Applicability of calcein as a chelating agent for simultaneous determination of Cd(II), Cu(II), Pb(II)and Zn(II) in water samples by adsorptive stripping voltammetry

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ABSTRACT

A sensitive and selective electrochemical procedure is presented for simultaneous determination of trace levels of Cd(II), Cu(II), Pb(II) and Zn(II) in water samples with calcein as a chelating agent. The method is based on the adsorptive accumulation of the complexes of Cd(II), Cu(II), Pb(II) and Zn(II) ions with calcein onto the surface of hanging mercury drop electrode for 60 seconds, Then the preconcentrated metals-complexes were analyzed by adsorptive stripping differential puls voltammetry. The effect of various parameters such as pH, concentration of calcein, accumulation potential and accumulation time were studied. The optimum conditions for simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) include pH 4.0, calcein concentration of 0.6 mmol/L, accumulation potential of -0,7 V (vs. Ag/AgCl) and accumulation time of 60 seconds. Under the optimum conditions the measurement peak current is proportional to the concentration of Cd(II), Cu(II), Pb(II) and Zn(II) in the range 0.2 - 110 µg/L ; 0.2 – 100 µg/L ; 0.2 – 110 µg/L ; and 0.2 – 100 µg/L respectively. The limits of detection were 1.38 µg/L for Cd(II), 0.76 µg/L for Cu(II), 1.43 µg/L for Pb(II) and 0.98 µg/L for Zn(II). The relative standard deviation were 0.84%, 0.25%, 0.43% and 0.54% for Cd(II), Cu(II), Pb(II) and Zn(II) for ten replicates (n = 10) measurements of mixture solution 10 µg/L of Cd(II), Cu(II), Pb(II) and Zn(II). The methods were successfully applied for simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) in sea water, tap water and lake water.

Keywords: selective and sensitive, calcein, simultaneous, adsorptive stripping voltammetry.
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INTRODUCTION

Over the last decade, Adsorptive Stripping Voltammetry (AdSV) has envolved into a very versatile and powerful electroanalytical technique. This method is aimed to extend the scope of the sensitivity and selectivity of stripping analysis toward numerous analytes (organic molecules and metal chelates exhibiting surface-active properties) [1]. Based on the wide range of applications reported during the recent years, the adsorptive concept appears to be the most universal stripping approach. When the given substance contains an electrochemically reducible or oxidisable group, the peak current on the voltammetric curve is recorded after completion of the accumulation period.

Electroanalytical procedures have been proposed for the determination of trace metals because they can be a valid option in the multicomponent analysis of several species; they can also be used for natural water, especially seawater [2]. The sensitivity and selectivity of these methods can be improved either by the introduction of new types of electrodes or by the development of new analysis procedures. Electrochemical methods include stripping voltammetric techniques, principally anodic stripping and adsorptive cathodic stripping voltammetry, which show numerous advantages, such as minimum sample pretreatment, high speed of analysis, good performance with saline matrix, low cost, and good selectivity and sensitivity [1,3].

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The development of AdSV methods has allowed the determination of many trace metals; this is not limited to amalgam-forming elements such as anodic stripping voltammetry. The method is based on the interaction between metal ion and a ligand, followed by the accumulation of the complex onto the electrode surface, from which it is subsequently stripped during the cathodic voltammetric scan. The ligand forms a complex with the target metal, which is adsorbed and later reduced on the electrode. The adsorption of the complex is often related to the solubility [4-5]. In addition, the size of the metal complex determines the surface coverage, and thus the sensitivity. Therefore, it is necessary to study the applicability of new ligands that may increase the sensitivity and selectivity of trace metal ion determination.

A possible limitation of AdSV is that sometimes only one or two metals can be determined in a single scan, depending on the metal specificity of the ligand used for the determinations [4]. Because of the great sensitivity enhancement obtained with AdSV methods, several complexing agents have been studied for use during the adsorptive collection of complexes, but there are only few studies about simultaneous metal determination [5-8].

These proposed methods use different ligands: for example, dimethyl glyoxime [8] to detect Co, Zn, Cd and Ni, but with high detection limits (2.3 – 5.1 µg/L) or substituted catechols [5] that lack much sensitivity (>5 µg/L). Moreover, a mixture of ligands has been proposed for the multi-elemental determinations of several elements (Cu, Pb, Cd, Ni, Co and Zn) in seawater [4]. The detection limits of 0.2 µg/L for Cu(II), 0.4 µg/L for Pb and 0.12 µg/ L for Cd were obtained using dimethyl glyoxime and 8-hydroxyquinoline as ligand by AdSV. Therefore, there is a great interest in developing new ligands with adequate complexing properties, which may perform the simultaneous determination of several metals at trace levels.

Trace heavy metals are very important in the environment due to their serious toxicity although present at very low concentrations. The presence of heavy metals in the environment, including food ingredients is very dangerous, because of high level of toxicity [9]. Adsorptive stripping voltammetry was chosen as an alternative method of analysis because it has many advantages such as: high salt content of sea water does not interfere, high sensitivity, low detection limit in ug/L, simple and easy sample preparation, rapid analysis, less infra structure [10-11]. In addition, with this method it is possible to learn chemical species of heavy metals [12-13] which can not be done with other methods.

This method can be used for the simultaneous determination of Cd and Pb in food samples by using 2-mercaptobenzothiazole as complexing agent [9]. Simultaneous determination of Cd, Cu and Pb in sea water by AdSV [14] in the presence of thymolphthalexone [15] and simultaneous determination of trace of cadmium and zinc by using 5-phenyl-1,2,4-triazol-3-tion (PTT) as complexes agent [16]. Simultaneous determination of ultra trace levels of copper and cadmium in food and water samples with luminal as a chelating agent [17], simultaneous determination of lead and cadmium in the presence of morin as a complexing agent [18] and simultaneous determination of copper, lead, cadmium and zinc by adsorptive stripping voltammetry in the presence of murexide [19]. Almost all methods of determination of metals in very small amounts require considerable time on pre-concentration step prior to measurement. In adsorptive stripping voltammetry pre-concentration time is short, generally less than one minute [20].

The   present   paper   describes   an   adsorptive   stripping   voltammetry   procedure   for determination cadmium, copper, lead and zinc in water samples   using is (N,N-bis(carboxymetyl)aminometyl) fluorecein or calcein as a complexing agent. The structure of calcein can be seen in Figure 1.
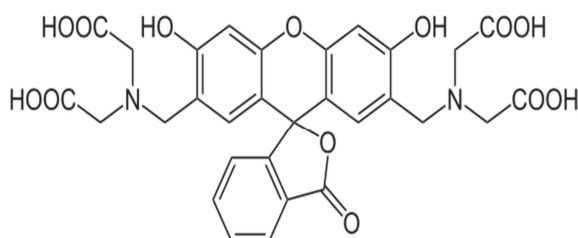

Figure 1. Structure of Calcein (Fluorexon)

To overcome the problem mentioned above, a better condition of adsorptive stripping voltammetry is needed to get a selective and sensitive method. The   parameters studied were: variations of calcein concentration, variation of pH solution, accumulation potential and accumulation time. To determine the precision, accuracy, sensivity and linearity of method, relative standard deviation, recovery, limit of detection and correlation coefficient were determined. The method at optimum condition was applied   for the direct determination

of Cd(II), Cu(II), Pb(II) and Zn(II) in sea water, tap water and lake water.

EXPERIMENTAL SECTION

2.1. Materials

The materials used for this study were: NH_4Cl, calcein, concentrated HNO_3, concentrated HCl, acetate buffer, NH_4OH, nitrogen gas, doubly distilled water, Whatman filter paper and samples (sea water, tap water and lake water). Various chemicals were purchased from Merck and the aqueous solution of Cd, Cu, Pb and Zn 1000 mg/L were prepared by using the analytical grade Merck product.

2.2. Instruments

The instrument used in this study were: 797 Computrace with Metrohm HMDE working electrode, a reference electrode Ag/AgCl/KCl 3 M and the Pt electrode as a counter electrode, pH meter models 80 Griffin (Griffin & George Loughborough, UK), analytical balance Mettler AE 200, Toledo OH-USA and glassware commonly used in laboratory.

2.3. Methods

The aim of this study was to obtain the optimum condition of each metal ion Cd, Cu, Pb and Zn by adsorptive stripping voltammetry. Therefore studied the effect of the following parameters namely, variations of concentration calcein of 0.2 mmol/L calcein to 0.9 mmol/L, variation of pH 3 to pH 9, accumulation potential of - 0.2 V to -1.1 V and accumulation time variation of 20 seconds to 100 seconds. To determine the precision and accuracy of method relative standard deviation (RSD) and recovery were determined. Procedure used in this study according were reported previously [10].

2.4. Sample preparation

Sea water samples were obtained from a beach around Bungus, Padang City. The samples were placed in plastic bottles previously washed with 1% HNO3 solution. In the laboratory, samples were filtered through a 0.45 µm membrane filter and acidified with HNO_3 to pH 2. Before the analysis, all the samples were digested by UV irradiation for 90 s at 90°C (10.0 mL of sample with 100 µL of 30 % H_2O_2).

RESULTS AND DISCUSION

Preliminary experiments were carried out to identify the general features which characterise the behaviour of the metal ions-calcein systems on a hanging mercury drop electrode (HMDE). Fig. 2 shows adsorptive stripping differential pulse voltammograms of Cd(II), Cu(II), Pb(II) and Zn(II)-calcein system. Curve (a) shows the voltammograms of mixed solution containing 10 µg/L of cadmium, copper, lead and zinc in the absence of calcein under optimum conditions. Very small cathodic peaks were found at - 0.13, - 0.38, -0.56 and -0.98 V for copper, lead, cadmium and zinc, respectively. Curve (b) shows the adsorptive stripping voltammmograms of mixture of metal ions (cadmium, copper, lead and zinc) and calcein system. Four separated peaks for the reduction of cadmium, copper, lead and zinc complexes with calcein were observed that was due to the difference in the thermodynamic stability of their complexes. Comparison of the voltammograms revealed that height of the cadmium, copper, lead and zinc peaks depends not only on the duration of the preconcentration step but, in the presence and absence calcein, also the stripping initial potential,which revealed the adsorptive nature of the response [9].

In this work, a new sensitive and rapid adsorptive voltammetric method for simultaneous determination of ultra trace levels of cadmium, copper, lead and zinc was developed based on adsorptive stripping voltammetry of cadmium, copper, lead and zinc complexes with calcein.
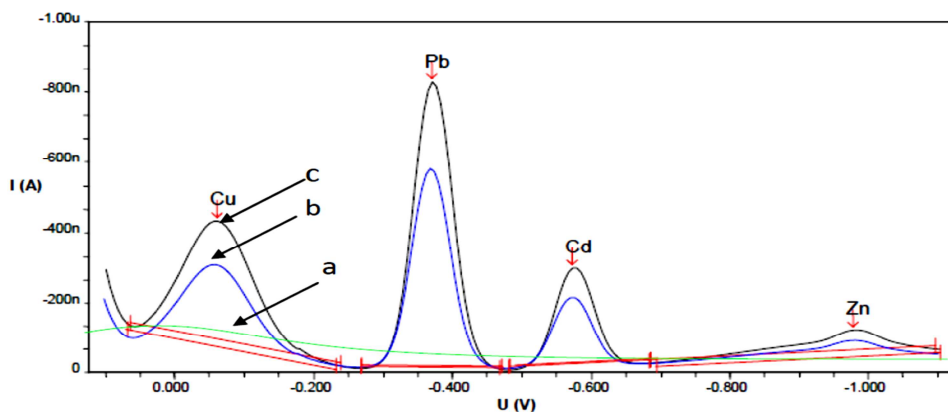
Fig. 2. Adsorptive stripping voltammetry for Cd(II)-calcein, Cu(II)-calcein, Pb(II)-calcein and Zn(II)-calcein system, (a) Voltammogram of calcein in the absence of trace metals, (b) Differential pulse adsorptive stripping voltammograms in mixed solution of Cd(II), Cu(II), Pb(II) and Zn(II) 10 µg/L in the absence of calcein, (c) The adsorptive stripping voltammmograms of mixture of metal ions (cadmium, copper, lead and zinc) in the presence calcein

3.1. Effects of callcein cconcentration

The effect of calcein concentration on the sensitivity of the proposed method was also studied cathodic stripping peak current of Cd- calcein, Cu-calcein, Pb-calcein and Zn-calcein complexes increased with increasing calcein concentration up to 0.6 mM, at concentration higher than 0.6 mM where both peaks diminish as a consequence of a full electrode surface coverage. An optimum calcein concentration of 0.6 mM was selected for further experiment (Fig.3)
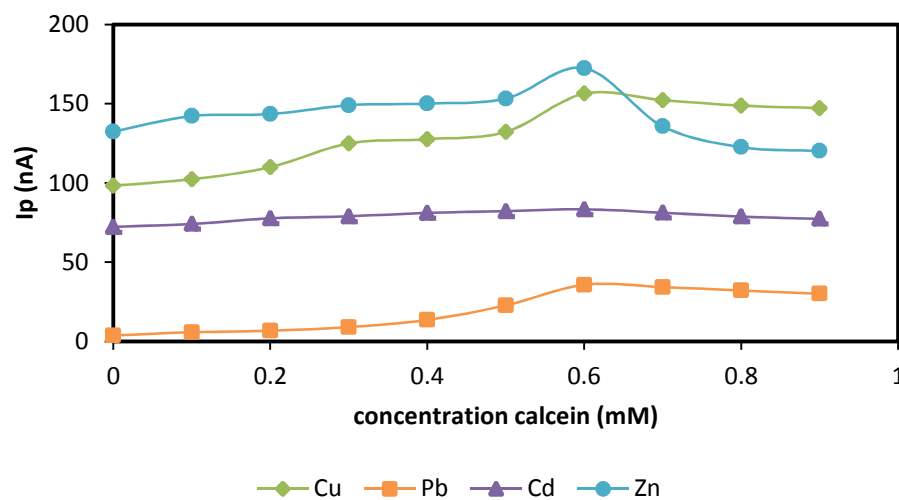

Figure 3. Effect variation of concentration calcein on peak current (Ip)
Condition : 10 mL mixed solution of Cd, Cu, Pb dan Zn 10 µg/L; 0.2 mL calcein; 0.2 mL KCl 0.1 M; accumulation time 60 s; accumulation potential -0.7 and pH 4.

3.2. Effects of pH

The inffluence of pH on the cathodic stripping peak currents of cadmium, copper, lead and zinc was studied in the pH range of 3.0 – 9.0. It was found that at pH 4.0 the peak currents of cadmium, copper, lead and zinc were at maximum value. Thus pH 4.0 was adopted for further studied (Fig. 4). The decrease in the response for cadmium, copper, lead and zinc, is due to competitive formation of metal hydroxide.
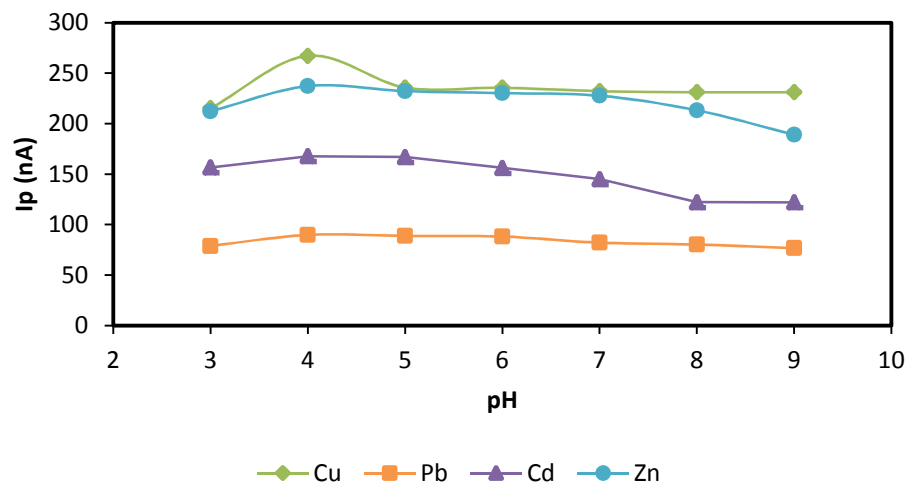
Figure 4. Effect variation of pH on peak current (Ip)
Condition : 10 mL mixed solution of Cd, Cu, Pb dan Zn 10 µg/L; 0.2 mL calcein   0.6 mM; 0.2 mL KCl 0.1 M; accumulation time 60 s and accumulation potential -0.7 V

3.3. Effect of accumulation potential

The effect of accumulation potential on the stripping peak current of the complex was examined over the potential range of -0.1 V to -0.9V. The voltammograms of the above system in the accumulation potential range indicate that the best peaks separation took place when the accumulation potential of -0.7 V was used for the pre-concentration step. As shown in Fig. 5, the peak current increased with changing potential from -0.1 V to -0.7 V, probably due to the increased accumulation of the complex on the electrode surface The peak current decreased at potentials more negative than -0.7 V, so this potential was used in all experiments.
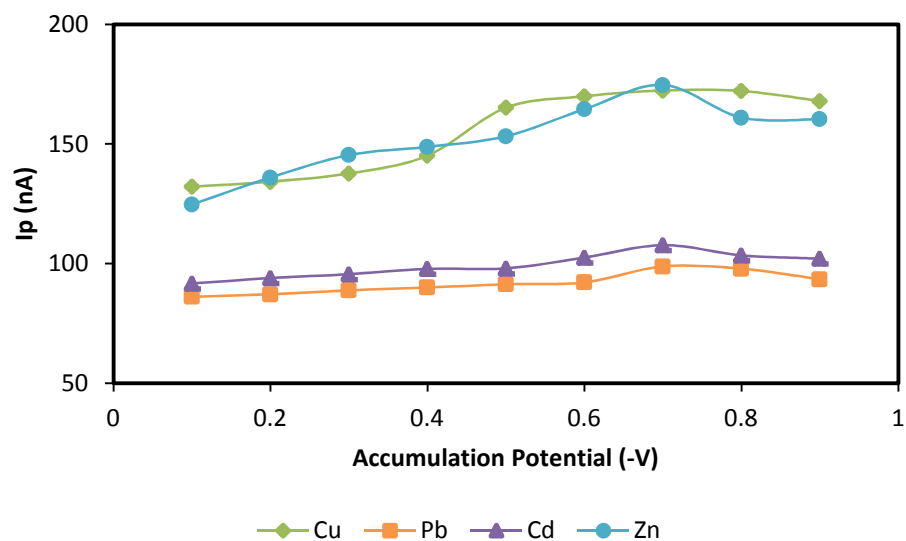

Figure 5. Effect variation of accumulation potential on peak current (Ip)
Condition: 10 mL mixed solution of Cd, Cu, Pb dan Zn 10 µg/L; 0.2 mL calcein 0.6 mM ; 0.2 mL KCl 0.1 M; accumulation time 60 s and pH 4

3.4. Effects of accumulation time

Fig. 6 shows plots of the cathodic peak current in adsorptive stripping voltammetry versus accumulation time for cadmium, copper, lead and zinc. The time required for accumulation of the metal complex, with less time required for higher concentration. At mentioned before, calcein concentrations that were higher than required may cause inhibition of the chelate adsorption by competitive coverage by the free ligand. An increase in the stripping peaks current with accumulation time was observed for Cd-calcein, Cu-calcein, Pb-calcein and Zn-calcein complexes. As was expected for adsorption processes, the dependence of the peak current on the accumulation time limited by saturation of electrode, resulting in the current reaching a plateau at high accumulation times, as shown in Fig. 6. Thus, deposition time of 60 s was used throughout, as it combines good sensitivity and relatively short analysis time.
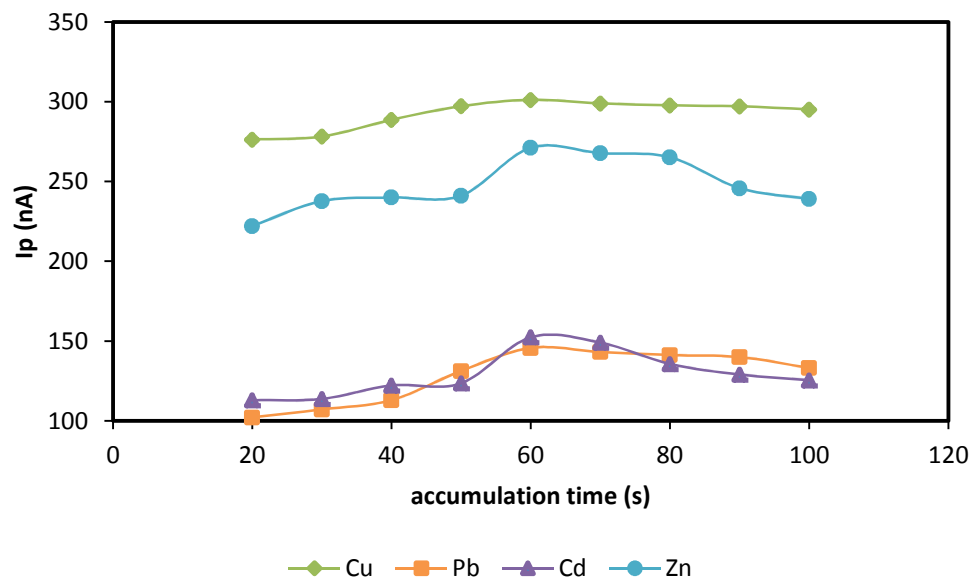
Figure 6. Effect variation of accumulation time on peak current (Ip)
Condition : 10 mL mixed solution of Cd, Cu, Pb dan Zn 10 µg/L; 0.2 mL calcein 0.6mM ; 0.2 mL KCl 0.1 M; accumulation potential – 0.7 V and pH 4

3.5. Linear range and limit of detection

Under the optimized conditions, a linear relationship between the reduction peak current of the cadmium, copper, lead and zinc complexes and the concentration of Cd(II), Cu(II), Pb(II) and Zn(II) were obtained (Table 1). To verify the linear relationship between peak current and metal concentrations, four calibration curves were plotted under optimum conditions. The calibration equation, obtained by least-squares method, is $Ip = 3.936C + 161.2$ ($r^2 = 0.986$) for Cd(II), $Ip = 4.327C + 303.6$ ($r^2 = 0.988$) for Cu(II), $Ip = 5.012C + 146$ ($r^2 = 0.988$) for Pb(II) and $Ip = 2.521C + 113.5$ ($r^2 = 0.985$) for Zn(II) determination, where C is the concentration of metal ions (µg/L) and Ip is the peak current (nA). The stripping peak current of Cd(II)-calcein, Cu(II)-calcein, Pb(II)-calcein and Zn(II)-calcein complexes were found to be directly proportional to be the cadmium, copper, lead and zinc concentration in the range of range 0.2 -1 35.0; 0.2 - 110.0; 0.2 - 100.0 and 0.2 - 110.0 µg/L, respectively. The limit of detection was calculated of three times standard deviation of blank solution devided with slope (3SD/B) under optimum conditions were obtained: 1.021, 0.987, 0.972 and 0.957 µg/L of Cd(II), Cu(II), Pb(II) and Zn(II), respectively.

Table 1. Analytical parameters obtained for simultaneous analysis for Cd(II), Cu(II), Pb(II) and Zn(II) ions

Simultaneous determination	pH	Slope/nA µg/L	Intercept/nA	R^2	Linier Range (µg/L)	Detection limit(µg/L)
Cd(II)	4	1.43	217.0	0.88	10-110	1.38
Cu(II)	4	1.40	203.5	0.94	10-100	0.76
Pb(II)	4	1.34	128.5	0.90	10-110	1.43
Zn(II	4	1.43	217.0	0.88	10-100	0.98

3.6. Relative standard deviation (RSD)

Relative standard deviation is used to look at the precision of the method at the same operating conditions. The determination of the relative standard deviation performed at optimum conditions have been defined above. At the optimum conditions with eight times replicates (n = 8) measurements of 10 µg/L standard solution of Cd(II), Cu(II), Pb(II) and Zn(II) were: 0.39, 0,74, 1.35 and 0.41%, respectively. According to the AOAC method [21], the value of relative standard deviation was smaller than 8 % at concentration of 10µg/L. That mean this method had a high degree of precision because the RSD obtained for each metals were smaller than 8 %.

3.7 Recovery

Recovery was used to determine the level of accuracy of this method. Samples of known concentration were added the amount of standard solution and then compared with the concentrations of samples and standards added. In this study the recovery of sea water samples studied was taken from Bungus Padang City. The results of recovery of Cd(II), Cu(II), Pb(II) and Zn(II) by adsorptive stripping voltammetry at the optimum conditions, its value obtained were: 99.11%; 99.88%; 98.76% and 99.79 %, respectively. According to the AOAC method [21], the percent recovery for the solution with the concentration of 50 µg/L was in the range of 70-110 %. The result can be seen in Table 2. show that method has high accuracy.

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Table 2. Simultaneous determination of Cd, Cu, Pb and Zn in sea water Bungus, Padang City

Added (µg/L)				Found (µg/L)				Recovery (%)			
Cu	Pb	Cd	Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd	Zn
0	0	0	0	18,7±2.7	12,2±1,3	38,1±1.7	184,3±1.7	-	-	-	-
10	20	30	40	27,8±1.4	31,8±1,6	69,3±1.2	225,4±2.3	97	99	102	101
20	30	40	10	37,9±3,5	41,5±1.8	78,9±2.1	193,7±2.2	98	98	101	99
30	40	10	20	48,6±2.3	51,2±2.8	48,9±2.3	203,1±1.3	99	98	102	99

Mean ± SD

3.8. Interference studies
Possible interference by other metals with the adsorptive stripping voltammetry of cadmium, copper, lead and zinc was investigated by the addition of the interfering ion to the solution containing 10.0 mg/L of these metals using the optimised conditions. The results of this study are summarised in Table 3. Based on the results, it were found that most of the foreign ions did not interfere for cadmium, copper, lead and zinc determination.

Table 3. Tolerance ratio of interfering ions in the determination of 10 µg/L of Cd, Cu, Pb and Zn

Ions	Tolerance limit (mg/L)			
	Cu	Pb	Cd	Zn
Na^+, Al^+, Ca^+, Li^+, K^+, Ba^{2+}, Cr^{+3}, Co^{2+}, Ni^{2+}, Cl^-, F^-, Br^-, SO_4^{2-}, I^-	10	10	10	10
Cu^{2+}	-	10	1	1
Pb^{2+}	10	-	10	10
Cd^{2+}	10	10	-	10
Zn^{2+}	1	1	10	-
Fe^{3+}	1	1	1	1

3.9. Application
The proposed method was successfully applied for determination of cadmium, copper, lead and zinc in several water samples. The Cd(II), Cu(II), Pb(II) and Zn(II) contents of water samples, such as sea water, tap water and lake water were determined using the recommended procedure under optimum conditions, using the standard addition method. The results presented in Fig. 7, show the high sensitivity of the proposed method.
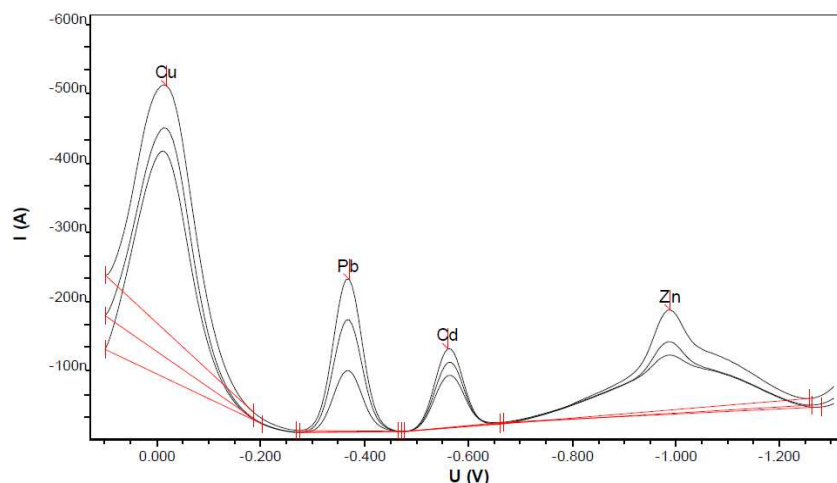

Fig. 7. Voltammogram of Cd, Cu, Pb and Zn in sea water samples from Bungus Padang City

CONCLUSION

It can be concluded that the optimum conditions for the simultaneous measurement of Cd(II), Cu(II), Pb(II) and Zn(II) by adsorptive stripping voltammetry were : concentration of calcein 0.6 mM, pH 4.0, accumulation potential of -0,7 V (*vs*. Ag/AgCl) and accumalation time of 60 s. Under the optimum conditions the measurement peak current proportional to the concentration of Cd(II), Cu(II), Pb(II) and Zn(II) in the range 0.2 - 110 µg/L ; 0.2 – 100 µg/L ; 0.2 – 110 µg/L ; and 0.2 – 100 µg/L respetively. The limit of detections of the method were 1.38 µg/L for Cd(II), 0.76 µg/L for Cu(II), 1.43 µg/L for Pb(II) and 0.98 µg/L for Zn(II). The relative standard deviation were 0.84%, 0.25%, 0.43% and 0.54% for Cd(II), Cu(II), Pb(II) and Zn(II) for ten replicates (n = 10) measurements of mixture solution 10 µg/L of Cd(II), Cu(II), Pb(II) and Zn(II). The method was successfully applied for simultaneous determination of Cd(II), Cu(II), Pb(II) and Zn(II) in sea water, tap water and lake water.

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