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Monitoring of Trace Metals in Biological Samples Using Electrochemical Nanosensors Prior to Anodic Stripping Votammetry

Rekha Dasari and Yugandha Sreedhar Neelam*

Department of chemistry, S.V.University, Tirupati 517502 A.P, India

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Abstract

The present paper, outline the simultaneous determination of lead and zinc using electrochemical nanosensors like electrochemical conjugated polymers (ECP's) which is prepared by polymerization process and its application in biological samples. In this work the metal ions were complexed with a newly sysnthesised analytical reagent 4-(2-hydroxy phenyl ethaminodiol), benzene-1, 3-diol (4-2-HPEDB-1,3,D) and analysis was carried out by anodic stripping voltammetry. Electrochemical characteristics like scan rate, deposition potential, deposition time, effect of pH were studied under optimized conditions. The calibration curve was constructed with in the range of 0.05–200 μg mL^{-1} at pH 4.0 (acetate buffer) having correlation factor as 0.9964 for lead, 0.9992 for zinc and RSD will found to be 5.3 %. The study of interfering ions during the analysis of lead and zinc with the error of >2%, shows the sensitivity and selectivity of the proposed method. The accuracy and precision of the proposed method was checked by the analysis of Certified Reference Material (CRM) and results were pertained in this paper.

Keywords:

Lead; zinc; nanosensors, (4-2-HPEDB-1,3,D); anodic stripping voltammetry; biological samples

1. Introduction

Nowadays the environment getting more pollution due to release of so many heavy metals which has considerable attention. Due to this more and more efforts have been put to control the metal exposure from the different fields of environment. According to this lead and zinc possess high toxic potential which are accumulated in living organisms and environmental samples.

Lead is highly toxic to human beings and animals even at low concentration. It involves the biosynthesis and affects the kidney, liver membrane, brain cells. When it accumulates in the body it causes some diseases like nausea, vomiting, diarrhea, sweating, convulsions, coma and some times finally it leads to the death. Apart from that the major use of lead is battery production; it is used in chemical industry for preparation of paints, pigments and colored inks. The main source of lead is lead mines, smelters and battery production.

Zinc plays an important role in exerting beneficial effects on cardio circulatory function and in prevention of black foot disease [1]. It is essential element for exceptional biology and public health and its deficiency causes growth retardation, delayed sexual maturation, infection susceptibility and diarrhea. Excessive absorption of zinc may lead the

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* **Corresponding Author**
E-mail: sreedhar_ny@rediff mail.com
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suppression of copper and iron absorption into living organisms. Application of zinc is used as catalyst in rubber manufacturing. Zinc mainly acts as antioxidant.

Several analytical techniques like spectrophotometry [2], AAS [3-5], NAA [6], ICPAES [7], ICPMS [8] and Chromatography [9] have been reported for the determination of zinc and lead but the above said techniques are expensive and required special laboratory conditions for analysis. In addition to that it requires time consuming and tedious reaction process. So there is a need to get approach some specific, sensitive and low expensive technique for the determination of lead and zinc at low concentration levels. For this purpose author used electrochemical nanosensors prior to analysis by anodic stripping voltammetry to get good reproducibility compare to other techniques.

Electrochemical conjugated polymers control the reactivity of the corresponding cations and enhance the efficiency of the electrode reactions in addition to that withdrawing groups can increase the oxidation potential up to limit of stability of the metal complex. Out of our knowledge, the literature shows that there is few more have been exist about trace metal determination in biological samples using electrochemical nanosensors [10-15] (Electrochemical conjugated polymers ECP's).

In the present study author reported an analytical technique anodic stripping voltammetry using newly synthesised reagent, 4-(2-hydroxy phenyl ethaminodiol), benzene-1.3-diol (4-2-HPEDB-1,3,D) for the complexation of metal ion and it is treated with electrochemical nanosensors like electrochemical conjugated polymers which is developed by polymerization process before injecting the sample into instrument. The proposed method is applied for the determination of lead and zinc in biological samples.

2. Experimental

2.1 *Apparatus*

Instrument used for analysis for anodic stripping voltammetry in differential pulse mode was an Elico CL-362 model polarographic system and Elico Li-129 model glass-calomel combined electrode was employed for measuring pH values.

Three electrode system consisting of hanging mercury drop electrode as working electrode, Ag/AgCl (salt KCl) was used as a reference electrode which provide a reversible half reaction with Nernstian behavior be constant over time and easy to assemble and maintain and a platinum wire as an auxiliary electrode which displays negative potential range.

2.2 Reagents

All reagents used were of analytical reagent grade. Double distilled water was used throughout the experiment. A stock solution of lead (II) and Zinc (II) was prepared by dissolving appropriate amount of their respective salts in double distilled water in volumetric flask. Working standard solution was freshly prepared by diluting the stock solution with double distilled water. 0.1 M concentration of 4-2-HPEDB-1,3,D was prepared by dissolving 2.48 g of 4-2-HPEDB-1,3,D in 100 mL of methanol.

2.2.1 Synthesis of 4-(2-hydroxy phenyl ethaminodiol benzene-1,3-diol (4-2-HPEDB-1,3,D)

Equimolar ratio of 2,4-dihydroxy acetophenone and 2-aminophenol in methanol mixture was refluxed for 3-4 hours and the contents were cooled at room temperature. It gives orange-red colour precipitate. The precipitate was filtered and washed with methanol to pure

Schiff base (M.P-115 ^{0}C, yield 97%) as shown in Scheme I. I.R: (KBr), 1601.8cm^{-}(C=N), 3304.0 cm^{-} (N-H), Free (OH) 3375.3 cm^{-} 1465.9 cm^{-}(O-H) and the spectrum was shown in Fig. 1.

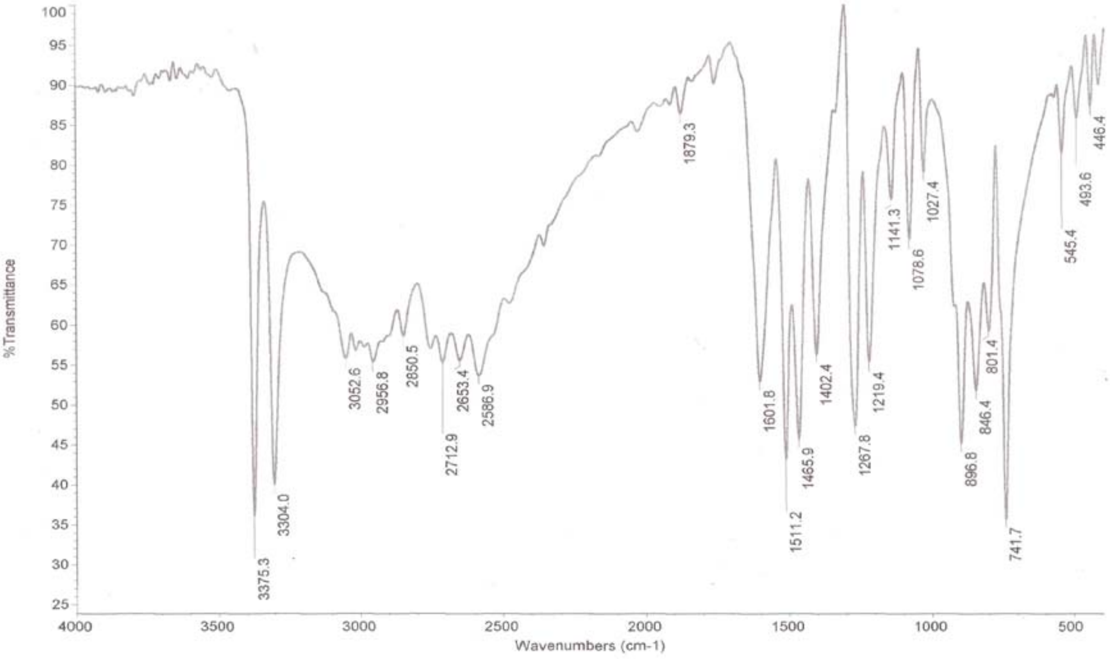

Fig.1 I.R spectrum for 4-(2-hydroxy phenyl ethaminodiol) benzene-1.3-diol (4-2-HPEDB-1,3,D).

2.3 Recommended analytical Procedure for the determination of metal ions

An aliquot of working standard solution containing 1-100 µL of metal ion is taken into 25 mL volumetric flask. To this 5 mL of acetate buffer solution (pH 4.0), 2 mL of reagent solution were added and transferred into electrolytictic cell and diluted with 9 mL of supporting electrolyte and then deoxygenated with nitrogen gas for ten min. A fresh drop of mercury of known area extruded from the micrometer of the hanging mercury drop electrode (HMDE) and the solution was stirred at constant and reproducible rate come out disturb the mercury drop . The two elements were determined in the ASV mode. Electrolysis was done at -0.80 V vs SCE deposition time 5 min, pulse amplitude of 50 mV, scan rate 2 mVs^{-1} and pH 4.0 for all two elements. The maximum peaks appearing for sample is at -0.56 V, -0.70 V for Lead and Zinc respectively. The relative standard deviation for both Pb and Zn was found to be found to be 5.3 % and the correlation coefficients were 0.9964 for Pb, 0.9992 for Zn respectively for 5 replicates. The voltammograms for the blank and sample solution was shown in Fig 2.

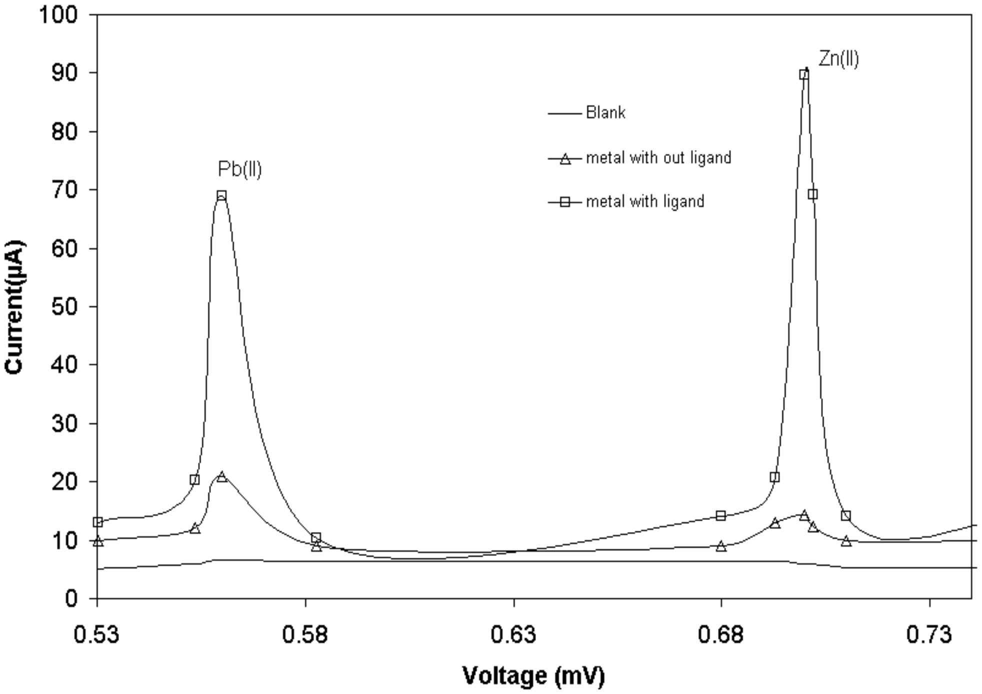

Fig.2 Voltammograms of Pb(II) and Zn (II). Peak at -0.56 mV and -0.70 mV

2.4.Preparation of electrochemical nanosensors (Electrochemical conjugated polymers- (ECP’s)

Polypyrole, polythiopene and polyaniline are generally used for the preparation of ECP’s because they are very active energy storage materials. They are synthesised by electropolymerisation process of conjugated polymers which are protanation to polymers by trifluoro acetatic acid (TEA) resulted in that absorption spectrum of Π – Π transition. Absorption spectrum of polymers did not shift doping-edoping of polymers in voltammetry were shifted positively by adding TEA and shifted negatively other functional group like pyrrole, thiopene and aniline etc.

2.5. Immobilization process

Metal complexes [M-4-2-HPEDB-1,3,D] (M=Pb and Zn) are immobilized on the surface of oxide electrodes by electrogeneratred ECP’s like the important groups polypyrole, polythiopene and polyaniline conjugated polymers with different internal pH and extraction by means of suitable extractor.

2.6. Analysis of biological samples

The samples were washed with acetone 2-3 times in a beaker with continuous stirring. Then they were dried in an electric oven at 70° C for 4 h. Two grams of the sample was weighed and taken in a beaker. To this a (1:1) mixture of nitric acid and perchloric acid was added, and the mixture was heated on a hot plate. The solution was evaporated to near dryness. The ash was taken up with 5 mL of HCl (1+9) and evaporated to dryness. The residue was taken up in 2 mL conc. HCI, filtered and made up to 25 mL with water. Suitable volumes of these solutions are taken for the determination of lead and zinc as described in above said procedure and the results were shown in Table1.

Table 1 Determination of lead and zinc in biological samples

Samples	Certified values (μg mL^{-1})		Present method (μg mL^{-1})	
	lead	Zinc	lead	Zinc
1.Human serum GBW 09135	0.17	1.03	0.173±0.07	1.05±0.8
2.Human hair GBW 09101	7.2	189	7.15±0.2	190±0.9
3.Bovine liver CZIM-LINER	0.71	162	0.73±0.02	165±0.9

3. Result and discussion

3.1 Voltammetric studies

3.1.1 Effect of pH

The effect of pH on the peak potential E_p and current intensity i_p, using voltammograms was examined for [M-(4-2-HPEDB-1,3,D)]. The pH was varied in the whole pH range 2.0 to 10.0 for [M-(4-2-HPEDB-1,3,D)] complex. It can be observed from Fig. 2, -56.0 mV, -70.0 mV that the maximum peak current obtained with pH 4.0. When the pH has been increased from 2.0 to 10.0 the peak potentials have been shifted towards more negative values, indicating proton participation in the reduction process and the results were shown in Fig. 3.

3.1.2 Effect of Scan Rate

As for the scan rate; the current response with increasing the scan rate of 40 mVs^{-1} gave the maximum response. Accordingly, the optimum conditions for recording a maximum developed and sharper voltammetry peak for 0.5 mM [M-(4-2-HPEDB-1,3,D)] are scan rate : 40 mVs^{-1} and pulse amplitude: 50 mV.

Other experimental parameters such as temperature and ionic strength were optimized. The stripping peak currents were not modified when the temperature varied between 20-50°C and the value chosen was 25°C.

3.1.3 Effect of deposition potential

It is generally recognised that the Pb and Zn accumulate on the mercury electrode in the anodic potential range as results of mercury oxidation followed by the formation of amalgam. Consequently it is expected that strong dependence of the accumulation efficiency on the factor determining the state of the electrode solution interface would be observed at deposition potential -80 mV vs. SCE, maximum current is observed for the two elements i.e -56.0 mV for Pb and -70.0 mV for Zn.

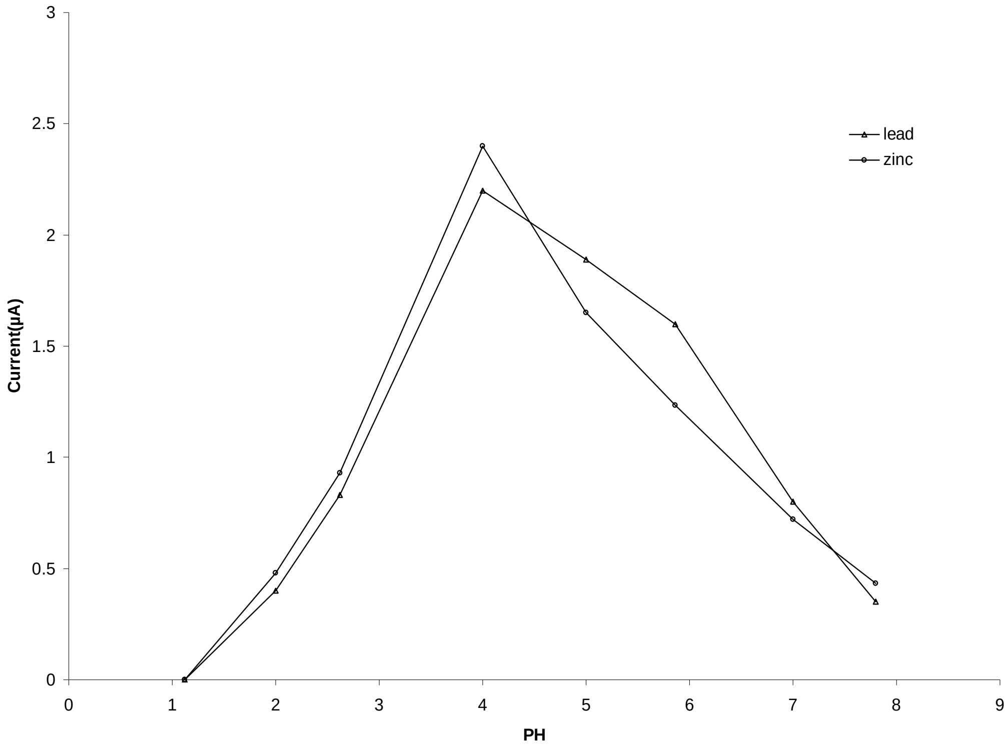

Fig.3 Effect of pH on determination of Pb and Zn

3.1.4 Effect of deposition time

The peak current increased markedly with increasing accumulation time. For accumulation time higher than 5 times the shape of voltammograms of Pb and Zn are changed and an addition peak is observed due to the reduction of amalgum.

3.1.5 Calibration

The calibration curve is constructed in the concentration range of 0.05 to 200 µg mL^{-1} with correlation coefficient of 0.9964 for lead, 0.9992 for zinc and RSD will found to be 5.3 % for both lead and zinc and calibration curve is prepared according to the general procedure under the optimized conditions as shown in Fig 4.

3.1.6 Stoichiometry of the complex

The composition of the complex was found to be 1:1 = M^{2+}: 4-2-HPEDB-1,3,D (M=Pb,Zn). The stoichiometry of the complex was verified by mole ratio method and Job's continuous variation method.

3.1.7 Effect of Foreign ions

The Study of the interfering ions during the analysis of lead and zinc in biological samples were shown the sensitivity and selectivity of the present method and the results were shown in Table 2. The metal ions are individually added to the lead and zinc ions having appropriate concentration and the general procedure was applied. The tolerable limits of various foreign ions are masked using suitable masking agents and recovery ranges with the error (<2%) are shown in Table 2. The results are almost quantitative in the presence of interfering ions to evaluate the feasibility of the present method.

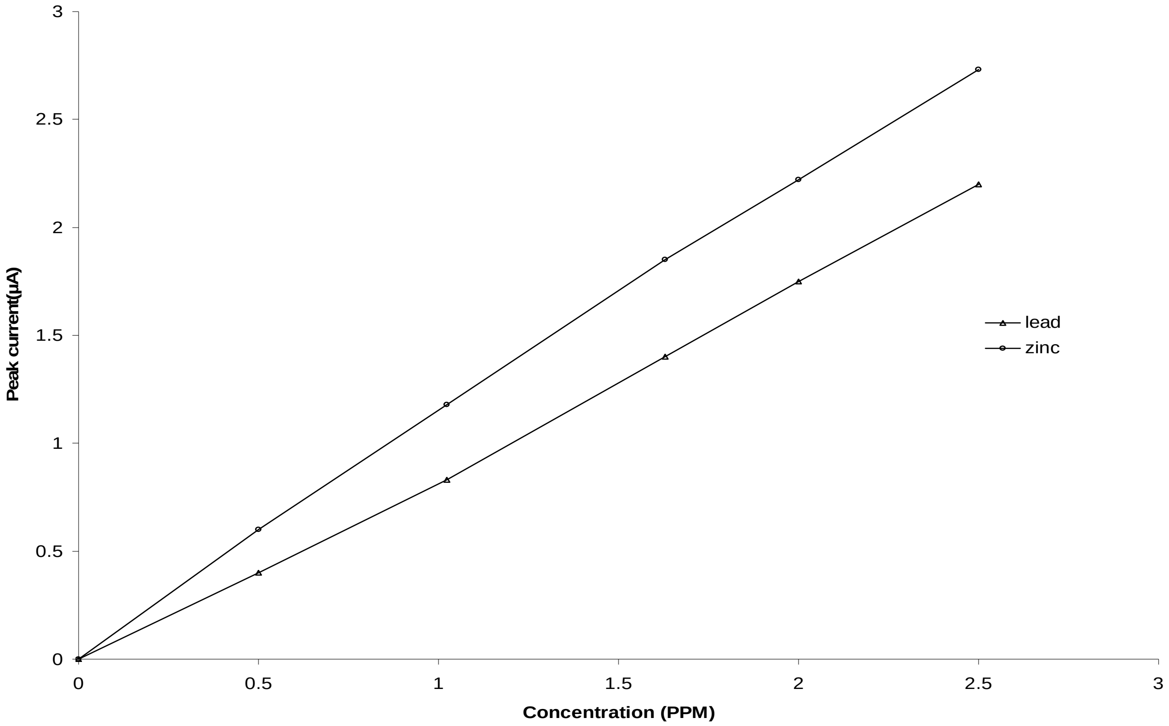

Fig.4 Calibration curve for Pb and Zn.

Table 2 Study of Interfering ions during the analysis of lead and zinc

Interfering species	Tolerance limit for lead (µg mL^{-1})	Tolerance limit for Zinc (µg mL^{-1})
K^+ , Na^+, Cs^+ , HCO_3^- , I^- , Br^- , Cl^- , N^- ^{3} ,ClO^- 4 ClO_2^- ^{3} NO^- ^{2} ,F− , IO , BrO , Mg^{2+} , Ca^{2+} , Ba^{2+} , Mn(II) , Al^{3+}	1000	1000
Zn^{2+}	1000	-
Pb^{2+}	500	1000
Sr^{2+}	500	500
Rh(III)	250	20
CrO	250	100
Co^{2+}	250	50
Ce(IV)	250	50
CN−	100	50
Cu^{2+} , Ni^{2+} , Fe(III) 50	50	50
Pd(II)	20	250
Fe(II) , Hg(II)	20	50

4. Conclusion

The present method for the simultaneous analysis of the lead and zinc using electrochemical nanosensors like electrochemical conjugated polymers (ECP's) by anodic stripping voltammetry is alternative method for the determination of lead and zinc. For this purpose a new analytical reagent 4-(2-hydroxy phenyl ethaminodiol), benzene-1.3-diol (4-2-HPEDB-1,3,D) was sysnthesised at ordinary laboratory conditions which is economically very cheep and simple. The accuracy and precision of the present method was checked by the Certified Reference Material (CRM) and the stastical data which is obtained from the proposed method is good agreement with Certified Materials. The present method is successfully applied for the determination of lead and zinc in biological samples.

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