APPLICABILITY OF CALCEIN IN THE SIMULTANEOUS DETERMINATION OF TRACE METALS COPPER, LEAD AND CADMIUM BY CATHODIC ADSORPTIVE STRIPPING VOLTAMMETRY

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ABSTRACT

The present study of Calcein as complexing agent for simultaneous determination of Cu(II), Pb(II) and Cd(II) by Cathodic Adsorptive Stripping Voltammetry had been conducted. This method based on adsorptive accumulation of Cu(II)-Calcein, Pb(II)-Calcein, and Cd(II)-Calcein complexes onto hanging mercury drop electrode (HMDE). Followed by reduction of adsorptive species onto work electrode. The Optimum conditions were achieved at calcein concentration 6 mM, pH 5, accumulation time 50 seconds and accumulation potential -0.7V. This method has been applied to determine Cu(II), Pb(II) and Cd(II) in tap water, river and sea water. Concentration of metal ion in tap water was obtained Cu(II) 226.12µg/L, Pb(II)8.84 µg/L, while Cd(II) undetected. For river was obtained Cu(II) 119.10µg/L, Pb(II) 6.65 µg/L, Cd(II) 33.67 µg/L and for sea water was obtained Cu(II) 804.63 µg/L, Pb(II) 80.86µg/L while Cd(II) undetected. Relative standard deviation was obtained Cu(II) 0.53%, Pb(II) 3.16% and Cd(II) 7.69% and recovery was obtained Cu(II) 99.97%, Pb(II) 96.68% and Cd(II) 99.31%.
Keyword: Cathodic adsorptive stripping voltammetry, simultaneous, copper, lead,cadmium, calcein

INTRODUCTION

Heavy metals such as copper, lead and cadmium are toxic when absorbed in the body, their accumulative behavior causes poisoning, cancer, brain damage, etc. Copper is a heavy metal that is needed by the body but in very small quantities. Copper contained in several enzymes. The maximum allowed in the body that is 0.5 mg/kg body weight (WHO technical report, No. 683.1982). In larger quantities of copper will cause anemia, systemic heart disease and bone disorders[1].

Lead is a general metabolic poison and enzyme inhibitor. It can cause mental redardation and semipermanent brain damage in young children. Lead has the ability to replace calcium in bone to form sites for long-term replace[2]. Lead is toxic to the metabolic and enzyme inhibitor, it can cause mental retardation and brain damage in children semipermanent[3]. Lead also can accumulate in bone because these metals can form Pb^{2+} ions which can replace the presence of Ca^{2+} on bone tissue[4].

Metal cadmium is a highly toxic substance and easily accumulates in soft tissues. The target cadmium poisoning is the kidney and liver[5]. Its toxicity of cadmium is influenced by the interaction between cadmium with sulfihidril group (-SH) of the protein which causes inhibition of enzyme activity[6]. Because of the increased industrial use of copper, lead and cadmium and its serious hazardous effect to human health, the development of new sensitive methods for quantifying trace amount of copper, lead and cadmium is required.

On the other hand, trace metal ions of copper, lead and cadmium frequently exist together in many real environmental samples. It is necessary to determine these trace metal ions simultaneously in most instances. The common methods for the detection of trace metal ions include spectrophotometry[7], electrophoresis[8], liquid chromatography[9], solid-phase extraction coupled with atomic absorption

spectroscopy[10],atomic emission spectroscopy[11] and inductively coupled plasma mass spectrometry.[12] However, even if these methods present high sensitivity and selectivity, they are expensive,time-consuming and can not be used for insitu analyses.Therefore,there is a growing interesting developing electrochemical methods for the detection of trace metal ions in different matrices. Electrochemical detection of trace metals presents many advantages[13], such as good sensitivity, high selectivity without any prior separation (due to complexation by organic molecules containing coordinating sites), fast analytical response making them useful for flow analysis[14] and alert systems, easy of use (simple and low cost equipment, few analytical steps) and it offers the possibility of outside laboratory analysis, using a portable "pocket" potensiostat.

Among the electrochemical methods for the detection of trace metals, the most used ones are Cathodic Adsorptive Stripping Voltammetry (CAdSV). For the above reason Cathodic 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 µg/L, simple and easy sample preparation, rapid analysis, less infra structure[15-17]. In addition, with this method it is possible to learn chemical species of heavy metals[18-19], which can not be done with other methods.

The purpose of this technique is to make the analysis more selective and lower detection limit. Selectivity can be improved by selecting the ligand and the electrolyte solution, which is used the more selective ligand selectivity. Detection limit lowered by increasing the concentration of the analyte adsorbed on the electrode surface[15,18].Cathodic Adsorptive Stripping Voltammetry method takes a ligand to form a complex with the metal to be analyzed. Several ligands have been used for the determination of trace metals simultaneously is 8-quinolinol[20], xylinol orange[21] and calcon[22]. Calcein use as a complexing agent in the method CAdSV ever done for titanium[23], aluminum and gallium[24], and lead[25]. Under these conditions, in this study used Calcein as a complexing agent in the simultaneous determination of Pb(II), Cu(II) and Cd(II) in CAdSV. Cathodic Adsorptive Stripping Voltammetry is a method used to determine the trace elements by combining the sensitivity, selectivity, accuracy and precision that is superior to the method proposed for the determination of Cu(II), Pb(II) and Cd(II) simultaneously using Calcein as a complexing agent.

Calcein first introduced and used by Diehl and Ellingboe in 1956. Calcein molecules have fluorescent properties and acid-base of fluorescent structures and properties of the acid chelates etilendiamintetraacetat. Fluorescent calcein rose from zero to maximum at pH 3 to pH 6.5 to 8.5, and dropped at a higher pH, and becomes zero at pH 12 and above.
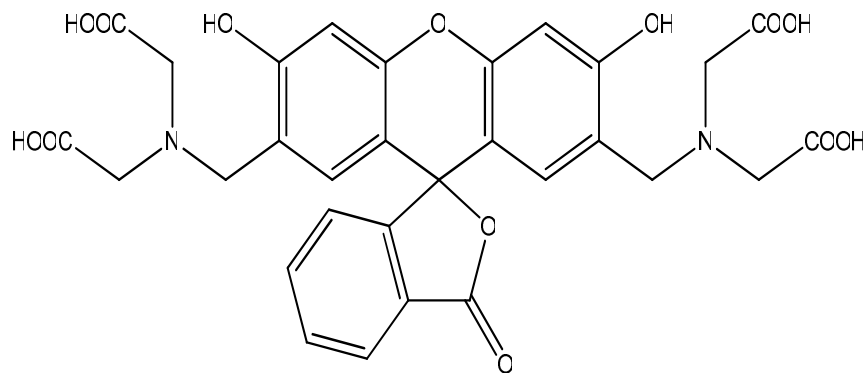

Fig.-1: Structure of Calcein

Calcein reacted differently to the metal ion. In a solution of pH 6.5 to 8.5, which indicates calcein maximum fluorescence will be lost with the addition of copper, cobalt, iron, nickel and many other metal ions. In the solution with a high pH, which did not show any fluorescence calcein but with the addition of calcium, strontium, barium and magnesium showed fluorescence but only at not too high pH to precipitate the cation be hydroxide.

Calcein (8-[N,N-bis(carboxymethyl)aminomethyl]-4-methylumbelliferone) at first use is as an indicator metalokrom titration determination of calcium and magnesium at pH 12 using EDTA. Calcein been used in direct determination flourimetric calcium, in addition calcein has also been used in the spectrophotometric method, but it is also used for the determination of non-metals such as cyanide and sulfuric.[24]

Calcein use as complexing has been carried out for the determination of titanium metal with catalytic adsorptive stripping voltammetry method and obtained the detection limit of 0.12 nM with a linearity of 1.7 to 160 nM[23]. Calcein determination of complex formation constants with metals Pb, Cd, Ag and Cu have also been carried out and obtained the constant formation of each complex[26] for $1.17x10^8$, $1.59x10^{12}$, $3.32x10^8$and $3.80x10^8$.

In this study, we have examined the application of Calcein to the simultaneous determination of copper, lead and cadmium by Cathodic Adsorptive Stripping Voltammetry on a hanging mercury drop electrode (HMDE). The method relies on the effective accumulation of the Cu(II), Pb(II) and Cd(II) complexes with Calcein onto the HMDE. The method is extremely sensitive, selective and simple for the determination of trace levels of copper, lead and cadmium in real samples(tap water, river water and sea water).

EXPERIMENTAL

Instrument
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 auxiliary electrode, pH meter models 80 Griffin(Griffin & George Loughborough, UK), analytical balance Mettler AE 200, Toledo OH-USA and glassware commonly used in laboratory.

Material
The materials used for this study were KCl, Calcein, HNO_3, Standard solutions of Cu(II) 1000 mg/L, Standard solutions of Pb(II) 1000 mg/L, Standard solutions of Cd(II) 1000 mg/L, NH_4OH, CH_3COONH_4, CH_3COOH, N_2 gas, doubly distilled water, Whatman filter paper and water samples (tap water, river water and sea water).

Sample Preparation
Tap water samples were taken from laboratory of Analytical Chemistry, Andalas University, river water taken from Batipuh Koto Tangah Padang City and sea water taken from Padang City. Samples have been taken with the added $HNO_3$with comparison HNO_3: sample = 1: 1000.

Work procedures
The aim of this study was to obtain the optimum condition of each metal ion Pb, Cu and Cd by cathodic adsorptive stripping voltammetry. Therefore studied the effect of the following parameters namely: variations of concentration calcein of 1.0 to 10.0 mM, pH at 3 - 8, accumulation potential at -1.0 to -0.5 V and accumulation time at 10 to 100 second. The precision and accuracy of method relative standard deviation (RSD) and recovery were determined.Procedure used in this study was: according to reported previously.[15]

RESULTS AND DISCUSSION

Optimum Conditionsof Measurement
This study aims to find the optimum condition of Cu(II), Pb(II), and Cd(II) simultaneously using cathodic adsorptive stripping voltammetry to be applied in tap water, river water and sea water samples. Use the solution of 0.1M KCl as supporting electrolyte and Calcein as a complexing agent. Parameters studied: pH,Calcein concentration, accumulation time and accumulation potential.

Preliminary experiments has been conducted as shown in Figure-2:(a) voltammogram of 1 mM Calcein without metal, (b) voltammogram of metal ions Cu(II), Pb(II) and Cd(II) without the addition of Calcein

and (c) voltammogram for the complex Cu-Calcein, Pb-Calcein and Cd-Calcein. At Calcein measurements without the metal is seen that there is no peak current in a potential range used, the measurement of metal ions in the absence of visible Calcein peak currents low in potential areas -0.08 V, -0.4 V and -0.55 V. Higher peak currents and clearly visible on the measurement of metal ions Cu(II) 10µg/L , Pb(II) 50µg/L, Cd(II) 30 µg/L supplemented with 1 mM Calcein on the potential area of -0.08 V, -0.4 V and -0.55 V. Each metal ion peak currents are at different E½. For Cu(II) on the potential -0.08 V, Pb (II) at a potential of -0.4 V and metals ion Cd (II) at potential -0.55 V. Determination of Cu(II), Pb(II) and Cd(II) simultaneously using Cathodic Adsorptive Stripping Voltammetry test method using Calcein as a complexing agent can be done because the three metals can be measured by a separate voltammogram for each metal.
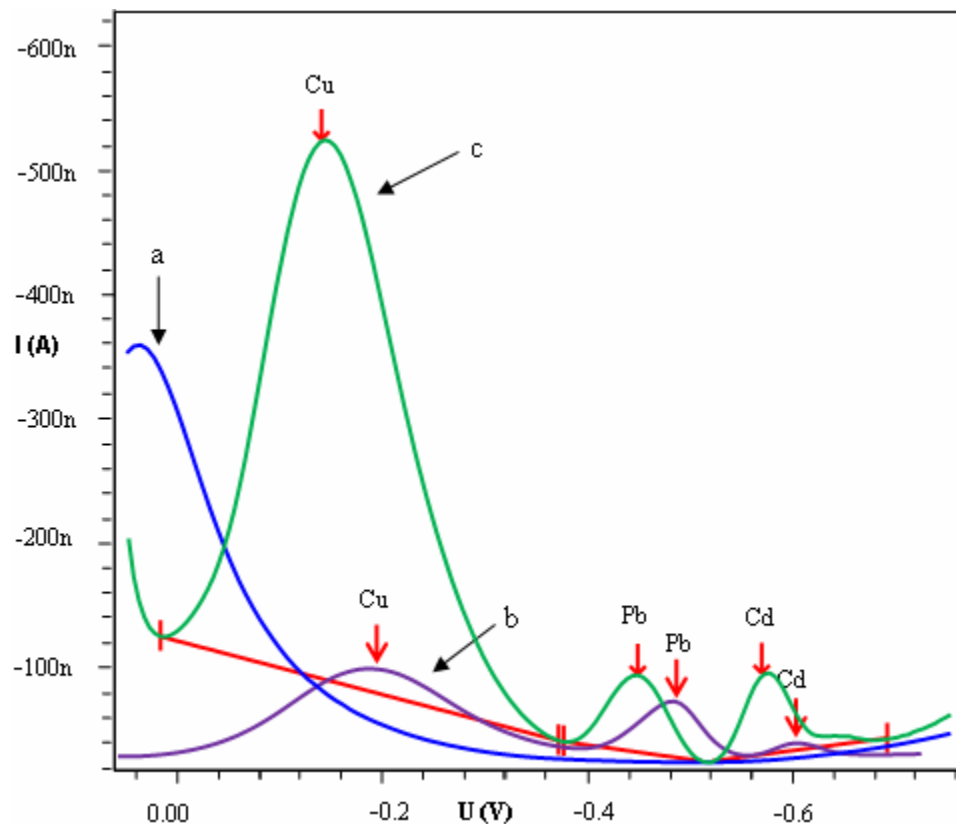

Fig.-2: Voltammogram adsorptive stripping voltammetry(a) Calcein without metal,(b) metal without Calcein,(c) Cu(II), Pb(II) and Cd(II) respectively is 10µg/L, 50µg/L, 30µg/L with 1mM Calcein

Effect of calcein concentration
Calcein influence on the sensitivity of the determination of the concentration of metal ions Cu(II), Pb(II) and Cd(II) simultaneously with Cathodic Adsorptive Stripping Voltammetry method studied at various concentration Calcein of 1 mM; 2mM; 3 mM; 4 mM; 5 mM; 6mm; 7 mM; 8 mM; 9 mM and 10 mM in the standard solution mixture of Cu (II) 10µg/L, Pb(II) 50 µg/L and Cd(II) 30 µg/L. The condition of -0.7 V accumulation potential, accumulation time of 70 seconds, pH 5 and 0.1 M KCl as supporting electrolyte.
The results (Figure-3) showed peak current Pb-Calcein and Cd-Calcein complex increased with increasing concentration until the concentrations of 6 mM and at concentrations greater peak current Pb-Calcein and Cd-Calcein complex decreased, whereas the Cu-Calcein complex peak current decreases with increasing concentration up to 5 mM and the peak current rise complex Cu-Calcein occur at

concentrations of 6 mM and declined in Calcein greater concentration of 6 mM. In the complex Cu-Calcein maximum peak current obtained at a concentration of 1 mM Calcein while for complex Pb-Calcein and Cd-Calcein at a concentration of 6 mM Calcein. This difference occurs because the Calcein more reactive to the metal ion Cu(II) than the metal ion Pb(II) and Cd(II) so that the Calcein small concentration of metal ions Cu(II) will first form a complex with Calcein.
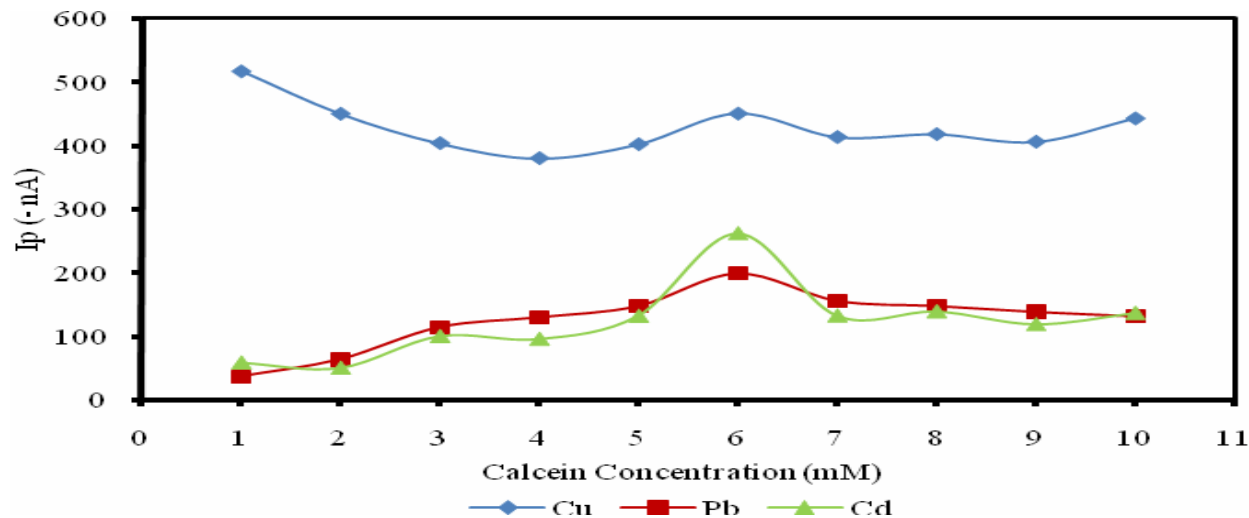

Fig.-3: Calcein concentration curve to the peak current(Ip). Measurement conditions: 10mL of a mixture of standard solution of Cu(II), Pb(II) and Cd(II) with a concentration of each of 10 µg/L, 50 µg/L and 30 µg/L, 0.2mL KCl 0.1M,-0.7V accumulation potential, accumulation time of 70 seconds, a potential scan from 0.172V to-0.75V and pH5.

The peak current resulting from the reduction of metal-ligand complexes are adsorbed on the surface of the working electrode on the stripping step. At Calcein small concentrations peak current complex and Cd-Calcein, Pb-Calcein low because there are metal ions which have not formed a complex with Calcein[27]. While the reduction in peak current at concentrations Calcein higher than the optimum concentration occurs because of competition ligands adsorbed on the surface of the electrode when step deposition (preconcentration) and form complexes with metal ions as well as competition adsorption between ligand with ligand-free so that the electrode surface is available for adsorption of metal-ligand complex into smaller, besides the high ligand concentration of metal-ligand complex would be difficult to be reduced so that the resulting peak currents lower. For the concentration of 6 mM Calcein chosen as the optimum conditions for simultaneous determination of metal ions Cu(II), Pb(II) and Cd(II) by Cathodic Adsorptive Stripping Voltammetry.

Effect of pH
The complex formation between the Cu(II), Pb(II) and Cd(II) ions with Calcein at accumulation step were affected by pH. The reaction process is described in reaction

Accumulation step
$M^{n+} + nL \rightarrow ML_n$
$ML_n \rightarrow ML_{nads}$
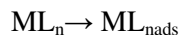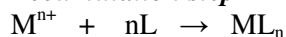
Stripping step
$ML_{nads} + ne \rightarrow M^{n+} + nL$
At the proper pH it was expected more and more Cu-calcein , Pb-calcein, and Cd-calcein complexes formed at the electrode surface, so that they would produce optimum peak current at the time of

measurement. The influences of pH on the adsorptive peak currents (Ip) of Cu, Pb and Cd were studied in the pH range of 3 to 8. The result are shown in Fig.-4. At accumulation (pre-concentration) step, the complex formation between the Cu(II), Pb(II) and Cd(II) ions with calcein were affected by pH. The results shown that the peak current Cu-Calcein and Pb-Calcein increased from pH 3 to 4 and decreased at higher pH, Cd-Calcein peak currents up from pH 3 to pH 5 and decreases at higher pH. At low pH there is an excess proton resulting in competition with metal ions to form a bond with the ligand so that the resulting current low, while at a pH greater than the pH optimum peak current decline due to OH⁻ ions so that metal ions can form hydroxide and will settle on electrode surface that is difficult to remove[27]. For the pH 5 chosen as the optimum pH in the simultaneous determination of Cu(II), Pb(II) and Cd(II).

pH plays a role in increasing the number of complex compounds or ion association formed in the process of adsorption on the electrode HMDE that would influence the formation of a complex, complex stability and absorption properties[28]. Effect of pH on peak current complex Cu-Calcein, Pb-Calcein and Cd-Calcein studied at various pH3; 4; 5; 6; 7 and 8 using acetate buffer with measurement conditions the concentration of metal ions Cu(II), Pb(II), Cd(II) respectively is 10µg/L, 50µg/L, 30µg/L,Calcein 6mM, accumulation potential of -0.7V, and accumulation time of 50s.
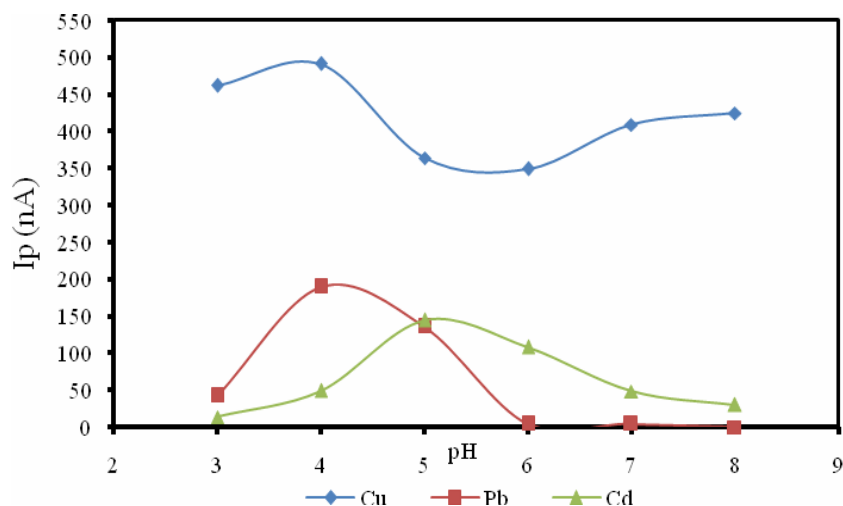

Fig.-4: pH curve on peak current(Ip). Measurement conditions: 10mL of a mixture of Cu(II), Pb(II), Cd(II) with a concentration of each of 10µg/L , 50µg/L and 30µg/L , 0.2mL Calcein 6mM, 0, 2mL of 0.1M KCl, accumulation potential -0.7V, the accumulated time of 70seconds, a potentials can from 0.172V to-0.75V.

Figure-4. shows the influence of pH on peak current complex Cu-Calcein, Pb-Calcein and Cd-Calcein, peak current Cu-Calcein and Pb-Calcein increased from pH 3 to 4 and decreased at higher pH, Cd-Calcein peak currents up from pH 3 to pH 5 and decreases at higher pH. At low pH there is an excess proton resulting in competition with metal ions to form a bond with the ligand so that the resulting current low, while at a pH greater than the pH optimum peak current decline due to OH⁻ ions so that metal ions can form hidroxide and will settle on electrode surface that is difficult to remove[27]. For the pH 5 chosen as the optimum pH in the simultaneous determination of Cu(II), Pb(II) and Cd(II).

Effect of AccumulationPotential
In the above figure-5 can be seen, in every variation of accumulation potential were tested, giving values of different peak currents. Accumulation potential is a potential at the time of the analyte deposited on the working electrode. Determination of the potential effects on the accumulation of peak current complex

Cu-Calcein, Cd-Calcein and Pb-Calcein tested the potential range -0.5 V to -1.0 V. The measurement conditions of the accumulated time of 70 seconds, the concentration of standard solution mixture of Cu(II), Pb(II) and Cd(II) each of 10µg/L, 50µg/L and 30µg/L, the concentration of 6 mM Calcein and at pH 5.

The potential effect on peak current complex accumulation of Cu-Calcein, Cd-Calcein and Pb-Calcein. Cu-Calcein and Cd-Calcein peak current highest accumulation potential obtained at -0.7 V, and for complex Pb-Calcein at a potential of -0.6 V. On a more positive potential than -0.6 V and -0, 7 V peak currents low due to the complex process of deposition on the surface of the working electrode has not reached the maximum, while the accumulation potential more negative than optimum potential analyte complex reduction process occurs during the deposition process takes place so that the peak current is obtained decreasing[27]. Potential -0.7V chosen as the optimum accumulation potential for the simultaneous determination of metal ions Cu(II), Pb(II) and Cd(II).
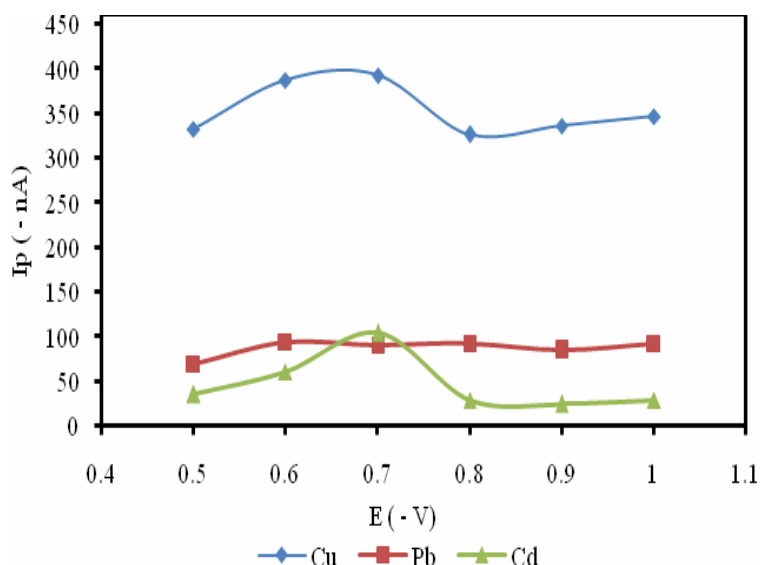

Fig.-5: Accumulation potential curve of the peak current (Ip). Measurement conditions: 10 mL of a mixture of Cu(II), Pb(II), Cd(II) with a concentration of each of 10µg/L, 50µg/L and30µg/L, 0.2 mL Calcein 6 mM, 0, 2 mL of 0.1 M KCl, accumulation time of 70 seconds, a potential scan from 0.172 V to -0.75 V and pH 5.

Effect ofAccumulationTime
Accumulation time is the time when the analyte accumulated or deposited on the surface of the working electrode HMDE (Hanging Mercury Drops Electrode). Effect on the deposition time during the accumulation step (pre-concentration). At the accumulation time pre-cocentration was useful to improve sensitivity and lower detection limit[28-29]. The length of time the accumulation of Cu(II), Pb(II) andCd(II) affects the stability of the complex formed on the electrode surface. In this study, to determine the relationship between accumulation time with a peak current tested, by varying the accumulation time as follows: 10, 20, 30, 40, 50, 60, 70, 80, 90 and100 seconds. The result can be seen in Fig.-6.

Figure-6 shows the relationship between peak current accumulation time with the metal complex. The peak current complex Cu-Calcein highest in accumulation time 10seconds, this was due to Cu(II) is more reactive to Calcein so that at no time can form complexes with Calcein and deposited on the working electrode than the metal ions Pb(II) and Cd(II). High flow metal ions Pb(II) and Cd(II) increases in the accumulated time that the longer, because the metal ions are bound to Calcein and accumulates on the electrode surface more and more and after reaching the accumulation time of 50 seconds has decreased due to saturation at the electrode surface work so that when the metal complex deposition process takes

place it will be difficult for the reduced[30]. At the optimum time all metal complexes have been deposited perfect that produce high peak currents and optimum accumulation time used for the determination of metal ions Cu(II), Pb(II) and Cd(II) simultaneously next is 50 seconds.

Relative Standar Deviation (RSD)
Relative standard deviation is used to see at the precision of the method is the same operating conditions. The determination of the relative standard deviation performed at optimum conditions have been defined above. At the optimum conditions with 10 times replicates (n = 10) measurements of 10 mL mixture standard solution Cu(II), Pb(II) and Cd(II) with concentration 10 µg/L, 50 µg/L, 30 µg/L, respectively were : 0.53%, 3.16%, and 7.69%. According to the AOAC method[31], the value of relative standard deviation was smaller than 8% at concentration of 10 µg/L . That mean this method has a high degree of precision because the RSD obtained for each metals was smaller than 8%.
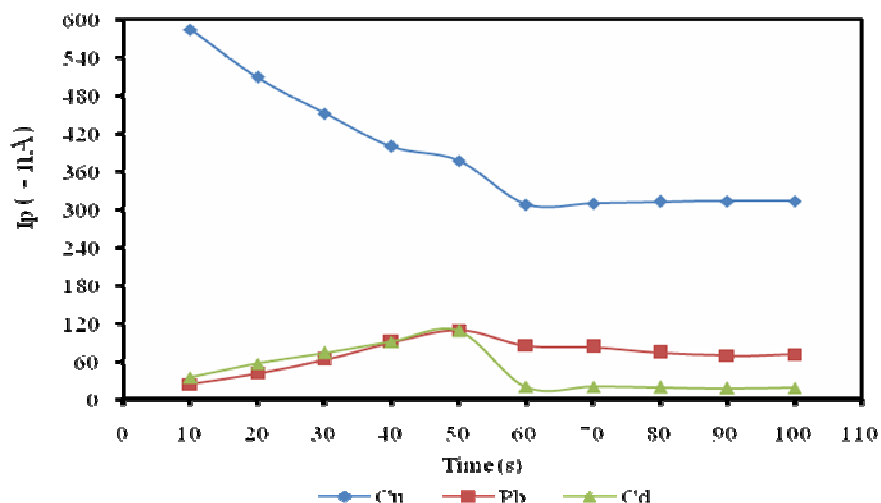

Fig.-6: The curve between the accumulated time versus the peak current (Ip), Conditions of measurement: 10 mL of a mixture of Cu(II), Pb(II), Cd(II) with a concentration of each 10µg/L , 50µg/L and 30µg/L , 6 mM Calcein 0.2 mL, 0.2 mL of 0.1 M KCl, accumulation potential -0.7 V, a potential scan from 0.172 V to -0.75 V and pH 5.

Determination of the relative standard deviation (RSD) was conducted to see the accuracy of the method in the same operating conditions in a short time interval. Determination RSD performed at a concentration of standard solution mixture of Cu(II), Pb(II), and Cd(II) respectively is 10µg/L, 50µg/L, 30µg/L with repetition as much as 10 times (n = 10). Conditions of measurement used are the optimum conditions that have been found previously, namely: Calcein optimum concentration of 6 mM, pH optimum solution of 5, the optimum accumulation potential -0.7 V and optimumaccumulation time of 50 seconds.

Analytical Application
The proposed method was successfully applied to the determination of Cu(II), Pb(II) and Cd(II) in tap water from laboratorium Analytical Chemistry of Andalas University, river water from Batipuh Koto Tangah Padang City and seawater from Padang City. It can be seen Fig.-8 and Table-1 using the optimum conditions for measurement that had been obtained for each of metals ion them. The standard addition method was used, in order to eliminate the matrix effect. The data obtained for samples spiked with known amounts of Cu(II), Pb(II) and Cd(II) showed good recoveries.

In Table-1, it can be seen the concentration of Cu(II) in tap water, river water and sea water was 226.12µg/L, 119.10µg/L, and 804.63 µg/L . The content of Cu(II) contained in seawater samples desert. Concentration of Pb(II) contained in tap water, river water and sea water was 8.84µg/L, 6.65µg/L,

80.86µg/L and metals Cd(II) only measured in river water samples 33.67 µg/L. This occurs because water sampling locations were around waste disposal.

Recovery

Determination of recovery was conducted to determine the level of accuracy of the method. Samples of known concentration added with a standard solution, then compared to the concentration of samples and standards are added. In this study, the recovery value of the studied samples of river water.

The determination of the recovery is done by measuring the10ml samples of known concentration in which there is a standard solution of Cu(II), Pb(II) and Cd(II) with a concentration of 1µg/L, 2µg/L and 3µg/L. Measurements were taken at the optimum conditions that have been obtained in the previous determination. The recovery by adsorptive stripping voltammetry method of Cu(II) was 99.97%, Pb(II) was 96.68% and Cd(II) was 99.31%.

Value recoveries of the three metal ions using adsorptive stripping voltammetry method has good accuracy and precision as it still is in the standard range of recovery AOAC is 80-110% for the analyte concentration of 100µg/L [31].
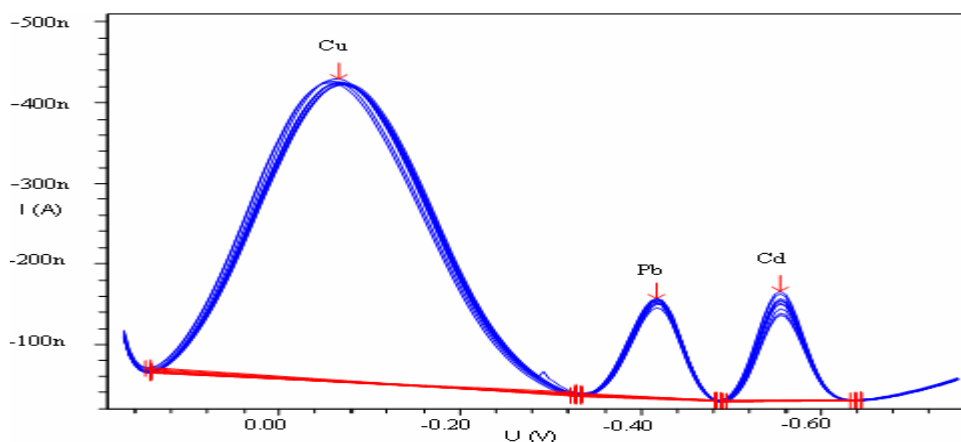

Fig.-7: Voltammogram for Cu(II), Pb(II) and Cd(II). Measurement conditions: concentration standard mixture of Cu(II), Pb(II), and Cd(II) respectively is 10 µg/L , 50 mg/L, 30 g/L, 0.2 mL of 0.2 mM Calcein, 0.2 mL of 0.1 M KCl, accumulated time of 50 seconds, accumulation potential -0.7V, pH 5 and repetition as much as 10 times
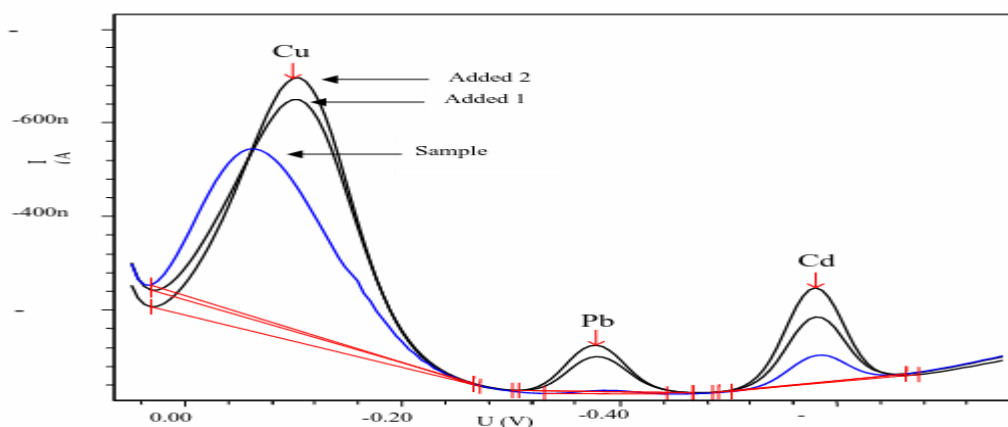

Fig.-8: Voltammogramat river water samples of Batipuh Koto Tangah Paandg City, the standard addition method at accumulation potential -0.7V, accumulation time of 50 seconds, pH5, 6mM,Calcein concentration and scan potential of 0.05V to-0.7V.

Pb
c =   6.651 ug/L
+/-   5.919 ug/L (89.00%)

19.099 ug/L
33.244 ug/L (27.91%)
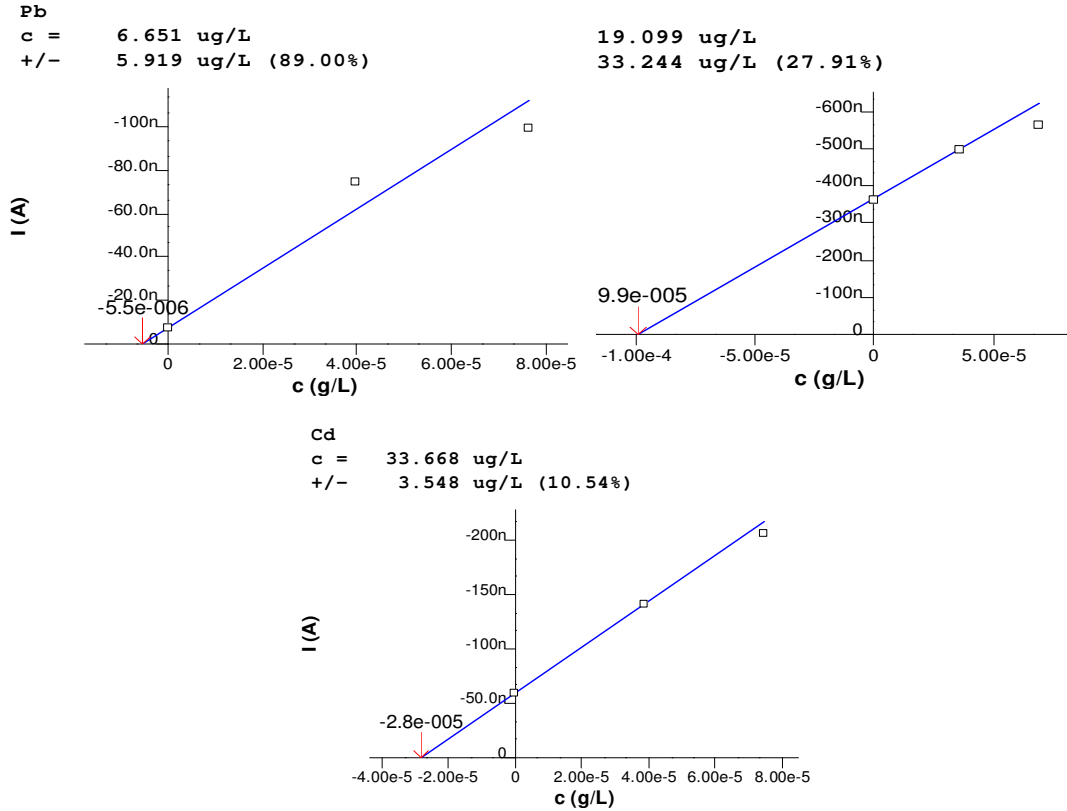

Cd
c =   33.668 ug/L
+/-   3.548 ug/L (10.54%)

Fig.-9: Curve addition of river water Batipuh Panjang Koto Tangah Paandg City.

Table-1: Results of measurements of Cu(II), Pb(II) and Cd(II) in water samples

No	Sample	Metal concentration (µg/L)		
		Cu(II)	Pb(II)	Cd(II)
1	Tap water	226.12	8.84	0
2	River water	119.10	6.65	33.67
3	Sea water	804.63	80.86	0

CONCLUSION

It can be concluded that the optimum conditions for the determination of Cu(II), Pb(II) and Cd(II) simultaneously with CAdSV, namely: Calcein concentration of 6 mM, pH 5, the accumulated time of 50 seconds and a potential accumulation of -0,7V. By using the optimum condition is the determination of relative standard deviation for each metal at 0.53% for Cu(II), 3.16% for Pb(II) and 7.69% for Cd(II).The optimum conditions of simultaneous measurement of all three metals are then applied to the three water samples. On tap water samples obtained measurement as follows: 226.12µg/L for Cu(II), 8,84µg/L for Pb(II) and found no metal Cd(II). While in the river water samples obtained measurement data as follows: 119.10µg/L for Cu(II), 6.65µg/L for Pb(II) and 33.67 µg/L for Cd(II). In the samples of sea water, the measurement data obtained as follows: 804.63 µg/L for Cu(II), 80.86µg/L for Pb(II) and no metal content of Cd(II).Data recovery for a third calculation of Cu(II), Pb(II) and Cd(II) is 99.97%, 96.68% and 99.31%.

ACKNOWLEDMENTS

REFERENCES

1.   S. Abbasi, K. Hussein, T. Reza, *Food Chem.,* **123**, 507, (2010).
2.   H.S. Sanusi,*The Content and Distribution of Heavy Metals in Various Marine Fish Commoditiae Transmitted Trough the TPI Fish Market Jakarta*, Faculty of Fisheries, Bogor Agricultural Institute(1985).
3.   E. Espada-Bellido, M.D. Galindo-Riano, M.Garcia-Vargas, *J. of Hazard. Mat.,* **166, 1326**(2009).
4.   A. Asghari, *The Malay. J. Anal. Sci.,* **12(2),** 410(2008).
5.   P. Suciu, V. Marisol, R. Liviu, *J.of Pharm. and Biomed. Anal.,* **23**,99(2009).
6.   E. Mohamad, *J.of Entrophy*.,**8**,1(2013)
7.   X. Wen, Q. Yang,Z. Yan and Q. Deng, *Microchem.J.,***97**, 249(2011).
8.   F.S.Lopes, O.A. Junior and G.R. Gutz, *Electrochem.Comm.,***12**,1387(2010).
9.   N. McGillicuddy, E.P. Nesterenko, P.N. Nesterenko, P. Jones and B. Paull. *J. of Chromatogr.A*, **1276**, 102(2013).
10. K. Ghanemi, Y. Nikpour, O. Omidvar and A. Maryamabadi, *Talanta,* **85**, 763(2011).
11. W. Li, P. Simmons, D. Shrader, T.J. Herrman and S.Y. Dai, *Talanta,* **12**, 43(2013).
12. B. Dai, M. Cao, G.Fang, B.Liu, X. Dong, M. Panand S. Wang, *J. of Haz. Mat.,* **103**, 219(2012).
13. A.J. Bard and L.R. Faulkner, *Electrochem.Methods: Fund.and Applic.*, 2nd Ed., John Wiley & Sons, NewYork (2001).
14. R. Nasraoui, D.Flonerand F. Geneste, *Electrochem. Comm.,***12**, 98(2010).
15. Deswati, H. Suyani and Safni, *Ind. J. Chem.,***12(1),**20(2012).
16. A. Ensafi, S. Abbasi and H. Rahimi-Mansour, *Anal. Sci.,* **17**,609(2001).
17. S. Zang and W. Huang, *Anal. Sci.,* **17**, 983(2001).
18. Deswati, H. Suyani, Imelda and Yulia, *J. Res. of Chem.,***3(1)**,22(2009).
19. R. Jugade and A. P. Joshi, *Anal. Sci.,* **22(4)**, 571(2006).
20. C. Collado-sanchez, J. Perez-pena, M.D. Gelado-caballero, J.A. Herrera-melion, *Anal. Chim. Acta*, **320**, 19(1996).
21. A.A. Ensafi, T. Khayamian, A. Benridi, E. Mirmomtaz, *Anal. Chim. Acta,* **561**, 225 (2006).
22. Deswati, H. Suyani, Safni, U. Lukman, H. Pardi, *Indo. J. Chem.,***13(3)**, 236 (2013).
23. M. Gawrys, J.Z. Mowski, *Anal. Chim. Acta*, **427,** 55 (2001).
24. S. Saito, S. Jun-ichi, Y. Keitaro, M. Mizuo, A. Masakazu, *J. Chromatogr. A*.,**1140**, 230(2007).
25. E. Fischer, M.G. Constant, Vanden Berg, *Anal. Chim. Acta,* **432**, 11(2001).
26. D. Phillips, B. Paul Jr, *Anal. Lett.,* **4(12),** 867(1971).
27. Deswati, E. Munaf, H. Suyani, R. Zein and H. Pardi, *Asian J. of Chem.***13(3)**,3978 (2015).
28. H. Palar, *Heavy Metal Contamination and Toxicology*, Rineka cipta, Jakarta, (2012).
29. S. Abbasi, B. Atousa, A. Freshteh, *Food Chem.,* **129,**1274 (2011).
30. Deswati, E. Munaf, H. Suyani, U. Loekman, H. Pardi,*Res. J. Pharm. Biol. and Chem. Sci*., **5(4)**, 990 (2014).
31. *AOAC Guidelinesfor Single Laboratory*, (Http://www.aoac.org/Official Methods/slv guidelines.pdf), 19/12/2002.

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