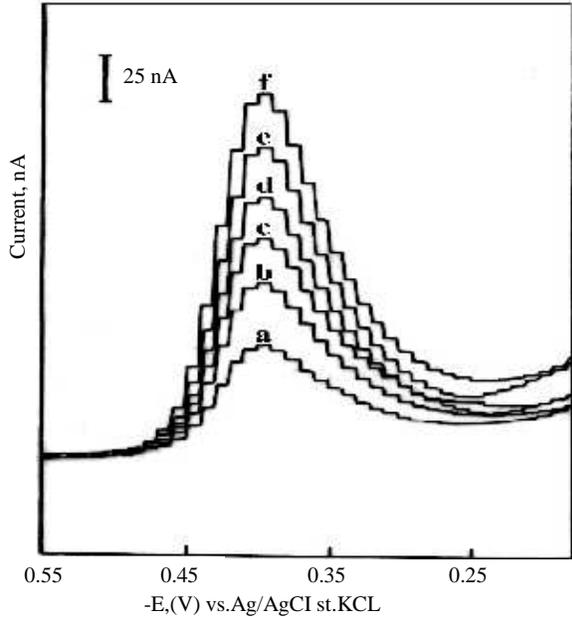


Fig. 2: DPAS Voltammograms of Pb(II) in B₃ sample spiked with different concentrations of Pb(II) ions in 0.028M Britton-Robinson buffer solution, pH ~2.1 at deposition potential -0.55 V and deposition time 30 sec. (a): Sample, S (b): S+10×10⁻⁸, (c): S+20×10⁻⁸, (d): S+30×10⁻⁸, (e): S+40×10⁻⁸, (f): S+5×10⁻⁸ M Pb(II)

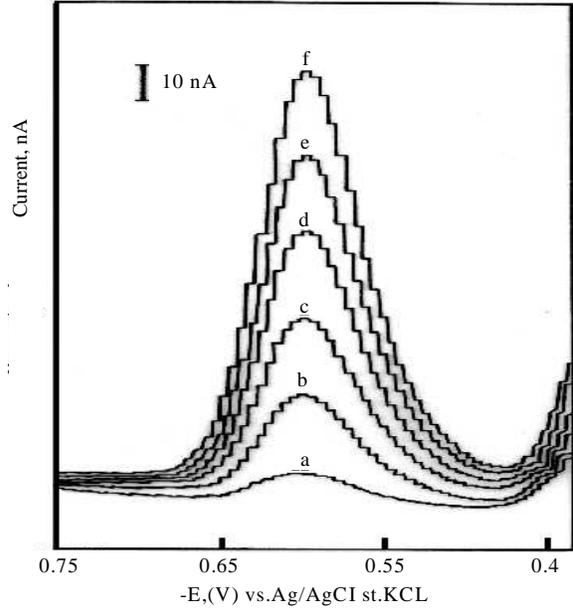


Fig. 4: DPAS Voltammograms of Cd(II) in B₂ sample spiked with different concentrations of Cd(II) ions in 0.028 M Britton-Robinson buffer solution, pH ~2.1 at deposition potential -0.75 V and deposition time 90 sec. (a): Sample, S, (b): S+10×10⁻⁹, (c): S+20×10⁻⁹, (d): S+30×10⁻⁹, (e): S+40×10⁻⁹, (f): S+50×10⁻⁹ M Cd(II)

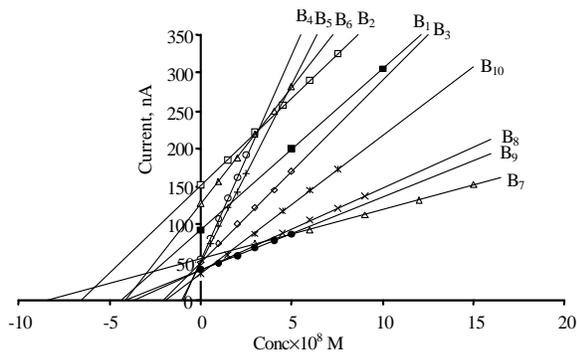


Fig. 3: Standard addition plot of Pb(II) in samples: (1): B₁ at 10 sec, (2): B₂ at 90 sec, (3): B₃ at 30 sec, (4): B₄ at 60 sec, (5): B₅ at 45 sec, (6): B₆ at 20 sec, (7): B₇ at 15 sec, (8): B₈ at 60 sec, (9): B₉ at 10 sec, (10): B₁₀ at 45 sec, at deposition potential -0.55V using (DPASV)

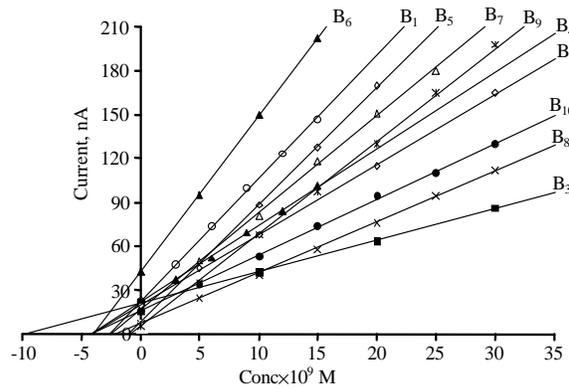


Fig. 5: Standard addition plot of Cd(II) in samples: (1): B₁ at 60 sec, (2): B₂ at time 90 sec, (3): B₃ at 30 sec, (4): B₄ at 120 sec, (5): B₅ at 90 sec, (6): B₆ at 120 sec, (7): B₇ at 90 sec, (8): B₈ at 60 sec, (9): B₉ at 60 sec, (10): B₁₀ at 60 sec, at deposition potential -0.75 V using (DPASV)

conditions, straight lines are obtained (standard addition method) as shown in Fig. 5. From the interceptions of these lines with the concentration axis at zero current signals, one can calculate the concentration of Cd(II) in

each sample. The result of concentration values in $\mu\text{g g}^{-1}$ are listed in Table 3. The results indicated that, the Cd(II) contents are ranging from 0.006-0.103 $\mu\text{g g}^{-1}$ and the cadmium content increases in the following

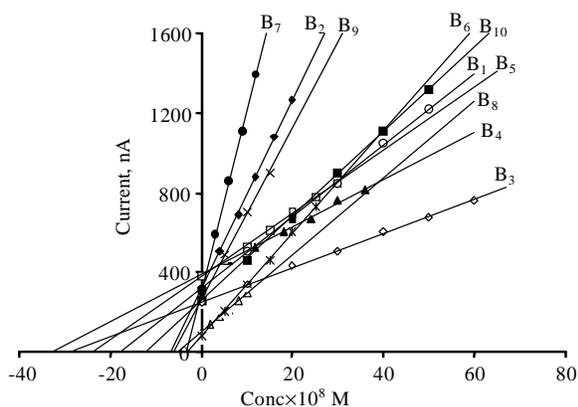


Fig. 6: Standard addition plot of Cu(II) in samples: (1): B₁ at 10 sec, (2): B₂ at 40 sec, (3): B₃ at 10 sec, (4): B₄ at 15 sec, (5): B₅ at 30 sec, (6): B₆ at 15 sec, (7): B₇ at 40 sec, (8): B₈ at 10 sec, (9): B₉ at 20 sec, (10): B₁₀ at 15 sec, at deposition potential $-0.25V$ using (DPASV)

order $B_5=B_9 < B_8 < B_7 < B_1 < B_6 < B_2 < B_4 < B_{10} < B_3$. Thus, cadmium content increases in the whole herb without roots and decreases in the leaves. The results also indicated that, cadmium content in the medicinal herb samples are less than the permissible values were given by WHO and FAO.

DPAS voltammetric determination of Cu(II):

Figure 6 shows the standard addition plots of ip against Cu(II) concentration for ten medicinal herb sample solutions in Briton-Robinson buffer solution of pH ~ 2.1 at deposition potential -0.25 volt. From the interceptions of these straight lines with the concentration axis at zero current signals, the concentration of each sample in $\mu g g^{-1}$ was calculated and the resulting concentration values are listed in Table 4. The results indicate that, the Cu(II) concentrations are ranging from $0.198-2.124 \mu g g^{-1}$, which are less than the permissible values are given by WHO and FAO.

The results also indicate that, the divalent copper content increases in the following order $B_6 < B_7 < B_8 < B_9 < B_2 < B_{10} < B_1 < B_5 < B_3 < B_4$. Thus Cu(II) contents increases in the leaves and decreases in the flowering plant without roots.

The precision and reproducibility of the selected procedure were investigated by measuring the concentration of Cd(II), Pb(II) and Cu(II) in all medicinal herb samples under consideration for (n = 5). The values of slopes, intercepts, confidence intervals, standard deviations and correlation coefficients obtained for all samples are listed in Table 2-4. These

statistical parameter values indicate the reproducibility of the procedure for determination of each of Cd(II), Pb(II) and Cu(II) in all samples in this Briton-Robinson buffer solution, pH ~ 2.1 .

Flame atomic absorption spectrometric determination of copper:

Copper was determined by atomic absorption spectroscopy of the treated sample solutions at 324.7 nm. The concentration values of each sample are listed in Table 4. It was found that the concentration of copper is ranged between $0.202-2.010 \mu g g^{-1}$. From Table 4, it was found that, the data obtained by stripping voltammetry are in a close agreement with those obtained by flame atomic absorption spectrometry. However, the slight differences that found in some cases are mainly due to the manipulation of the analyst and the use of the calibration curves in the case of flame atomic absorption spectrometry.

Flame atomic absorption spectrometric method was not obeyed for determination of cadmium and lead, so the concentration of each cadmium and lead is less than the detection limits of the FAAS technique.

Graphite furnace atomic absorptionspectrometric determination of cadmium and lead:

Cadmium and lead were determined by graphite furnace atomic absorption spectrometry at 228.9 and 283.2 nm respectively. The resulting data were listed in Table 2 and 3. From Table 2, it was found that, the resulting data obtained by stripping voltammetry are in a close agreement with those obtained by graphite furnace atomic absorption spectrometry. However, the slight difference that found in some cases are mainly due to manipulation of the analyst and the use of the calibration curve in the case of graphite furnace atomic absorption spectrometry instead of the standard addition method, which is more accurate than the calibration curves.

From Table 3, it was found that the resulting data obtained by stripping voltammetry is mainly less than that obtained by graphite furnace atomic absorption spectrometry due to the same reasons discussed above for cadmium as well as there is an another factor: in case of stripping voltammetry only divalent lead was detected but in the case of GFAAS, all lead species in the sample solution were detected.

The foregoing results reveal that, the stripping voltammetric approach accurate, low maintain cost, rapid reproducible, highly sensitivity and selective method for monitoring of the trace elements, cadmium, lead and copper in medicinal herbs. The results also indicate that, copper, cadmium and lead contents in the

samples are less than that permissible values which given by WHO and FAO and differ from each other's according to its environment contamination, production and storage.

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