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Simultaneous Voltammetric Detection of Lead and Zinc via Chloroform Extraction Using 4-(2-Hydroxyphenylethaminodiol) Benzene-1,3-diol

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***Abstract*: This study presents a new method for detecting very small amounts of lead and zinc metals in medicine and biological samples. Metals like these can pollute the environment due to human activities and industrial processes. To find these metals, the research introduces a newly made chemical, named 4-(2-hydroxyphenylethaminodiol), benzene-1,3-diol (4-2-HPEDB-1,3,D), which helps in identifying the metals more clearly. The method involves a technique called anodic stripping voltammetry, which is used after the metals are extracted into a solvent called chloroform. This technique helps in understanding how the metals behave in different conditions, such as in medicines and biological materials. The researchers were able to measure concentrations of lead and zinc ranging from 0.01 to 200 micrograms per milli-litre, with very high accuracy. They also studied how other substances might interfere with the detection of lead and zinc, and found the method to be both sensitive and selective. Factors like the acidity (pH), the intensity of the electric pulse used, and the speed of the scan were adjusted to get the best results. The researchers determined that the metal and the newly introduced chemical combine in a one-to-one ratio. This finding was confirmed using two different methods: the molar ratio method and Job's continuous variation method. This method was successfully applied to detect lead and zinc in biological samples, showing that it works well and is accurate when compared to other existing methods. This study offers a new approach to monitoring metal pollution in pharmaceutical and biological samples.**

***Keywords*: lead, zinc, extraction procedure, anodic stripping voltammetry, biological samples.**

1. Introduction

The growing concern over environmental pollution, particularly from heavy metals, has intensified research and remediation efforts. The discharge of metals such as lead and zinc into the environment has drawn considerable attention due to their substantial toxicity and propensity to accumulate in biologically active soil regions. This accumulation poses risks to living organisms and can lead to the uptake of these metals by crops, directly impacting human health.

Lead is notable for its soft, malleable nature and chemical stability, characteristics that allow it to mimic calcium and accumulate in the bone matrix. Its toxicity is profound, affecting humans and animals even at low concentrations and interfering with vital biological processes. The exposure to lead can lead to severe health issues, ranging from nausea, vomiting, and diarrhoea to more extreme outcomes such as convulsions, coma, and potentially death. Industrially, lead is predominantly used in battery production and is a key component in the chemical manufacturing of paints, pigments, and colored inks. The primary sources of lead contamination are mining, smelting, and the production of batteries.

Zinc, in contrast, is crucial for human health, supporting cardiovascular function and preventing diseases like black foot disease [1]. It is a necessary element that, when deficient, can cause growth retardation, delayed sexual maturation, increased susceptibility to infection, and diarrhea. However, excessive zinc intake can interfere with the absorption of other essential minerals, such as copper and iron. In the industrial sphere, zinc serves as a catalyst in the manufacturing of rubber and acts as an antioxidant.

Traditional analytical methods for detecting zinc and lead, such as spectrophotometry [2], Atomic Absorption Spectroscopy (AAS) [3]-[5], Neutron Activation Analysis (NAA) [6], Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) [7], Inductively Coupled Plasma Mass Spectrometry (ICPMS) [8], and Chromatography [9], [10], have been well-documented. However, these techniques are often costly, require specialized laboratory conditions, and are associated with time-consuming and complex preparation processes.

In light of these challenges, the development of an efficient, accessible analytical technique for monitoring lead and zinc in environmental samples becomes imperative. This study introduces a novel approach utilizing anodic stripping voltammetry, paired with a newly synthesized reagent, 4-(2-hydroxyphenyl ethaminodiol), benzene-1,3-diol (4-2-HPEDB-1,3,D), for the complexation of metal ions. A streamlined extraction procedure leveraging chloroform, chosen for its high dielectric constant, precedes the electroanalytical detection. This method has been successfully applied to the quantification of lead and zinc in pharmaceutical and biological samples, showcasing its potential as an effective tool for environmental metal pollution assessment.

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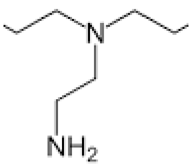


Tren is a C3-symmetric, tetradentate chelating ligand that forms stable complexes with transition metals, especially those in the 2+ and 3+ oxidation state.

2. Methodology

1) Apparatus

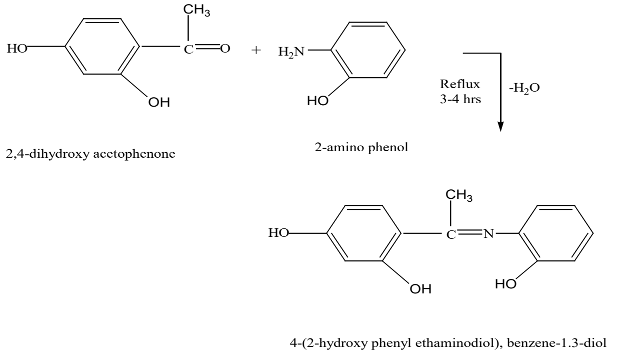
In the analysis conducted by anodic stripping voltammetry in differential pulse mode, the Elico CL-362 model polarographic system was utilized. The Elico Li-129 Model glass-calomel combined electrode served for pH value measurements.

The experimental setup incorporated a three-electrode system: the working electrode was a hanging mercury drop, the reference electrode was an Ag/AgCl (salt KCl), chosen for its stable and reversible half-reaction characteristics, ease of assembly, and maintenance, and the auxiliary electrode was a platinum wire, selected for its performance in negative potential ranges.

2) Reagents

All chemicals utilized in this study were of analytical grade. Throughout the experiments, double-distilled water was employed. Stock solutions of lead (II) and zinc (II) were prepared by dissolving precise amounts of their respective salts in double-distilled water in a volumetric flask. Working standard solutions were freshly made by diluting the stock solutions with double-distilled water. A 0.1 M solution of 4-2-HPEDB-1,3,D was prepared by dissolving 2.48 g of the compound in 100 ml of methanol.

3) Synthesis of 4-2-Hydroxy phenyl ethaminodiol Benzene-1,3-diol (4-2-HPEDB-1,3,D)



A mixture containing equimolar amounts of 2,4-dihydroxy acetophenone and 2-aminophenol in methanol was refluxed for 3-4 hours. Upon cooling to room temperature, an orange-red colored precipitate formed. This precipitate was filtered, then washed with methanol to obtain a pure Schiff base (Melting Point: 115°C, Yield: 97%), as depicted in Scheme I. The IR spectrum showed peaks at 1601.8 cm^-1 (C=N), 3304.0 cm^-1 (N-H), 3375.3 cm^-1 (free OH), and 1465.9 cm^-1 (O-H), illustrated in Figure 1.

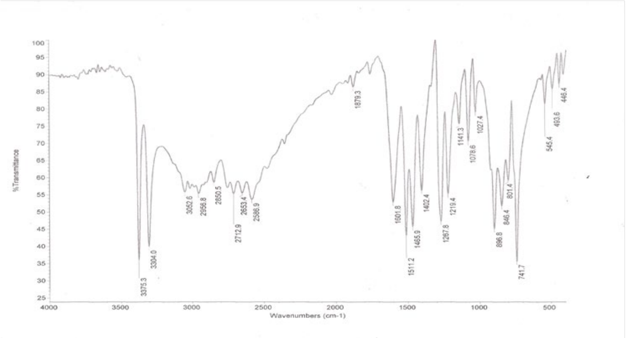


Fig. 1. I.R. spectrum for 4-(2-hydroxy phenyl ethaminodiol) benzene-1.3-diol (4-2- HPEDB-1,3,D)

4) Recommended Analytical Procedure for Metal Ion Determination

An aliquot containing 1-100 µl of metal ion was taken into a 25 mL volumetric flask. To this, 5 mL of acetate buffer (pH 4.5) and 2 mL of the reagent solution were added. The mixture was then shaken with 5.0 mL of chloroform for 30 seconds and allowed to settle for 5-10 minutes. The organic phase was collected and transferred into an electrolytic cell, diluted with 9 mL of a supporting electrolyte, and deoxygenated with nitrogen gas for ten minutes. A fresh mercury drop was extruded from the hanging mercury drop electrode (HMDE) micrometer, and the solution was stirred at a constant rate to avoid disturbing the mercury drop. Lead and zinc were determined using the ASV mode. Electrolysis conditions were set at -0.80 V versus SCE with a deposition time of 5 minutes, pulse amplitude of 50 mV, scan rate of 2mV/s, and pH 4.0 for both elements. The peak potentials for lead and zinc were observed at -0.56V and -0.70V, respectively, as shown in Figure 2.

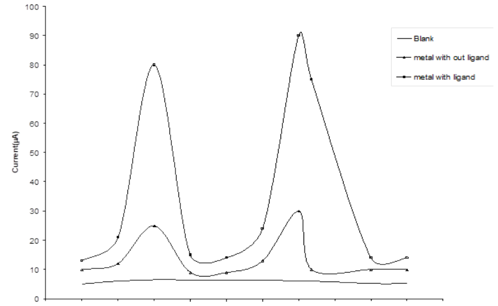


Fig. 2. Differential pulse polarogram of Pb and Zn (II). Peak at -56.0 mV and -70.0mV

Table 1
Determination of Lead and Zinc in biological samples

Samples	Certified values		Present method	
	Lead	Zinc	Lead	Zinc
Human serum (GBW 09135)	0.17	1.03	0.172±0.06	1.05±0.7
Human hair (GBW 09101)	7.2	189	7.25±0.19	191±0.8
Bovine liver (CZIM-LINER)	0.71	162	0.73±0.02	164±0.9

5) Collection of Samples

Samples were collected from various locations in and around Tirupati, taking necessary precautions at every stage, including container selection, sample collection and storage, processing, and analysis.

6) Analysis of Biological Samples

Hair samples were washed 2-3 times with acetone in a beaker under continuous stirring, then dried in an electric oven at 70°C for 4 hours. Two grams of the dried sample was taken in a beaker, to which a (1:1) mixture of nitric acid and perchloric acid was added. The mixture was heated on a hot plate until nearly dry. The resultant ash was dissolved in 5 mL of HCl (1+9) and evaporated to dryness. The residue was dissolved in 2 mL of concentrated HCl, filtered, and made up to 25 mL with water. These solutions were used for lead and zinc determination as described previously, and the results were summarized in Table 1.

3. Result and Discussion

A. Voltammetric Studies

1) Effect of pH

The influence of pH on the peak potential (Ep) and current intensity (ip) was investigated for the [M-(4-2-HPEDB-1,3,D)] complex (where M = Pb, Zn) across a pH range of 2.0 to 10.0. Figure 2 shows that the maximum peak current was observed at pH 4.0, with peak potentials shifting towards more negative values as the pH increased from 2.0 to 10.0. This shift indicates the involvement of protons in the reduction process, as depicted in Figure 3.

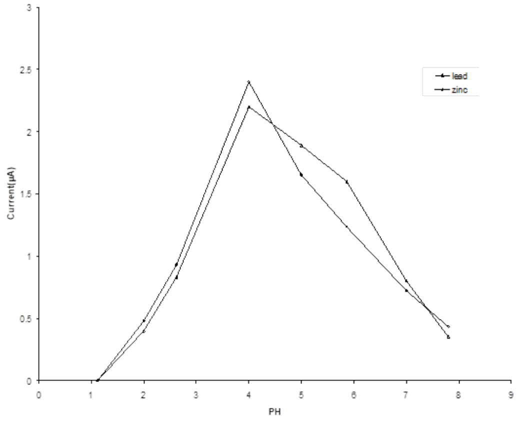


Fig. 3. Effect of pH on determination of Pb and Zn

2) Effect of Scan Rate

The relationship between the scan rate and current response was also examined. An increase in the scan rate to 40 mV/s resulted in the highest current response. Therefore, the optimal conditions for achieving a maximum and more defined voltammetry peak for 0.5 mM [M-(4-2-HPEDB-1,3,D)] were determined to be a scan rate of 40 mV/s and a pulse amplitude of 50 mV. Additional experimental parameters, including temperature and ionic strength, were optimized. It was found that the stripping peak currents remained unchanged when the temperature was varied between 20°C and 50°C, leading to the selection of 25°C as the standard temperature for these experiments.

3) Effect of deposition potential

The accumulation of Pb and Zn on the mercury electrode within the anodic potential range is known to occur through mercury oxidation and subsequent amalgam formation. It is anticipated that the efficiency of accumulation would significantly depend on factors affecting the electrode-solution interface state. At a deposition potential of -0.80 V versus SCE, the maximum current for both elements is observed, indicating optimal conditions for their accumulation.

4) Effect of deposition time

An increase in accumulation time significantly enhances the peak current. When the accumulation time exceeds five times the initial duration, alterations in the voltammograms of Pb and Zn are observed, along with an additional peak attributed to the reduction of amalgam. This highlights the impact of deposition time on the detection process.

5) Effect of Solvent

The extraction efficiency of the [M-(4-2-HPEDB-1,3,D)] complex was tested using various organic solvents, including dimethyl formaldehyde, CCl4, cyclohexane, chloroform, xylene, toluene, n-butanol, 1-pentanol, 1-amyl alcohol, and nitrobenzene. Chloroform was found to be the most effective solvent for extracting the [M-(4-2-HPEDB-1,3,D)] complex compared to the other tested solvents. Thus, chloroform has been selected as the preferred solvent for extracting the complex in subsequent experiments.

6) Stoichiometry of the complex

The complex's composition was determined to be in a 1:1 ratio (M2+: 4-2-HPEDB-1,3,D). The stoichiometry of the complex was confirmed using the mole ratio method, providing a clear understanding of the complex formation between the metal ions and the 4-2-HPEDB-1,3,D reagent.

7) Calibration

A calibration curve was established using the general procedure within the optimized conditions, covering a concentration range from 0.01 to 200 μg/mL. The correlation coefficients obtained were 0.9998 for lead and 0.9995 for zinc, indicating high precision in the measurements. The relative standard deviation was calculated at 5.3%, demonstrating consistency in the results. The corresponding calibration curve is illustrated in Figure 4.

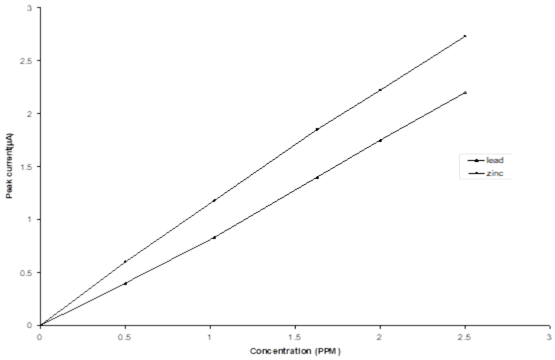


Fig. 4. Calibration curve for Pb and Zn

Table 2
Study of interfering ions during the analysis of Lead and Zinc

Interfering species	Tolerance limit for Lead	Tolerance limit for Zinc
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

8) *Effect of Foreign ions*

An investigation into the impact of interfering ions on the analysis of lead and zinc in pharmaceutical and biological samples demonstrated the method's sensitivity and selectivity. This analysis is detailed in Table 2. Metal ions were individually added to samples containing lead and zinc at specific concentrations, followed by the application of the general analytical procedure. The interference from various foreign ions was mitigated using appropriate masking agents, ensuring that recovery rates remained within an error margin of less than 2%. The data, presented in Table 2, suggest that the method provides nearly quantitative results even in the presence of interfering ions, thereby affirming the viability of the proposed analytical approach.

4. Conclusion

This study introduces a novel analytical method for the simultaneous determination of lead and zinc in biological samples using anodic stripping voltammetry, facilitated by chloroform extraction at a hanging mercury drop electrode. Central to this method is the synthesis of a new analytical reagent, 4-(2-hydroxyphenylethaminodiol) benzene-1,3-diol (4-2-HPEDB-1,3,D), which was developed under ordinary laboratory conditions, offering an economical and straightforward approach for metal detection.

The calibration curve, constructed under optimized conditions, spans a concentration range from 0.01 to 200 µg/mL, with correlation coefficients of 0.9998 for lead and 0.9995 for zinc, illustrating the method's high precision and accuracy. This accuracy was further validated against certified reference materials, demonstrating excellent agreement and underscoring the method's reliability. Additionally, the method proved resilient against interference from foreign ions, highlighting its selectivity and sensitivity in complex sample matrices.

The study's findings indicate that the method is not only economically viable but also practical for routine application, thanks to the synthesized reagent's simplicity and cost-effectiveness. The successful application of this method for detecting lead and zinc in biological samples, coupled with its potential for broader use in environmental monitoring and pollution control, showcases its significance.

By synthesizing the technical robustness of the analytical method with practical considerations such as economic benefits and simplicity of reagent synthesis, this integrated conclusion emphasizes the comprehensive value of the study. The method's validation against certified standards and its demonstrated application in real-world samples further reinforce its utility and effectiveness in addressing the challenges associated with metal pollution analysis.

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