APPENDIX

Publications & presentations
LIST OF PUBLICATIONS

1. A Simple spectrophotometric method for the determination of Arsenic in industrial and environmental samples using 2, 4-Dihydroxy benzophenone-2-Amino thiophenol

   **K. Deepa, Y. Lingappa**

2. Spectrophotometric determination of Selenium in Industrial and Environmental samples using vanillin-2-aminonicotinicacid (VANA)

   **K. Deepa** and **Y. Lingappa**
   International journal of Der Pharma Chemica **2014, 6(1)**,52-60.

3. Spectrophotometric determination of mercury in environmental samples using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED)

   **K. Deepa**, **Y. Paul Raj** and **Y. Lingappa**
   International journal of Der Pharma Chemica, **(Accepted)**.

4. Spectrophotometric determination of Lead in medicinal leaf and environmental samples using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED)

   **K. Deepa**, **Y. Paul Raj** and **Y. Lingappa**
   Journal of Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. **( Under review).**

5. Synthesis, Characterization and Biological Activity of 5 - Methyl, Thiophene -2-Carboxaldehyde Derivatives of Copper (II), Cobalt (II) and Nickel (II) Complexes


A simple spectrophotometric method for the determination of arsenic in industrial and environmental samples using 2,4-Dihydroxy benzophenone-2-amino thiophenol

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Highlights
- The synthesis and characterization and chromogenic properties of ligand.
- The ligand is stable more than 6 months and it is more sensitivity and selectivity.
- BPBT method, it was applied for determination of AS in some environmental samples.
- As (III) – BPBT complex showed maximum absorbance at 343 nm.

Abstract
2,4-Dihydroxy benzophenone-2-amino thiophenol (BPBT) has been proposed as a new analytical reagent for the direct non-extractive spectrophotometric determination of arsenic. The reagent reacts with arsenic in acidic medium (pH = 6.0, sodium acetate–acetic acid buffer) to form light greenish yellow colored 1:1 (M:L) complex. Maximum absorbance was obtained at 343 nm and remains constant for over 24 h. The molar absorptivity and Sandell’s sensitivity of BPBT are found to be $6.01 \times 10^4 \text{L mol}^{-1}\text{cm}^{-1}$ and 0.0016 $\mu\text{g cm}^{-2}$ respectively. The system obeys Beer’s law in the range of 0.125–2.637 $\mu\text{g/ml}$ of As (III). Since BPBT method is more sensitive, it was applied for the determination of arsenic in some environmental water samples.

Introduction
Arsenic compounds are widely used and have long been recognized as toxicants [1–3]. Arsenic is widely distributed in the nature. It occurs as inorganic and organic compounds as trivalent. Animals vary in their arsenic accumulation depending upon the type of food they consume [5,6]. Acute arsenic exposure can give symptoms with rapid onset of headache, nausea and severe gastro-intestinal irritation [7]. Due to increased industrialization more and more industrial waste get accumulated in various regions and make their passage through soil cause severe environmental pollution and wide life toxicity [4] and also enter into animal body especially in their liver, kidney and lean meat.

Arsenic occurs naturally in the Earth’s crust in its inorganic form, trivalent (arsenite) or pentavalent (arsenate) form. Erosion

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of arsenic containing surface rocks probably accounts for a significant amount of arsenic in water supplies. It is a ubiquitous element in water, soil and sediments. The occurrence of arsenic in plants and animals generally reflects its accumulation from the environment. The presence of arsenic in drinking water has reached calamitous proportions in many parts of the world. There are numerous reports in the literature based on past and ongoing experience in various countries in Asia and South America concerning the higher risks of skin, bladder, lung, liver, and kidney cancer that result from continued consumption of elevated levels of arsenic in drinking water [8]. Consumption of even low levels of arsenic over a long period can cause a multitude of diseases. The maximum permissible limit for As (III) drinking water is 0.05 mg/L as recommended by WHO [9]. In certain areas in India, Bangladesh, China, and Mongolia [10], arsenic levels in groundwater exceed 1 ng/mL. Regarding inorganic arsenic, As (III) is appreciably more toxic than As (V). Usually these species of arsenic in natural water are found at the trace levels [10].

There are only a few analytical techniques available, which have sufficient sensitivity and selectivity to directly determine arsenic at the trace levels in natural water. Therefore, the development of sensitive and accurate methods for speciation and preconcentration of trace amounts of As (III) and As (V) is necessary. Recently many kinds of conventional analytical techniques such as hydride generation coupled plasma atomic emission spectrometry (HG-ICP-AES) [5], capillary electrophoresis inductively coupled plasma mass spectrometry (CE-ICP-MS) [4], high performance liquid chromatography–inductively coupled plasma mass spectrometry [11], electro thermal atomic absorption spectrometry (ETAAS) [12], hydride generation – atomic absorption spectrometry [13], hydride generation – atomic fluorescence spectrometry [14], cathodic stripping Voltammetry [15], anodic stripping voltammetry [16], neutron activation analysis [17], photometric analysis [18], ion selective electrodes [19] and energy dispersive X-ray fluorescence spectrometry [20] have been used for the determination of low concentrations of arsenic. But all these techniques are costly and require trained staff. Recently most of the spectrophotometric methods have been developed as an alternative for the determination of arsenic instead of conventional techniques.

We used an ICP-OES machine which converts all arsenic forms into inorganic arsenic. Inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) are very feasible techniques for quantitative determination of the As (III). The most important advantages of these techniques are high sample throughput, simplicity and good sensitivity in comparison to other techniques [21,22] used for As (III) determination.

Nowadays, due to the higher sensitivity achieved with axially viewed plasma and better spectral resolution given by high-resolution monochromators, it is expected that low concentrations of all naturally occurring arsenic may be directly quantified by ICP OES. Furthermore, depending on the nebulizer used to introduce the sample solution in the plasma, sensitivity improves.

This paper describes synthesis, characterization and analytical properties of new reagent viz., 2,4-dihydroxy benzophenone–2-amino thiophenol (BPBT). Since the reagent is more sensitive, it is used for the determination of arsenic in various water samples.

**Experimental**

**Apparatus**

A Shimadzu (Model-1601) UV–VIS spectrophotometer (Perkin Elmer Singapore Private Limited, Singapore) and ELICO model Li-610 pH meter (M/s ELICO private limited, Hyderabad, India) with combination electrodes were used for measurements of absorbance and pH respectively. ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry Model-7000) methods were used for the quantitative analysis of As (III). To determine the As (III), AOAC methods were used (AOAC 1986, 2003; Jorhem 1993). In this method, the samples were dissolved at 190°C and 400 psi pressure in Mars 5 apparatus (Vessel Type KXP 1500, CEM, Matthews, USA). As (III) was analyzed by inductively coupled plasma-optical emission Spectrometry (Varian Vista-MPX CCD Simultaneous Spectrophotometer, Mug rave-Victoria, Australia) [23,24].

**Reagent and solutions**

All chemicals used were of analytical-reagent grade of the highest purity available procured from Merck. Doubly distilled de-ionized water was used throughout the experiment. Glass vessels were cleaned soaking in acidified solutions of K2Cr2O7 followed by washing with conc. HNO3 and were rinsed several times with high purity de-ionized water. Stock solutions and environmental water samples were kept in polypropylene bottle containing 1 ml of conc. HNO3.

**Preparation of reagent (BPBT)**

2,4-Dihydroxy benzophenone (5 g, 0.0233 mol) dissolved in 20 ml of methanol, 2-amino thiophenol (2.5 ml, 0.0233 mol) dissolved in 20 ml of methanol were taken in 250 ml round bottom flask. Suitable quantity (10 ml) of 1 M sodium acetate was added to the reaction mixture and refluxed for 12 h. On cooling the reaction mixture brown colored product was separated out. It was collected by filtration and washed several times with hot water followed by n-hexane. This compound was recrystallised from methanol and dried in vacuum. The ligand is stable for more than 6 months. Yield is 8.48 g; m.p. 118–120°C. The structure of BPBT is shown in (Fig. 1).

**Characterization of BPBT**

The reagent has been characterized by IR, 1H NMR and Mass spectral data. Infrared spectrum (Fig. 2) of BPBT shows bands at 3374, 3063, 2593, 1628, 1598, 1584, 1220, 1122, 699 cm−1 respectively corresponding to υ (O–H) symmetric stretch, υ (C–H) Aromatic stretch (sp²–sp³–C–H), υ (S–H) stretch (weak) (δ), υ (C=C) Aromatic stretch, υ (C=N) Schiff base, Aromatic ring υ (C–C) stretch, υ (C=O) stretch, υ (C=N) stretch, υ (C=S) stretch. H1 NMR spectrum of BPBT (CDCl3 + DMSO-d6) showed signals at 6.30–7.54 (12H), 3.30 (1H) due to benzene or aromatic protons, –SH (thiolic protons) (Fig. 3). Mass spectrum of BPBT (Fig. 4) shows signal at 322 (M + 1) corresponding to its molecular ion peak. The molecular formula of the reagent is C19H15N3O2S (M.Wt. 321).

**pKa values of reagents**

The pKa values were determined by recording the UV–Visible spectra of 1 × 10−4 M solutions of the reagent at various pH values using Philips and Merrit method. The values of deprotonation obtained for BPBT were 6.86 (pK1) and 6.86 (pK2).

![Fig. 1. BPBT-structure.](image-url)
BPBT solution

A $1 \times 10^{-2}$ M solution was prepared by dissolving 0.3215 gm of BPBT in 100 ml of methanol. The reagent solution is stable for at least 24 h.

As (III) standard solution

A 100 ml amount of stock solution ($1 \times 10^{-2}$ M) was prepared by dissolving 0.3120 gm of sodium arsenate heptahydrate $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (E. Merck pre analysis) in de-ionized water.
1000 ppm stock solution of arsenic was prepared by dissolving 0.416 gm of sodium arsenate heptahydrate in 100 ml distilled water.

**Buffer solution**

1 M sodium acetate +0.1 M hydrochloric acid (pH = 0.5–3.0), 0.2 M sodium acetate +0.2 M acetic acid (pH = 3.5–6.0), 1 M sodium acetate +0.2 M acetic acid (pH = 6.5–7.5), 2 M ammonia +2 M ammonium chloride (pH = 8.0–12.0) buffer solutions were prepared in distilled water. Suitable portions of these solutions were mixed to get the desired pH.

**Potassium permanganate solution**

A 1% potassium permanganate solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid.

**Tartrate solution**

A 100 ml stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS grade (99%) potassium sodium tartrate tetra hydrate in (100 ml) de-ionized water. Suitable portions of these solutions were prepared in distilled water. Aliquots of this solution were standardized with oxalic acid.

**Aqueous ammonia solution**

A 100 ml solution of aqueous ammonia was prepared by diluting 10 ml concentrated NH₃ (28–30%, ACS grade) to 100 ml with de-ionized water. The solution was stored in a polypropylene bottle.

**Preparation of sample solutions**

**Preparation of water samples**

Different water samples (Ground water and Tap water) were collected from various places around Tirupati, A.P., India. The samples (150 ml) were stored at 5 °C in metal free polyethylene bottles. Water samples were filtered through what man filter paper No. 41 and collected into 250 ml beakers. All the filtered environmental water samples were evaporated nearly to dryness with a mixture of 10 ml con HNO₃ and 5 ml of con H₂SO₄ in a fume cupboard and then cooled to room temperature. The sample was digested in the presence of an excess potassium permanganate solution according to the method recommended by Fiffled et al. [5] The residues were then heated with 10 ml of deionized water in order to dissolve the salts. The solutions were cooled and neutralized with dilute NH₄OH. The digest was transferred into a 25 ml calibrated flask and diluted up to the mark with deionized water.

The reaction of As (III) was tested with (BPBT) at different pH values. The samples were prepared in 10 ml of the solution containing constant volume of 25 ml of As (III), 2 ml of buffer solution (pH = 6.0), 1 ml of 1 × 10⁻⁴ M BPBT, 2 ml lithium chloride solution. The solution was then shaken with 10 ml portions of methanol for two minutes and then allowed to stand. Each time the combined organic phase of aliquots were taken in 10 ml standard flasks and made up to the mark with methanol. The absorbance was measured in 200–800 nm range against reagent blank.

**Recommended procedure**

A known aliquot of the sample solution was taken in a 25 ml standard flask containing constant volume of 10 ml of buffer solution (pH = 6.0), 1 ml of 1 × 10⁻⁴ M BPBT and 1 ml of As (III) solution made up to the mark with distilled water. Absorbance of the solution was measured at 343 nm against the reagent blank. The absorbance values were referred to the predetermined calibration plot to compute the amount of Arsenic.

**Results and discussion**

The reagent BPBT may be easily prepared. The reagent solutions (1 × 10⁻⁴ M) are found to be stable for 24 h. The absorption band at 343 nm indicates the formation of As (III)–BPBT complex in solution. As (III) reacts with BPBT in acidic medium (pH = 6.0) to give

**Table 1**

<table>
<thead>
<tr>
<th>Physico-chemical and analytical characteristics of As (III)–BPBT complex.</th>
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<tbody>
<tr>
<td>Characteristic property</td>
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<td>--------------------------</td>
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<tr>
<td>Absorbance maximum (λmax (nm))</td>
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<tr>
<td>pH-range (optimum)</td>
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<tr>
<td>Mole of reagent required mole of metal ion for full color developed</td>
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<tr>
<td>Beer’s law validity range (µg/ml)</td>
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<tr>
<td>Molar absorptivity (L mol⁻¹ cm⁻¹)</td>
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<tr>
<td>Specific absorptivity (ml g⁻¹ cm⁻¹)</td>
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<tr>
<td>Sandell’s sensitivity (µg/cm²)</td>
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<td>Composition of complex as obtained in Job’s and molar ratio methods (M:L)</td>
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<td>Stability constant of the complex</td>
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<td>Relative standard deviation (RSD)%</td>
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<tr>
<td>Y-intercept</td>
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<tr>
<td>Angular coefficient (m)</td>
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<td>Correlation coefficient (r)</td>
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**Table 2**

<table>
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<th>Effect of foreign ions.</th>
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<td>Ion added</td>
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<tr>
<td>Citrate</td>
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<tr>
<td>Tartrate</td>
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<tr>
<td>Urea</td>
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<tr>
<td>Iodate</td>
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<tr>
<td>Bicarbonate</td>
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<tr>
<td>Thiocyanate</td>
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<tr>
<td>Sulphate</td>
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<td>Oxalate</td>
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<td>Thiourea</td>
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<tr>
<td>Nitrate</td>
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<td>Acetate</td>
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<tr>
<td>Phosphate</td>
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<tr>
<td>Bromide</td>
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<tr>
<td>Chloride</td>
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<tr>
<td>Fluoride</td>
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</table>

**Fig. 5.** Absorption spectra of (a) As (III)–BPBT complex (λmax = 343 nm) in aqueous solution, (b) BPBT vs water blank (1 × 10⁻⁴ M).
water soluble complexes. The colour reactions are instantaneous at room temperature. The change in the order of addition metal ion, reagent BPBT and buffer has no effect on the absorbance on the complexes. Analytical characteristics of the complexes were summarized in Table 1. The stoichiometry of the complexes (M:L = 1:1) was determined by Job’s continuous variation and molar ratio methods. Sodium acetate (0.2 M)–acetic acid (0.2 M) buffer solution (pH = 6.0 and T = 300 K) and equimolar solutions of As (III) and BPBT were used in the calculation of stability constants of the complexes.

Absorption spectra of the reagent and As (III)–BPBT complex

Absorption spectra of As (III)–BPBT complex and reagent shows maximum absorbances at 343 nm and 320 nm, respectively (Fig. 5). Hence all the spectral measurements of the complex have been carried out at 343 nm.

The study of the effect of pH on the color intensity of the reaction mixture showed that the constant and maximum color is obtained in the pH range 5.0–7.0, the complex has maximum absorbance in buffer solution of (pH = 6.0). The analytical studies were therefore carried out at (pH = 6.0).

Different molar excess of BPBT were added to As (III) concentration and the absorbances were measured adopting the standard procedure. It was observed that 10-fold molar excess of reagent with respect to metal ion is necessary to get maximum absorbance.

Hence a 10-fold molar excess of reagent was used for further experimental studies.

The absorbance of the solution was measured at different time intervals to ascertain the time stability of the color complex. It was observed that the color development was instantaneous and remained constant for more than 24 h. Physicochemical and analytical properties of As (III) complex of BPBT are summarized in Table 1.

Adherence of the As (III)–BPBT complex system to Beer’s law

For the possible determination of As (III) at micro level, the absorbance of the solution containing different amounts of the metal ions was measured at 343 nm. The linear plot between the absorbance and the amount of As (III) is drawn and the straight line obtained with the equation $A_{343} = 0.21333C + 0.00847$. Further Beer’s law is obeyed in the range of 0.125–2.637 µg/ml, the molar absorptivity and Sandell’s sensitivity are found to be 6 L mol$^{-1}$ cm$^{-1}$ and 0.0016 µg/cm$^{-2}$ respectively. The standard deviation of the method for ten determinations of 2.34 µg/ml is ±0.00821.

To assess the precision and accuracy of the method estimations are carried out for a set of five determinations of As (III) under optimum conditions. The results showed that standard deviation of the method was not more than 0.00821 and relative standard deviation was less than 0.99545. These results indicate that the method has good precision, besides being accurate.

Interference of foreign ions

The effect of various cations and anions which are generally associated with the metal ion in the determination of As (III) was studied by measuring the absorbance of arsenic. The complex contains 2.34 µg/ml of As (III) in solution. The colour of the reaction was developed as described in the standard procedure. An error of ±2% in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions and cations in the BPBT methods respectively were presented in Table 2. Higher amounts of Fe$^{3+}$ do not interfere in the presence of 70 ppm of fluoride. Larger amounts of Hg$^{2+}$ do not interfere in the presence of 600 ppm of iodide.

The present method (2,4-Dihydroxy benzophenone-2-amino thiophenol) was applied for the determination of arsenic when present alone and present in water samples.

The present ligands containing aromatic ring are found to be potential and cost effective for the determination of As (III) without the need for extraction using the toxic solvents. Further the reagents are easy to synthesize using commercially available

### Table 3

<table>
<thead>
<tr>
<th>Sample name</th>
<th>AS (III) found</th>
<th>R.D (%)</th>
<th>S.D</th>
<th>R.S.D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICP-OES</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ground water (well water)</td>
<td>1.7</td>
<td>0.0008</td>
<td>.049</td>
<td>1.68</td>
</tr>
<tr>
<td>Ground water (river water)</td>
<td>1.5</td>
<td>0.0013</td>
<td>0.0865</td>
<td>1.47</td>
</tr>
<tr>
<td>Industrial waste water (tap water)</td>
<td>1.06</td>
<td>0.0008</td>
<td>0.0791</td>
<td>1.07</td>
</tr>
<tr>
<td>Ground water</td>
<td>1.04</td>
<td>0.0013</td>
<td>0.1247</td>
<td>1.06</td>
</tr>
<tr>
<td>Ground water (upper)</td>
<td>1.02</td>
<td>0.001</td>
<td>0.0978</td>
<td>1.03</td>
</tr>
<tr>
<td>Ground water (lower)</td>
<td>1.03</td>
<td>0.0011</td>
<td>0.1114</td>
<td>1.05</td>
</tr>
</tbody>
</table>

* a Average of the five determination.
* b Collected at Pollur (Palamaneru-Chittoor), A.P., India.
* c Collected at Angerpet, A.P., India.
* d Collected at Amarraja industry, Karakamadhi A.P., India.
* e Collected at Manjani, A.P., India.
* f Collected at Yaganti (upper), A.P., India.
* g Collected at Ammaraja industry, Karakamadhi A.P., India.
precursors. Moreover the present method is simple, rapid and very sensitive for non-extractive spectrophotometric determination of As (III) in aqueous medium.

Effect of foreign ions on the extraction of the As (III)–BPBT complex

The effect of foreign ions is studied by measuring the absorbance of the reaction mixture containing 2.34 µg/ml of As (III) in the presence of different amounts of foreign ions. The results are presented in Table 2. An error of ±2% in the absorbance value caused by foreign ions is considered as a tolerable limit.

Composition and stability constant of the complex

Job’s method of continuous variation and molar-ratio methods were applied to ascertain the stoichiometric composition of the complex. It is found that BPBT forms 1:1 complex with As (III) as shown in (Fig. 7).

Application

The proposed extractive spectrophotometric method is applied for the determination of As (III) in water samples. A known aliquot of the sample solutions were taken into a 25 ml separating funnel and the Arsenic content was determined as described in the general procedure. The results are checked with parallel determinations by direct ICP-OES. The data obtained in the analyses of water samples are given in Table 3.

Conclusion

The author has introduced a new sensitive reagent BPBT for the extractive spectrophotometric determination of trace amounts of As (III). The proposed spectrophotometric method is simple, highly sensitive and selective for the determination of As (III) in water samples when compared with other spectrophotometric methods.

The proposed method is simple, rapid and common metal ions such as Fe^{3+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Mn^{2+}, Cr^{3+} do not interfere. Urea, bicarbonate, citrate, sulphide, SO_{4}^{2-}, and PO_{4}^{3-} also do not interfere. It also offers advantages like reliability and reproducibility in addition to its simplicity instant color development and less interference effect. The results obtained through UV–Visible spectrophotometer have been compared with those obtained through the ICP-OES. The method has been successfully applied for the determination of arsenic in various environmental samples.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.12.030.

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[23] AOAC International, AOAC 985.01, 984.27, 1996.
Spectrophotometric determination of mercury in environmental samples using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED)

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method was developed for the determination of Hg (II) using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) as an analytical reagent. The reagent has been synthesized and characterized using IR, 1H NMR and mass spectral data. The metal ion in aqueous medium forms light yellow colored complex with MTCED at (pH = 2.0) (sodium acetate-hydrochloric acid buffer solution) showing maximum absorbance at 385 nm. Hence, analytical studies were further carried out at 385 nm. The reagent reacts with mercury in acidic medium to form light yellow colored 1:1 (M:L) complex. The color of the reaction is instantaneous and absorbance values remain constant for 24 hours. The composition of the Hg (II) complex with MTCED was studied by the method of Job’s continuous variation and molar ratio methods. Beer’s law was obeyed in the range of 0.83-8.6 µg ml$^{-1}$ of Hg (II). The molar absorptivity and Sandell’s sensitivity of the method were found to be 5.58 x $10^4$ L mol$^{-1}$cm$^{-1}$ and 0.00179 µg/ cm$^2$ respectively. Since MTCED method is more sensitive, it was applied for the determination of mercury in environmental samples.

Key words: Mercury determination, non-extractive spectrophotometry, 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED), environmental samples.

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INTRODUCTION

Mercury is one of the most toxic heavy metals in the earth and it exists in nature at trace and ultra trace amounts in three valence states [1]. Mercury (0, I, II) species and are able to combine with most inorganic and organic ligands to form various complexes, e.g. HgX_{4-2} (where X= Cl, Br and I) and methyl mercury [2,3]. Mercury can accumulate in animals and plants and also enters into human body through the food chain causing damage to central nervous system [4]. Due to the toxicological effects and potential accumulation of mercury onto human bodies and aquatic organisms, the determination of mercury (II) or organo mercury (II) has seen an upsurge of interest in the last few years [5]. According to WHO, the allowed limits of mercury in drinking water are less than (1.0 ngmL^{-1}) [6].

Mercury is a serious environmental pollutant because of its toxic effects on all living organisms [7]. Mercury and its compounds cause serious diseases such as leukemia [8]. Mercury compounds can be present as a result of anthropogenic activities in various environmental samples [9]. They are usually present in natural waters at trace levels [9] and [10]. The lakes, rivers in vicinity of the industrial areas are the important indicators for mercury pollution. So, there is a need to develop new, selective, effective, cheap methods for determination of mercury [11].

A serious problem in the determination of mercury is related to low concentrations of target species. The main species of mercury in natural waters are inorganic mercury (Hg_{2}^{2+}, Hg^{2+}) and methyl mercury (CH_{3}Hg^{+}). Recent reports estimate that total mercury concentration is in the range of 0.2-100 ng L^{-1} and methyl mercury concentrations are lower (ca. 0.05 ngL^{-1}) in natural waters [12].

Mercury compounds have been used as catalysts, fungicides, herbicides, disinfectants, pigments and for other purposes. The world production was about 10,000 t in 1973 [13] and about 6,500 t in 1980 [14]. In addition to the production of pure mercury by industrial processes, mercury is released into the environment by human activities such as the combustion of fossil fuels, waste disposal and by industry. Recent estimates of anthropogenic emissions are in the order of 2,000 to 3,000 t year^{-1} [15-18].

Mercury is one of the most toxic heavy metals. It enters the environment as metallic, inorganic and organic mercury compounds through various industries like pulp and paper
industry, chlor-alkali plants, gold and silver mining, electrical industry, paints, fungicides and pharmaceuticals. Fossil fuel burning and cement manufacture cause emission of mercury in air [19–21]. The average value of mercury reported in oil is 10 ppm and sludge from sewage treatment plant is reported to contain up to 25 ppm mercury [22]. WHO has suggested intake of 0.3 mg of mercury of which not more than 0.2 mg should be in the form of methyl mercury and maximum permissible value in drinking water is 0.001 mg^{-1} [23]. The toxicity of mercury depends on its chemical species and it is found that organomercurials are more toxic than inorganic mercury compounds [24,25]. Mercury and its compounds are reported to be mutagenic and teratogenic in nature [26]. The present study was planned to determine the prevalence of selected trace elements in environmental samples.

We used an ICP-OES machine which converts all lead forms into inorganic lead. Inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS) are very feasible techniques for quantitative determination of the Hg (II). The most important advantages of these techniques are high sample throughput, simplicity and good sensitivity in comparison to other techniques [27- 28] used for Hg (II) determination. Nowadays, due to the higher sensitivity achieved with axially viewed plasma and better spectral resolution given by high-resolution monochromators, it is expected that low concentrations of all naturally occurring mercury may be directly quantified by ICP OES. Furthermore, depending on the nebulizer used to introduce the sample solution in the plasma, sensitivity improves.

This paper describes synthesis, characterization and analytical properties of new reagent viz., 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED). Since the reagent is more sensitive, it is used for the determination of mercury in various water samples.

MATERIALS AND METHODS

Experimental

Apparatus

Schimadzu 160A UV-Visible spectrophotometer (PerkinElmer Singapore Private Limited, Singapore) equipped with 10 cm quartz cell and an ELICO model L1-610 pH meter
M/s ELICO private limited, Hyderabad, India) were used in the present study. ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry Model-7000) methods were used for the quantitative analysis of Hg (II). To determine the Hg (II), AOAC methods were used (AOAC 1986, 2003; Jorhem 1993). In this method, the samples were dissolved at 190°C and 400 psi pressure in Mars 5 apparatus (Vessel Type XKP 1500, CEM, Matthews, USA). The Hg (II) was analyzed by inductively coupled plasma-optical emission spectrometry (Varian Vista-MPX CCD Simultaneous Spectrophotometer, Mugrave-Victoria, Australia) [29-30] (Mandal & Suzuki 2002).

Reagent and solutions

All chemicals used were of analytical-reagent grade of the highest purity available procured from Merck. Doubly distilled de-ionized water was used throughout the experiment. Glass vessels were cleaned soaking in acidified solutions of K₂Cr₂O₇ followed by washing with con. HNO₃ and were rinsed several times with high purity de-ionized water. Stock solutions of environmental water samples were kept in polypropylene bottle containing 1ml of conc. HNO₃.

Preparation of reagent (MTCED)

5-methylthiophene-2-carboxaldehyde (1 ml, 0.0092 mol) in 50 ml of methanol, ethylenediamine (0.55 g, 0.0092 mol) dissolved in 50 ml of methanol were taken in a 250 ml round bottom flask. Suitable quantity (1ml) of 1 molar sodium acetate was added to the reaction mixture and refluxed for 10 hours. On cooling the reaction mixture wheat colored product was separated out. It was collected by filtration and washed several times with hot water followed by n-hexane. This compound was recrystallised from methanol and dried in vacuum. The ligand was stable for more than 6 months. Yield is 93; m.p. 82-83 °C. The structure of MTCED as shown in the (Fig.1).

![Fig.1 MTCED - Structure](image-url)
Characterization of reagent (MTCED)

The reagent has been synthesized and characterized by IR, \(^1\)HNMR and Mass spectral data. Infrared spectrum of MTCED shows bands at 3087, 2915, 2830, 1634, 1476, 1375, 1305, 706 and 593 respectively corresponding to \(\nu\) (C-H) stretch (sp\(^2\)--C-H) (thiophene), \(\nu\) (C-H) stretch (methyl and methylene), \(\nu\) (C-H) stretch (aldehyde), \(\nu\) (C = N) schiff base, \(\delta\) (-CH\(_2\)) symmetrical stretch, \(\delta\) (-CH\(_3\)) symmetrical stretch, \(\nu\) (C - N) stretch, \(\nu\) (C - S) stretch and hetero cyclic ring C – C oop bend. \(^1\)HNMR spectrum of MTCED (CDCl\(_3\)+DMSO-d\(_6\)) showed signals at 2.45 (3H), 6.74-7.16 (2H), 3.30(2H) due to methyl protons, thiophene protons, methylene protons. Mass spectrum of MTCED shows signal at 277 (M+1) corresponding to its molecular ion peak. The molecular formula of the reagent is C\(_{14}\)H\(_{16}\)N\(_2\)S\(_2\) (M.Wt 276).

pKa values of reagents

The pKa values were determined by recording the UV-Visible spectra of 1X10\(^{-4}\)M solutions of the reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurements at different wave lengths determined spectrophotometrically using Phillips and Merrit method. The values of deprotonation of MTCED were 9.0 (pK\(_1\)= pK\(_2\)).

MTCED solution

A (1X10\(^{-2}\)M) solution was prepared by dissolving 0.276 gm of MTCED in 100 ml of methanol. The reagent solution is stable for at least 24 h.

Hg (II) standard solution

A (1X10\(^{-2}\)M) stock solution of mercury was prepared by dissolving 0.272 g of mercuric chloride (HgCl\(_2\)) (Merck Darmstadt) in double distilled water containing few drops of conc. Hcl and made up to the mark in a 100 ml volumetric flask.

1000 ppm stock solution of mercury was prepared by dissolving 0.13535 gm of mercuric chloride in 100 ml distilled water or 1.3535 gm of mercuric chloride in 1000 ml distilled water.

Buffer Solution

1M Sodium acetate + 0.1M hydrochloric acid (pH= 0.5 – 3.0), 0.2M Sodium acetate + 0.2M acetic acid (pH = 3.5 – 6.0), 1M Sodium acetate + 0.2M acetic acid (pH= 6.5 – 7.5), 2M
Ammonia + 2M ammonium chloride (pH= 8.0 – 12.0) buffer solutions are prepared in distilled water. Suitable portions of these solutions are mixed to get the desired pH.

**Potassium permanganate solution**

A 1% potassium permanganate solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid.

**Tartrate solution**

A 100 ml stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS grade (99%) potassium sodium tartrate tetrahydrate in (100 ml) de-ionized water.

**Aqueous ammonia solution**

A 100 ml solution of aqueous ammonia was prepared by diluting 10 ml concentrated NH₃ (28–30%, ACS grade) to 100 ml with de-ionized water. The solution was stored in a polypropylene bottle.

**Preparation of sample solutions**

**Preparation of water samples**

Different water samples were collected from various places around Tirupati, A.P., India. The samples (150 ml) were stored at 5°C in metal free polyethylene bottles. Water samples were filtered through Whatman filter paper no. 41 and collected into 250 ml beakers. All the filtered environmental water samples were evaporated nearly to dryness with a mixture of 10 ml of con HNO₃ and 5ml of con H₂SO₄ in a fume cupboard and then cooled to room temperature. The sample was digested in the presence of an excess potassium permanganate solution according to the method recommended by Fifiled et al.,⁵ The residues were then heated with 10 ml of deionized water in order to dissolve the salts. The solutions were cooled and neutralized with dilute NH₄OH. The digest was transferred into a 25 ml calibrated flask and diluted up to the mark with deionized water.

The reaction of Hg (II) was tested with 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) at different pH values. The samples were prepared in 10 ml of the solution containing constant volume of 2 ml of Hg (II), 2 ml of buffer solution (pH= 2.0), 1 ml of 1x10⁻² M MTCED, 2 ml lithium chloride solution and different metal ions. The solution was then shaken with 10 ml portions of methanol for two minutes and then allowed to stand. Each time the combined organic phase of aliquots were taken in 10 ml standard
flasks and made up to the mark with methanol. The absorbance was measured in 200-800 nm range against reagent blank.

**Recommended procedure**

An aliquot of the sample solution was taken in 25 ml standard flask containing 10 ml of buffer solution of (pH = 2.0) and 1 ml of 1x10^{-4} MTCED reagent solution and 1 ml of Hg (II) solution made up to the mark with distilled water. The absorbance of the complex was measured against the reagent blank at 385 nm. The absorbance values were referred to the predetermined calibration plot to compute the amount of mercury.

The author has introduced a new sensitive reagent MTCED for the extractive spectrophotometric determination of trace amounts of Hg (II).

The proposed method is more sensitive and selective when compared with other spectrophotometric methods. It also offers advantages like reliability and reproducibility in the addition to its simplicity instant color development and less interferences. The results obtained through UV-Visible spectrophotometer have been compared with those obtained through the ICP-OES.

**RESULTS AND DISCUSSION**

Mercury reacts with 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) in sodium acetate-hydrochloric acid buffer solution of pH 2.0 and gives 1:1 light yellow colored complex. The complex has a maximum absorbance at 385 nm. The optimum reaction conditions for the quantitative determination of the metal-ligand complex were established through a number of preliminary studies, such as the effect of pH, reagent concentration, interference of foreign ions, in order to develop a rapid, selective and sensitive extractive spectrophotometric method for the determination of Hg (II) at microgram levels.

**Absorption spectra of the reagent and Hg (II)-MTCED complex**

Absorption spectra of Hg (II)-MTCED complex and reagent show maximum absorbance at 385 nm and 340 nm respectively (Fig. 2). The reagent showed minimum absorbance at the wavelength of maximum absorbance of the complex. Hence, all the spectral measurements of the complex have been carried out at 385 nm.
The study of the effect of pH on the color intensity of the reaction mixture showed that the constant and maximum color is obtained in the pH range 1.0-3.0, the complex has maximum absorbance in buffer solution of (pH = 2.0). The analytical studies were therefore, carried out at (pH = 2.0).

![Absorption Spectra](image)

**Fig. 2. Absorption spectra of (a) Hg (II) – MTCED complex (λmax=385 nm) in aqueous solution, (b) MTCED Vs Water blank (1 x 10⁻⁴ M)**

Different molar excess of MTCED were added to Hg (II) concentration and the absorbance values were measured adopting the standard procedure. It was observed that 5 fold molar excess of reagent with respect to metal ion is necessary to get maximum absorbance. Hence, a 5 fold molar excess of reagent was used for further experimental studies.

The absorbance of the solution was measured at different time intervals to ascertain the time stability of the color complex. It was observed that the color development was instantaneous and remained constant for more than 24 hrs. Physicochemical and analytical properties of Hg (II) complex of MTCED were summarized in Table 1.
Adherence of the Hg (II) – MTCED complex system to Beers law

For the possible determination of Hg (II) at micro level, the absorbance of the solution containing different amounts of the metal ion is measured at 385 nm. The linear plot between the absorbance and the amount of Hg (II) is drawn and the straight line obtained with the equation $A_{385}=0.09993C+0.01827$ (Fig. 3). Further Beers law is obeyed in the range of 0.83-8.6 µg/ml, the molar absorptivity and Sandell’s sensitivity were found to be $5.58 \times 10^4$ L. mol$^{-1}$ cm$^{-1}$ and $0.00179$ µg/cm$^2$ respectively. The standard deviation of the method for ten determinations of 1.91 µg/ml is $\pm 0.00158$. The results showed that standard deviation of the method was not more than 0.00158 and relative standard deviation was less than 0.27052. These results indicate that the method has good precision, besides being accurate.
The effect of various cations and anions which are generally associated with the metal ion in the determination of Hg (II) was studied by measuring the absorbance of mercury. The complex contains 1.91 µg/ml of Hg (II) in solution. The colour of the reaction was developed as described in the standard procedure. An error of ±2% in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions and cations in the MTCED methods respectively were presented in Table 2. Higher amounts of Fe²⁺ do not interfere in the presence of 70 ppm of fluoride. Larger amounts of Hg²⁺ do not interfere in the presence of 600 ppm of iodide.

The present method was applied for the determination of mercury when present alone and present in water samples.

The present ligands containing thiophene ring are found to be potential and cost effective for the determination of Hg (II) without the need for extraction using the toxic solvents. Further the reagents are easy to synthesize using commercially available precursors. Moreover the

**Fig.3. Calibration plot for Hg (II) determination**

![Calibration plot for Hg (II) determination](image-url)
present method is simple, rapid and very sensitive for non-extractive spectrophotometric
determination of Hg (II) in aqueous medium.

Effect of foreign ions on the extraction of the Hg (II) –MTCED complex

The effect of foreign ions is studied by measuring the absorbance of the reaction mixture
containing 1.91 µg/ml of Hg (II) in the presence of different amounts of foreign ions. The
results presented in the Table 2. An error of ± 2 % in the absorbance value caused by foreign
ions is considered as a tolerable limit.

Table 2. Effect of foreign ions

<table>
<thead>
<tr>
<th>Ion Added</th>
<th>Tolerance limit µg/ml</th>
<th>Ion Added</th>
<th>Tolerance limit µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tartrate</td>
<td>594</td>
<td>W (V)</td>
<td>365</td>
</tr>
<tr>
<td>Iodate</td>
<td>509</td>
<td>Mn (II)</td>
<td>2.3</td>
</tr>
<tr>
<td>Urea</td>
<td>288</td>
<td>Pb (II)</td>
<td>8.3</td>
</tr>
<tr>
<td>Citrate</td>
<td>386</td>
<td>Cr (VI)</td>
<td>1.2</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>245</td>
<td>Ti (III)</td>
<td>0.41</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>234</td>
<td>Cd (II)</td>
<td>0.23</td>
</tr>
<tr>
<td>Sulphate</td>
<td>386</td>
<td>Hg(II)</td>
<td>0.40</td>
</tr>
<tr>
<td>Oxalate</td>
<td>351</td>
<td>Ni (II)</td>
<td>0.23</td>
</tr>
<tr>
<td>Thiourea</td>
<td>303</td>
<td>Fe (II)</td>
<td>0.21</td>
</tr>
<tr>
<td>Nitrate</td>
<td>249</td>
<td>Au (III)</td>
<td>0.42</td>
</tr>
<tr>
<td>Acetate</td>
<td>236</td>
<td>Pt (IV)</td>
<td>0.38</td>
</tr>
<tr>
<td>Phosphate</td>
<td>38</td>
<td>Pd(II)</td>
<td>0.24</td>
</tr>
<tr>
<td>Bromide</td>
<td>34</td>
<td>Ag (I)</td>
<td>0.21</td>
</tr>
<tr>
<td>Chloride</td>
<td>15</td>
<td>V (V)</td>
<td>0.11</td>
</tr>
<tr>
<td>Fluoride</td>
<td>7.9</td>
<td>Cu (II)</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Composition and stability constant of the complex

Job’s method of continuous variation and molar-ration methods were applied to ascertain the stoichiometric composition of the complex. It was found that MTCED forms 1:1 complex with Hg (II) as shown in the (Fig.4).

![Absorbance vs. V_L / (V_M + V_L)]

**Fig.4.** Job’s method of continuous variation method Hg (II)-MTCED. Hg (II) and MTCED 1 x 10^{-4}M: solvent-Methanol; (pH=2.0).

Application

The proposed extractive spectrophotometric method is applied for the determination of Hg (II) in water samples. A known aliquot of the above sample solutions were taken into a 25 ml separating funnel and the lead content was determined as described is given in the general procedure. The results were checked with parallel determinations by direct ICP-OES. The data obtained in the analyses of water samples were given in Table 3.
Table – 3. Determination of trace amount of Hg (II) in water samples.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Hg (II) found µg/ml</th>
<th>ICP-OES</th>
<th>S.D</th>
<th>R.S.D(%)</th>
<th>Proposed Methoda</th>
<th>S.D</th>
<th>R.S.D(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea waterb</td>
<td>1.07</td>
<td>0.00114</td>
<td>0.10640</td>
<td>1.013</td>
<td>0.00011</td>
<td>0.01085</td>
<td></td>
</tr>
<tr>
<td>River waterc</td>
<td>1.26</td>
<td>0.00011</td>
<td>0.00885</td>
<td>1.242</td>
<td>0.00011</td>
<td>0.00885</td>
<td></td>
</tr>
<tr>
<td>Drain waterd</td>
<td>3.44</td>
<td>0.00008</td>
<td>0.00240</td>
<td>3.328</td>
<td>0.00011</td>
<td>0.00330</td>
<td></td>
</tr>
<tr>
<td>Tap watere</td>
<td>1.03</td>
<td>0.00011</td>
<td>0.00977</td>
<td>1.023</td>
<td>0.00011</td>
<td>0.01075</td>
<td></td>
</tr>
<tr>
<td>River waterf</td>
<td>0.963</td>
<td>0.00011</td>
<td>0.01155</td>
<td>0.952</td>
<td>0.00008</td>
<td>0.00840</td>
<td></td>
</tr>
</tbody>
</table>

a. Average of the five determination
b. Collected at Bay of Bengal, (Chennai), India.
c. Collected at Tungabhadra river (Kurnool), A.P, India.
d. Collected at Anantapur town, A.P, India.
e. Collected at Anantapur Municipal Storage tank, A.P, India.
f. Collected at Swarnamuki (Srikalahasti), A.P, India.

CONCLUSION

The proposed spectrophotometric method is simple, rapid in expensive highly sensitive and selective for the determination of Hg (II) in water samples. The proposed method is free from interference of common metal ions such as Fe$^{3+}$, Pb$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Cr$^{3+}$. Urea, bicarbonate, citrate, sulphide, SO$_4^{2-}$, and PO$_4^{3-}$ also do not interfere. The proposed method is more sensitive than some reported methods in the literature and has been successfully applied for the determination of mercury in various environmental samples.

It offers also a very efficient procedure for speciation analysis. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES, and ICP-MS are available for the determination of lead at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of mercury in real samples down to ng g$^{-1}$ levels in aqueous medium at room temperature (25±5°C).
Acknowledgement

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Research paper

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Synthesis, Characterization and Biological Activity of 5-Methyl Thiophene-2-carboxaldehyde Derivatives of Copper (II), Cobalt (II) and Nickel (II) Complexes


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ABSTRACT

The Copper (II), Cobalt (II) and Nickel (II) complexes of 5 methyl thiophene-2-carboxaldehyde-thiosemicarbazone have been synthesized by a template method. The complexes are characterized by elemental analysis, conductivity measurements and electronic, IR, VSM and ESR spectral techniques. The molar conductivity data show them to be non-electrolytes. The electronic spectral data suggest square planar geometry for all the complexes. Various ESR parameters have been calculated. The bacterial and anti fungal activities of the complexes have been screened in vitro against the organisms Bacillus, E.coli, pseudomonas, Staphylococcus and fungi species such as F. oxysporum, M.phaseolina, A.flavus, A.niger.

Keywords: Copper (II) complex, Cobalt (II) complex, Nickel (II) complex, 5-methyl thiophene-2-carboxaldehyde derivative, Biological activity, ESR and VSM.

1. INTRODUCTION

Transition metal coordination compounds with multi-donor ligands containing Nitrogen and Sulphur atoms have versatile chelating ability with transition metal ions. These complexes have attracted considerable interest in biological systems because of the presence of copper in enzymes. It is both micro-nutrient as well as toxic element for living beings, depending up on the concentration level. The significance of Cobalt as a transition metal lies in its wide spectrum of applications covering many frontier areas of study, particularly in medicine. Even though Cobalt is not considered to be as toxic as most of the heavy metals, it is an equally harmful element. Among various ligands that form complexes with copper ion, Thiosemicarbazone derivatives possess broad pharmacological activity [1]. It is also highly interesting to study copper (II) complexes, which are important anticancer drugs because of their ability to adopt different coordination numbers, which leads to different geometries [2]. Copper, in divalent oxidation state, has been found to exhibit many types of coordination ranging from square planar to distorted trigonal bipyramidal, square pyramidal and octahedral. Copper complexes have attracted much attention in bioinorganic chemistry because of their structure and spectral properties in the solid state [3]. Earlier investigations on Cu(II) complexes containing tridentate and tetradentate ligands have given interesting result [4-6]. It should be of interest to study ligands containing amine as ligands, which show interesting structural and functional properties [7,8]. For this reason, we have studied Cu(II), Co(II) and Ni(II) complexes of 5-methyl-thiophene-2-carboxaldehyde-thiosemicarbazone (TCATS). The free TCATS ligand could not be isolated in pure state. Hence metal complexes of TCATS are derived from metal assisted Schiff base condensation (Template synthesis) of Thiosemicarbazide with 5-Methyl-thiophene-2-carboxaldehyde. Metal ion template assistance has been a boon to the rational design of complex multidentate ligand systems [9-13]. The synthetic utilization of metal ions have been particularly valuable [14-17] in Schiff base condensations. Therefore in this type of situation that metal ions have been so useful in providing template for directing the course of reaction. These complexes have been characterized by elemental analysis and analytical, ESR, electronic, VSM and antimicrobial activities.

2. Experimental

All chemicals of AR grade are used without further purification.5-methyl-Thiophene-2-carboxaldehyde and Thiosemicarbazone compounds are Aldrich chemicals. The metal
chelates were prepared using in situ reaction (Template synthesis) as the free ligand (TCATS) could not be isolated in the pure state.

Copper complex was prepared by reacting 5-methyl-thiophene-2-carboxaldehyde, Thiosemicarbazide and Cu(NO₃)₂ in 1:2:2 molar proportions. To a boiling solution of Thiosemicarbazide (1.690 g, 0.01854 mol) in methanol, a solution of 5-methyl-thiophene-2-carboxaldehyde (2.34 g, 0.01854 mol) in methanol was added and the reaction mixture was refluxed for 9 hours. A solution of Cu(NO₃)₂ (2.23 g, 0.00927 mol) in minimum quantity of methanol was added to the boiling mixture, which was further refluxed for 4 hours and then cooled. The complex was separated out and washed with hot water for several times and finally with Hexane. Cobalt and Nickel complexes of TCATS also prepared accordingly. Elemental (C, H, N and S) analysis is performed. The molar conductance is measured using a systronics 303 direct reading conductivity bridge. The electronic spectrum of the sample dissolved in DMF solvent is recorded at room temperature on Cary 5000 in UV-Vis, N-IR region. The powder samples are put into quartz tubes and ESR spectra are recorded at LNT on a Varian E-112 X-band spectrometer.

3. RESULTS AND DISCUSSION

The metal complexes are stable at room temperature, non-hygroscopic, insoluble in water, but slightly soluble in ethanol and methanol, and readily soluble in DMF and DMSO. The colour, molecular weight and molar conductance data are summarized in TABLE-1. The analytical data of the complexes indicate that the metal to ligand ratio is 1:2. The molar conductance values of these complexes in DMF are in the range 4-6 ohm⁻¹ cm² mol⁻¹ indicate their non-electrolytic nature [18].

3.1. Infrared spectra

Infrared spectra of the copper and cobalt complexes of TCATS are presented in Table-5. As free ligand could not be isolated, the spectra of metal complexes cannot be compared with the free ligand. The >C=N (imine band) is observed at 1670 cm⁻¹, 1583 cm⁻¹ and 1570 cm⁻¹ in the IR spectra of Cu-(TCATS)₂, Co-(TCATS)₂ and Ni-(TCATS)₂ respectively [19]. Additional bands are observed in Far IR spectra of metal complexes in 445-430 and 345-335 cm⁻¹ regions due to V(M-N) and V(M-S) modes respectively [20-22].

3.2. Magnetic susceptibility and Electronic spectra

The magnetic moment values of Cu (TCATS)₂ and Co (TCATS)₂ were observed as 1.77 BM and 2.6 BM respectively. In the electronic spectra of Cu (II) complex of TCATS bands observed at 15,952 cm⁻¹ and 21,946 cm⁻¹ may be assigned as, probably, the transitions ²A₁g ← ²B₁g and ²Eg ← ²B₁g respectively [23-26]. For Co (II) complex of TCATS a strong band is observed at 19,230 cm⁻¹ and low intensity band is at 7692 cm⁻¹ are in favour of square planar geometry [27-29].

The diamagnetic character of Ni (II) complex of TCATS probably indicates its square planar geometry. The electronic spectral data display three bands at 12,807 cm⁻¹ (ν₁), 25,568 cm⁻¹ (ν₂) and 29,337 cm⁻¹ (ν₃). Thus the obtained data confirm a square planar geometry around the Ni (II) ion [29-31].

Fig: 1. ESR spectra of Cu-TCATS at LNT

The spin Hamiltonian, orbital reduction and bonding parameters of Cu (TCATS)₂ complex is presented in Table-6. Kivelson and Neiman [32] have reported that g⊥ value is less than 2.3 for covalent character metal ligand bond and is greater than 2.3 for ionic character. Applying this criterion, the covalent character of the metal ligand bond in complex under study can be predicted. The trend g parallel > g perpendicular > g∞ observed for the complex suggests that the unpaired electron is localized in dz²-y² orbital [33] of the Copper (II) ion.

The ESR parameters g(parallel), g(perpendicular), A(parallel) and A(perpendicular) of complex, the energies of d-d transition are used [32-38] to evaluate the orbital reduction parameters, the bonding parameters (α²) and the dipolar interaction (P). The observed K parallel > K perpendicular relation indicates the presence of significant in-plane Pi-bonding [39]. The α² value for the present chelate lie at 0.6971 supporting the covalent nature of the complex. Giordano and
Bereman \cite{40} suggest the present complex is consistent with bonding of copper through sulphur donor atom. The shape \cite{41} of ESR lines, ESR data (Table-6) together with electronic spectral data suggest a square planar geometry for the copper complex.

\begin{table}[h]
\centering
\caption{Analytical data of Metal complexes of TCATS}
\begin{tabular}{|l|l|l|l|l|l|l|l|l|l|}
\hline
Compound & Color & M.P(\degree C) & Yield(\%) & Molecular Weight & C & H & N & S & M \\
\hline
Cu-(TCATS)$_2$ & Black & $>300^\circ$C & 262.5 & 35.25 & 3.50 & 17.90 & 25.65 & 13.01 \\
\hline
Co-(TCATS)$_2$ & Dirty Green & $>300^\circ$C & 257.9 & 36.22 & 3.02 & 17.95 & 26.95 & 12.02 \\
\hline
Ni-(TCATS)$_2$ & Greenish blue & $>300^\circ$C & 257.69 & 36.02 & 3.07 & 17.96 & 27.25 & 12.01 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Molar Conductance data of TCATS metal complexes}
\begin{tabular}{|l|l|}
\hline
Complex & Conductance (ohm$^{-1}$ cm$^2$ mol$^{-1}$) \\
\hline
Cu-(TCATS)$_2$ & 4.0 \\
Co-(TCATS)$_2$ & 6.0 \\
Ni-(TCATS)$_2$ & 4.0 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Selected IR bands (cm$^{-1}$) with tentative assignments}
\begin{tabular}{|l|l|l|l|l|l|l|l|}
\hline
Compound & $\nu$C=N & $\nu$C=S & $\nu$C-CH$_3$ & $\nu$M-N & $\nu$M-S \\
\hline
Cu-(TCATS)$_2$ & 1670 & 1250 & 621.25 & 1450.70 & 445 & 345 \\
Co-(TCATS)$_2$ & 1583 & 1233 & 707 & 1444.88 & 440 & 340 \\
Ni-(TCATS)$_2$ & 1570 & 1240 & 650 & 1440.50 & 430 & 335 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Magnetic moment data of metal complex of TCATS}
\begin{tabular}{|l|l|}
\hline
Complex & Magnetic moment(B.M) \\
\hline
Cu-(TCATS)$_2$ & 1.77 \\
Co-(TCATS)$_2$ & 2.60 \\
Ni-(TCATS)$_2$ & Diamagnetic \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Electronic spectral data and ligand field parameters of a Nickel complex}
\begin{tabular}{|l|l|l|l|l|l|l|l|l|l|}
\hline
Complex & Method of evaluation & $v_1$ & $v_2$ & $v_3$ & B & B$_{35}$ & 10Dq & $v_2-v_1$ & $v_2/v_1$ & h \\
\hline
Ni-(TCATS)$_2$ & A & 12807 & 25568 & 29337 & 965.2 & 0.85 & 12807 & 10755 & 1.839 & 1.25 \\
\hline
\end{tabular}
\end{table}
The antifungal and antibacterial activity of the Co(II), Ni (II) and Cu(II) complexes of TCATS were tested in vitro for the growth inhibiting potential against various fungal and bacterial strains using Food Poison Technique [42] and Paper Disc Technique[41] respectively. Fungal strains Fusarium oxysporum, Macrophomina phaseolina, A. flavus and A. niger and bacterial strains Bacillus, Escherichia coli, Psuedomonas and Stephilococcus were used. The concentrations of respective metal complexes were 25, 50, 100 and 200 ppm. DMF was used as solvent for preparing solutions [43].

The data is given in the Table-5 and 6. It is known that chelation reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor groups of ligands. \( \pi \) -Electron delocalization over the chelate ring also reduces the polarity of metal ion. Such chelation increases the lipophilic character of the metal complex which is necessary to cross the permeability barrier of cells resulting in interference with normal cell process [44].

In general the toxicity of metal complexes decreased as the radius of metal ion increases. The growth inhibition against all the fungi and bacteria is found to be in the order 

\[
Cu > Co \text{ and Ni.}
\]

The results recorded from the fungal activity were also further compared with the standard fungicide Grisofluvin. The results are quite promising.

### 3.3. Biological activity

The Table-6: Spin Hamiltonian and orbital reduction parameters of Cu-TCATS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cu (TCATS)₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>g (parallel)</td>
<td>2.341</td>
</tr>
<tr>
<td>g (perpendicular)</td>
<td>1.7541</td>
</tr>
<tr>
<td>( g_{avg} )</td>
<td>1.9497</td>
</tr>
<tr>
<td>G</td>
<td>1.3646</td>
</tr>
<tr>
<td>A (parallel)</td>
<td>0.0145</td>
</tr>
<tr>
<td>A (perpendicular)</td>
<td>0.0042</td>
</tr>
<tr>
<td>K (parallel)</td>
<td>0.9796</td>
</tr>
<tr>
<td>K (perpendicular)</td>
<td>0.7813</td>
</tr>
<tr>
<td>( \alpha^2 )</td>
<td>0.6971</td>
</tr>
<tr>
<td>P</td>
<td>0.0351</td>
</tr>
</tbody>
</table>

### Structure of Metal chelate (M-(TCATS)₂)

M = Cu (II), Co (II), Ni (II)

### Table-7: Fungal activity of Metal complexes of TCATS

<table>
<thead>
<tr>
<th>Ligand/complex</th>
<th>concentration</th>
<th>F. Oxysporum</th>
<th>M. phaseolina</th>
<th>A. flavus</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 ppm</td>
<td>83.33%</td>
<td>83.33%</td>
<td>82.22%</td>
<td>83.33%</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>88.88%</td>
<td>90%</td>
<td>86.66%</td>
<td>86.66%</td>
</tr>
<tr>
<td>Cu(TCATS)₂</td>
<td>100 ppm</td>
<td>90%</td>
<td>90%</td>
<td>91.11%</td>
<td>91.11%</td>
</tr>
<tr>
<td></td>
<td>200 ppm</td>
<td>92.22%</td>
<td>94.44%</td>
<td>92.22%</td>
<td>92.22%</td>
</tr>
<tr>
<td></td>
<td>25 ppm</td>
<td>77.77%</td>
<td>75.55%</td>
<td>77.77%</td>
<td>77.77%</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>82.22%</td>
<td>81.11%</td>
<td>77.77%</td>
<td>77.77%</td>
</tr>
<tr>
<td>Co-(TCATS)₂</td>
<td>100 ppm</td>
<td>84.44%</td>
<td>84.44%</td>
<td>84.44%</td>
<td>84.44%</td>
</tr>
<tr>
<td></td>
<td>200 ppm</td>
<td>88.88%</td>
<td>90%</td>
<td>86.66%</td>
<td>86.66%</td>
</tr>
<tr>
<td></td>
<td>25 ppm</td>
<td>78.88%</td>
<td>78.88%</td>
<td>72.22%</td>
<td>73.33%</td>
</tr>
<tr>
<td></td>
<td>50 ppm</td>
<td>81.11%</td>
<td>80.00%</td>
<td>80.00%</td>
<td>74.44%</td>
</tr>
<tr>
<td>Ni-(TCATS)₃</td>
<td>100 ppm</td>
<td>85.55%</td>
<td>82.22%</td>
<td>82.22%</td>
<td>84.44%</td>
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<tr>
<td></td>
<td>200 ppm</td>
<td>87.77%</td>
<td>88.88%</td>
<td>84.44%</td>
<td>85.55%</td>
</tr>
</tbody>
</table>
4. CONCLUSIONS

Metal complexes of Cu-(TCATS)$_2$, Co-(TCATS)$_2$ and Ni-(TCATS)$_2$ are derived from metal template assisted Schiff base condensation (Template synthesis) of 5-methyl-2-thiophenecarboxaldehyde and Thiosemicarbazide. The complexes were characterized by spectral and analytical data. From the spectral and analytical analyses, a square-planar geometry was assigned for the metal complexes. The ESR results of the complex show that $g_{\parallel} > g_{\perp}$, which indicate an axial elongation of the ligands. The covalency parameter ($\alpha^2$) confirms the covalent nature of the metal ligand bond. The antimicrobial activity against four fungal and four bacterial isolates are determined.

5. ACKNOWLEDGEMENT

The authors are thankful to CDIR; Lucknow, for the C, H and N analysis. Thanks are due to CIL, Central University, Hyderabad for VSM and ESR analysis. Our special thanks to Prof. Subba Ram Reddy, Department of Statistics, S.V. University, Tirupati for providing statistical analysis for Biological activity. Finally the authors wish to express special thanks to Prof. J. Lakshmana Rao, Department of Physics, SV University, Tirupati, for providing ESR spectra.

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23. Introduction to Ligand fields-BN. Figgis.
Spectrophotometric determination of selenium in industrial and environmental samples using vanillin-2-aminonicotinic acid (VANA)

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method was developed for the determination of Se (IV) using Vanillin-2-aminonicotinic acid (VANA) as an analytical reagent. The reagent has been synthesized and characterized using IR, $^1$H NMR and mass spectral data. The metal ion in aqueous medium forms light greenish yellow colored complex with VANA at (pH= 5.0) (sodium acetate- acetic acid buffer solution) showing maximum absorbance at 360 nm. Hence, analytical studies were further carried out at 360 nm. The reagent reacts with selenium in acidic medium to form light greenish yellow colored 1:1 (M:L) complex. The color reactions are instantaneous and absorbance values remain constant for 24 hours. The composition of the Se (IV) complex with VANA was studied by the method of job’s continuous variation and molar ratio method. Beer’s law was obeyed in the range 0.06-3.48 µg ml$^{-1}$ of Se (IV). The molar absorptivity and Sandell’s sensitivity of the method were found to be 7.48 x 10$^3$ Lmol$^{-1}$ cm$^{-1}$ and 0.0013µg/cm$^{-2}$ respectively. Since VANA method is more sensitive, it was applied for the determination of selenium in environmental samples.

Key words: Selenium determination, non-extractive spectrophotometry, Vanillin-2-aminonicotinicacid (VANA), Environmental samples.

INTRODUCTION

Selenium is an essential trace nutrient and selenium deficiency diseases are well known in veterinary medicine. Above trace levels, ingested selenium is toxic to animals and may be toxic to humans. The selenium concentration of most drinking waters and natural waters is less than 10 µg/ml$^{-1}$. Selenium is widely distributed in nature, in relatively small concentrations in rocks, plants, coal and other fossil fuels. Selenium enters into natural water through seepage from splendiferous soil and industrial waste. Selenium compounds have extensive applications and because of its significance, several analytical techniques have been reported for the determination of selenium, which includes spectro-fluorometry, electro thermal atomic absorption spectrometry, hydride generation, polarography, cathode-stripping voltammetry, radiochemical neutron activation analysis, and flow- injection techniques [1]. There are many reagents available for the spectrophotometric determination of selenium., among which, the recently used were J-acid [2], Leuco crystal violet [3], resazurin [4], sodium salt of hexamethyleneimine carbodithioate [5], 1- naphthylamine-7-sulphonic acid [6], variamine blue [7]. When selenium is present in animal feeds at a concentration less than 0.1 mg 1$^{-1}$, deficiency symptoms develop, but when present at a higher concentration, exceeding 5 mg1$^{-1}$, chronic selenosis occurs. Selenium tends to weaken the toxic action of some heavy metals in animal and human organisms [8-9].

Selenium is widely distributed in the environment (waters, soil, and air) albeit generally in very low concentrations (≤1µg/g). The selenium content sometimes reaches 0.5 mg/g in limonite rocks and 2.6 mg/g in vanadium-uranium rocks [10]. Selenium is a naturally occurring element found in rocks, soil, water, air and animals. Selenium is a trace mineral that is essential to good health but required only in small amounts [11-12]. Selenium is incorporated into
proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help to prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases such as cancer and heart disease [13]. Other selenoproteins help regulate thyroid function and play a role in the immune system [14,16]. Selenium compounds are widely used in paints, dyes, glass electrical, rubber, insecticides, and many other industries. Some industrial and agricultural processes release selenium as a by-product and selenium from such sources has caused environmental disaster [17]. The threshold limit value for selenium compounds in air is 0.1-0.2 mg dm$^{-3}$ in water it is 4.0 ppm. The toxicity, availability and environmental mobility of selenium are very much dependent on its chemical form [18]. Selenium can occur in different oxidation states in organic and inorganic compounds. In many environmental matrices, e.g. natural water and soils, the predominant oxidation state of selenium are Se (IV) and Se (VI). Water drained from such soil cause severe environmental pollution and wide life toxicity. Selenium is also reported to be present in cigarette paper, tobacco [19] and various cosmetic samples [20]. Because of its significance, several analytical techniques have been reported concerning the determination of selenium [21-24].

Plant foods are the major dietary sources of selenium in most countries throughout the world. The content of selenium in food depends on the selenium content of the soil where plants are grown or animals are raised. For example, researchers know that soils in the high plains of northern Nebraska and the Dakotas have very high levels of selenium. People living in those regions generally have the highest selenium intakes in the United States (U.S.). In the U.S., food distribution patterns across the country help prevent people living in low-selenium geographic areas from having low dietary selenium intakes. Soils in some parts of China and Russia have very low amounts of selenium. Selenium deficiency is often reported in those regions because most food in those areas is grown and eaten locally.

In the present study, we are reporting rapid, simple, sensitive and selective methods for the determination of traces of selenium (IV) with VANA, a new reagent.

This paper describes synthesis, characterization and analytical properties of new reagent viz., Vanillin-2-aminonicotinicacid (VANA). Since the reagent is more sensitive, it is used for the determination of selenium in various water and soil samples.

**MATERIALS AND METHODS**

**Apparatus**

A Shimadzu (Model-1601) UV-VIS spectrophotometer (PerkinElmer Singapore Private Limited, Singapore) and ELICO model LI-610 pH meter (M/s ELICO private limited, Hyderabad, India) with combination electrodes were used for measurements of absorbance and pH respectively. ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry Model-7000) methods were used for the quantitative analysis of Se (IV). To determine the Se (IV), AOAC methods were used (AOAC 1986, 2003; Jorhem 1993). In this method, the samples were dissolved at 190°C and 400 psi pressure in Mars 5 apparatus (Vessel Type XKP 1500, CEM, Matthews, USA). Se (IV) was analyzed by inductively coupled plasma-optical emission Spectrometry (Varian Vista-MPX CCD Simultaneous Spectrophotometer, Mug rave-Victoria, Australia) [25 – 26].

**Reagent and solutions**

All chemicals used were of analytical-reagent grade of the highest purity available procured from Merck. Doubly distilled de-ionized water was used throughout the experiment. Glass vessels were cleaned soaking in acidified solutions of K$_2$Cr$_2$O$_7$ followed by washing with conc. HNO$_3$ and were rinsed several times with high purity de-ionized water. Stock solutions and environmental water and soil samples were kept in polypropylene bottle containing 1ml of conc. HNO$_3$.

**Preparation of reagent (VANA)**

Vanillin (1.5g, 0.0098 mol) in 60 ml of methanol, 2-Aminonicotinic acid (1.36 g, 0.0098 mol) dissolved in 60 ml of methanol were taken in 250 ml round bottom flask. Suitable quantity (1ml) of 1molar sodium acetate and 2 or 3 drops of conc. H$_2$SO$_4$ were added to the reaction mixture and refluxed for 8 hours. On cooling the reaction mixture, ash colored product was separated out. It was treated with water and ethyl acetate. It was collected by filtration and washed several times with hot water followed by n-hexane. This compound was recrystalised from methanol and dried in vacuum. The ligand is stable for more than 6 months. Percent of Yield is 91; m.p.84-86 °C. The structure of VANA is shown in the Fig.1.
Characterization of reagent (VANA)
The reagent has been synthesized and characterized by IR, \textsuperscript{1}HNMR and mass spectral data. Infrared spectrum of VANA shows bands at 3375, 3094, 2863, 1714, 1695, 1665, 1662, 1588, 1510, 1464, 1299, 1266, 1067 and 927 respectively corresponding to $\nu$(O-H) symmetric stretch, $\nu$(C-H) stretch (pyridine) (sp\textsuperscript{2}---C-H), $\nu$(-OCH\textsubscript{3}) stretch, $\nu$(C = O) Aromatic stretch, $\nu$(HC = N) schiffbase, $\nu$(C =C) Aromatic stretch, $\nu$(C - C) stretch (pyridine ring), $\delta$(C - H) (Aromatic ring), $\nu$(C = N) stretch (pyridine), $\nu$(C - O) stretch, $\nu$(C - N) stretch and $\delta$(O-H)oop bend(carboxylic acid). H\textsuperscript{1}NMR spectrum of VANA (CDCl$_3$+DMSO-d$_6$) showed signals at 6.75 – 7.43 (6H), 9.73 (1H), 4.87 (3H), 5.26 (1H) due to benzene and pyridine protons, acid protons, methoxy protons, =C- H. Mass spectrum of VANA shows signal at 273 (M+1) corresponding to its molecular ion peak. The molecular formula of the reagent is C\textsubscript{14}H\textsubscript{12}N\textsubscript{2}O\textsubscript{4} (M.Wt, 273).

pKa values of reagents
The pKa values were determined by recording the UV-Visible spectra of 1X10\textsuperscript{-4}M solutions of the reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurements at different wave lengths determined spectrophotometrically using Phillips and Merrit method. The values of deprotonation of VANA were 7.0 (pK\textsubscript{1}) and 10 (pK\textsubscript{2}).

VANA solution
A 1X10\textsuperscript{-2}M solution was prepared by dissolving 0.2722 gm of VANA in 100 ml of methanol. The reagent solution is stable for at least 24 h.

Se (IV) solution
A (1X10\textsuperscript{-2}M) stock solution of selenium was prepared by dissolving 0.1890 g of sodium selenate (Na\textsubscript{2}Seo4) (Merck Darmstadt) in double distilled water containing few drops of conc.H\textsubscript{2}SO\textsubscript{4} and made up to the mark in a 100 ml volumetric flask. Aliquots of this solution were standardized with EDTA titration using xylene orange as an indicator. Dilute solutions were prepared from this stock solution.

1000 ppm stock solution of selenium was prepared by dissolving 0.2394 gm of sodium selenate in 100 ml distilled water or 2.394 gm of Sodium Selenate in 1000 ml distilled water.

Buffer Solution
1M Sodium acetate + 0.1M hydrochloric acid (pH = 0.5 – 3.0), 0.2M Sodium acetate + 0.2M acetic acid (pH = 3.5 – 6.0), 1M Sodium acetate + 0.2M acetic acid (pH = 6.5 – 7.5), 2M Ammonia + 2M ammonium chloride (pH = 8.0 – 12.0) buffer solutions were prepared in distilled water. Suitable portions of these solutions were mixed to get the desired pH.

Potassium permanganate solution
A 1% potassium permanganate solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid.

Tartrate solution
A 100 ml stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS grade (99%) potassium sodium tartrate tetra hydrate in (100 ml) de ionized water.

Aqueous ammonia solution
A 100 ml solution of aqueous ammonia was prepared by diluting 10 ml concentrated NH\textsubscript{3} (28–30%, ACS grade) to 100 ml with de-ionized water. The solution was stored in a polypropylene bottle.
Preparation of Sample solutions

Preparation of water samples

Different water samples (Ground water and Tap water) were collected from various places around Tirupati, A.P., and India. The samples (150 ml) were stored at 5°C in metal free polyethylene bottles. Water samples were filtered through whatman filter paper no. 41 and collected into 250 ml beakers. All the filtered environmental water samples were evaporated nearly to dryness with a mixture of 10 ml con HNO₃ and 5ml of con H₂SO₄ in a fume cupboard and then cooled to room temperature. The sample was digested in the presence of an excess potassium permanganate solution according to the method recommended by Fifield et al., ⁵. The residues were then heated with 10 ml of deionized water in order to dissolve the salts. The solutions were cooled and neutralized with dilute NH₄OH. The digest was transferred into a 25 ml calibrated flask and diluted up to the mark with deionized water.

Recommended procedure

An aliquot of the metal solution was taken in 25ml standard flask containing 10 ml of buffer solution of (pH=5.0) and 1ml of VANA reagent solution and made up to the mark with distilled water. The absorbance of the complex was measured against the reagent blank at 360 nm.

General procedure

A known aliquot of the sample solution was taken in a 25 ml standard flask containing constant volume of 10 ml of buffer solution (pH= 5), 1.0 ml of 1x10⁻³ M VANA and 1ml of Se (IV) solution made up to the mark with distilled water. Absorbance of the solution was measured at λ_max against the reagent blank. The absorbance values were referred to the predetermined calibration plot to compute the amount of Selenium.

RESULTS AND DISCUSSION

Selenium reacts with Vanillin-2-aminonicotinic acid (VANA) in sodium acetate-hydrochloric acid buffer solution of (pH = 5.0) and gives 1:1 light greenish yellow colored complex. The complex has a maximum absorbance at 360 nm. The optimum reaction conditions for the quantitative determination of the metal-ligand complex was established through a number of preliminary studies, such as the effect of pH, reagent concentration, interference of foreign ions, in order to develop a rapid, selective and sensitive extractive spectrophotometric method for the determination of selenium (IV) at microgram levels.

Absorption spectra of the reagent and Se (IV)-VANA complex

Absorption spectra of Se (IV)-VANA complex and reagent show maximum absorbance at 360 nm and 320 nm, respectively (Fig.2). The reagent showed minimum absorbance at the wavelength of maximum absorbance of the complex. Hence, all the spectral measurements of the complex were therefore carried out at 360 nm.

The study of the effect of pH on the color intensity of the reaction mixture showed that the constant and maximum color is obtained in the pH range 4.0-6.0, the complex has maximum absorbance in buffer solution of (pH = 5.0). The analytical studies were therefore, carried out at (pH= 5.0).

Different volume of molar excess of VANA was added to fixed Se (IV) concentration and the absorbances were measured adopting the standard procedure. It was observed that 10 fold molar excess of reagent with respect to metal ion is necessary to get maximum absorbance. Hence, a 10 fold molar excess of reagent was used for further experimental studies.

The absorbance of the solution was measured at different time intervals to ascertain the time stability of the color complex. It was observed that the color development was instantaneous and remained constant for more than 24 hrs. Physicochemical and analytical properties of Se (IV) complex of VANA were summarized in Table 1.
Fig. 2. Absorption spectra of (a). Se (IV) – VANA complex (λ max=360 nm) in aqueous solution, (b). VANA Vs Water blank (1x10^{-3} M)

Table 1. Physico – chemical and analytical characteristics of Se (IV) – VANA complex

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Characteristic Property</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absorbance Maximum λ max (nm)</td>
<td>360</td>
</tr>
<tr>
<td>2</td>
<td>pH – range (optimum)</td>
<td>4.0 – 6.0</td>
</tr>
<tr>
<td>3</td>
<td>Mole of reagent required mole of metal ion for full color developed</td>
<td>10 Fold</td>
</tr>
<tr>
<td>4</td>
<td>Beer’s law validity range (µg/ml)</td>
<td>0.06- 3.48</td>
</tr>
<tr>
<td>5</td>
<td>Molar absorptivity (L mol^{-1} cm^{-1})</td>
<td>7.48x10^3</td>
</tr>
<tr>
<td>6</td>
<td>Specific absorptivity (ml g^{-1} cm^{-1})</td>
<td>0.0213</td>
</tr>
<tr>
<td>7</td>
<td>Sandell’s sensitivity (µg/cm²)</td>
<td>0.0013</td>
</tr>
<tr>
<td>8</td>
<td>Composition of complex as obtained in Job’s and molar ratio methods (M:L)</td>
<td>1 : 1</td>
</tr>
<tr>
<td>9</td>
<td>Stability constant of the complex</td>
<td>1.005x10^4</td>
</tr>
<tr>
<td>10</td>
<td>Relative standard deviation (RSD)%</td>
<td>2.209302</td>
</tr>
<tr>
<td>11</td>
<td>Y-intercept</td>
<td>-0.03118</td>
</tr>
<tr>
<td>12</td>
<td>Angular coefficient (m)</td>
<td>0.23329</td>
</tr>
<tr>
<td>13</td>
<td>Correlation coefficient(υ)</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

Adherence of the Se (IV) – VANA complex system to Beers law

For the possible determination of Se (IV) at micro level, the absorbance of the solution containing different amounts of the metal iron is measured at 360 nm. The linear plot between the absorbance and the amount of Se (IV) is drawn and the straight line obtained with the equation A_{360} = 0.23329-0.03118 (Fig.3). Further Beers law is obeyed in the range of 0.06- 3.48 µg/ml, the molar absorptivity and sandell’s sensitivity were found to be 7.48x10^3 L.mol^{-1}cm^{-1} and 0.0013 µg/cm² respectively. The standard deviation of the method for ten determinations of 2.12 µg/ml is ± 0.00167. The results showed that standard deviation of the method was not more than 0.00167 and relative standard deviation was less than 2.20930.
Interference of Foreign Ions
The effect of various cations and anions which are generally associated with the metal ion in the determination of Se (IV) was studied by measuring the absorbance of Selenium. The complex contains 2.12 µg/ml of Se (IV) in solution. The colour of the reaction was developed as described in the standard procedure. An error of ±2% in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions and cations in the VANA methods respectively were presented in Table 2. Higher amounts of Fe²⁺ do not interfere in the presence of 70 ppm of fluoride. Larger amounts of Hg²⁺ do not interfere in the presence of 600 ppm of iodide.

The present method Vanillin-2-aminonicotinicacid (VANA) was applied for the determination of selenium when present alone and present in water and soil samples. The present ligands containing aromatic ring are found to be potential and cost effective for the determination of Se (IV) without the need for extraction using the toxic solvents. Further the reagents are easy to synthesize using commercially available precursors. Moreover the present method is simple, rapid and very sensitive for non-extractive spectrophotometric determination of Se (IV) in aqueous medium.

### Table 2. Effect of foreign ions

<table>
<thead>
<tr>
<th>Ion Added</th>
<th>Tolerance limit µg/ml</th>
<th>Ion Added</th>
<th>Tolerance limit µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tartrate</td>
<td>594</td>
<td>W(V)</td>
<td>365</td>
</tr>
<tr>
<td>Iodate</td>
<td>509</td>
<td>Mn (II)</td>
<td>2.3</td>
</tr>
<tr>
<td>Urea</td>
<td>288</td>
<td>Pb (II)</td>
<td>8.3</td>
</tr>
<tr>
<td>Citrate</td>
<td>386</td>
<td>Cr (VI)</td>
<td>1.2</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>245</td>
<td>Ti (III)</td>
<td>0.41</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>234</td>
<td>Cd (II)</td>
<td>0.23</td>
</tr>
<tr>
<td>Sulphate</td>
<td>386</td>
<td>Hg(II)</td>
<td>0.40</td>
</tr>
<tr>
<td>Oxalate</td>
<td>351</td>
<td>Ni (II)</td>
<td>0.23</td>
</tr>
<tr>
<td>Thiourea</td>
<td>303</td>
<td>Fe (II)</td>
<td>0.21</td>
</tr>
<tr>
<td>Nitrate</td>
<td>249</td>
<td>Au (III)</td>
<td>0.42</td>
</tr>
<tr>
<td>Acetate</td>
<td>236</td>
<td>Pt (IV)</td>
<td>0.38</td>
</tr>
<tr>
<td>Phosphate</td>
<td>38</td>
<td>Pd(II)</td>
<td>0.24</td>
</tr>
<tr>
<td>Bromide</td>
<td>34</td>
<td>Ag (I)</td>
<td>0.21</td>
</tr>
<tr>
<td>Chloride</td>
<td>15</td>
<td>V (V)</td>
<td>0.11</td>
</tr>
<tr>
<td>Fluoride</td>
<td>7.9</td>
<td>Cu (II)</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Effect of foreign ions on the extraction of the Se (IV) –VANA complex

The effect of foreign ion is studied by measuring the absorbance of the reaction mixture containing 2.12 µg/ml of Se (IV) in the presence of different amounts of foreign ions. The results presented in the Table 2. An error of ± 2 % in the absorbance value caused by foreign ions is considered as a tolerable limit.

Composition and stability constant of the complex

Job’s method of continuous variation and molar-ratio methods were applied to ascertain the stoichiometric composition of the complex. It was found that VANA forms 1:1 complex with Se (IV) as shown in the (Fig.4).

Application

The proposed extractive spectrophotometric method is applied for the determination of Se (IV) in water samples. A known aliquot of the above sample solutions were taken into a 25 ml separating funnel and the selenium content was determined as described is given in the general procedure. The results were checked with parallel determinations by direct ICP-OES. The data obtained in the analyses of water samples were given in Table 3 and 4.

![Fig.4. Job’s method of continuous variation method Se (IV)-VANA. Se (IV) and VANA 1X10^{-3}M: solvent-Methanol; (pH=5.0)](image-url)

Table 3. Determination of trace amount of Se (IV) in water samples

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Se (IV) found µg/ml</th>
<th>ICP-OES</th>
<th>S.D</th>
<th>R.S.D (%)</th>
<th>Proposed Methoda</th>
<th>S.D</th>
<th>R.S.D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground waterb</td>
<td>1.722</td>
<td>0.00013</td>
<td>0.07548</td>
<td>1.713</td>
<td>0.0001</td>
<td>0.00583</td>
<td></td>
</tr>
<tr>
<td>Ground waterc</td>
<td>1.103</td>
<td>0.00011</td>
<td>0.00997</td>
<td>1.210</td>
<td>0.00011</td>
<td>0.00908</td>
<td></td>
</tr>
<tr>
<td>Industrial waste waterd</td>
<td>0.63</td>
<td>0.00158</td>
<td>0.25</td>
<td>0.68</td>
<td>0.00114</td>
<td>0.16730</td>
<td></td>
</tr>
<tr>
<td>Ground watere</td>
<td>0.28</td>
<td>0.00114</td>
<td>0.40482</td>
<td>0.25</td>
<td>0.00114</td>
<td>0.45310</td>
<td></td>
</tr>
<tr>
<td>Ground water (upper)f</td>
<td>0.13</td>
<td>0.00114</td>
<td>0.86626</td>
<td>0.16</td>
<td>0.00114</td>
<td>0.70544</td>
<td></td>
</tr>
<tr>
<td>Ground water (lower)g</td>
<td>0.21</td>
<td>0.0011</td>
<td>0.51935</td>
<td>0.198</td>
<td>0.00011</td>
<td>0.05551</td>
<td></td>
</tr>
</tbody>
</table>

a. Average of the five determination
b. Collected at Pollur (Palamaneru-chittoor), A.P, India.
c. Collected at Raniyep, A.P, India.
d. Collected at Karakambadi, A.P, India.
e. Collected at Mahanandi, A.P, India.
f. Collected at Yaganti (upper), A.P, India.
g. Collected at Yaganti (lower), A.P, India.
Table 4. Determination of trace amount of Se (IV) in soil samples

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Se (IV) found µg/mg</th>
<th>S.D</th>
<th>R.S.D (%)</th>
<th>Proposed Method*</th>
<th>S.D</th>
<th>R.S.D (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polluted soil$^b$</td>
<td>1.36</td>
<td>0.00114</td>
<td>0.0807</td>
<td>1.32</td>
<td>0.00114</td>
<td>0.0862</td>
</tr>
</tbody>
</table>

a. Average of the five determination
b. Collected at Pollur (Palamaner-chittoor), A.P, India.

CONCLUSION

The author has introduced a new sensitive reagent VANA for the extractive spectrophotometric determination of trace amounts of Se (IV). The proposed spectrophotometric method is simple, highly sensitive and selective for the determination of Se (IV) in water and soil samples when compared with other spectrophotometric methods. The proposed method is simple, rapid and common metal ions such as Fe$^{3+}$, Pb$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, Cr$^{3+}$ do not interfere. Urea, bicarbonate, citrate, sulphide, SO$_4^{2-}$, and PO$_4^{3-}$ also do not interfere. It also offers advantages like reliability and reproducibility in addition to its simplicity instant color development and less interference effect. The results obtained through UV-Visible spectrophotometer have been compared with those obtained through the ICP-OES. The method has been successfully applied for the determination of selenium in various environmental samples.

In this paper a new simple, sensitive, selective, and inexpensive method with the Se (IV)–VANA complex was developed for the determination of selenium in industrial, environmental, for continuous monitoring to establish the trace levels of selenium in difficult sample matrices. It offers also a very efficient procedure for speciation analysis. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES, and ICP-MS, are available for the determination of selenium at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables, and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of selenium in real samples down to ng g$^{-1}$ levels in aqueous medium at room temperature (25±5°C).

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Spectrophotometric determination of Lead in medicinal leaf and environmental samples using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED)

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method was developed for the determination of Pb (II) using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) as an analytical reagent. The reagent has been synthesized and characterized using IR, ¹H NMR and Mass spectral data. The metal ion in aqueous medium forms light yellow colored complex with MTCED at pH=3.0 (sodium acetate-hydrochloric acid buffer solution) showing maximum absorbance at 380 nm. Hence, analytical studies were further carried out at 380 nm. The reagent reacts with lead in acidic medium to form light yellow colored 1:1 (M:L) complex. The color reactions were instantaneous and absorbance values remain constant for 24 hours. The composition of the Pb (II) complex with MTCED was studied by the method of Job’s continuous variation and molar ratio method. Beer’s law was obeyed in the range of 0.27-2.4 µg ml⁻¹ of Pb (II). The molar absorptivity and sandell’s sensitivity of the method were found to be 5.86 x 10⁴ Lmol⁻¹cm⁻¹ and 0.0017 µg/cm² respectively. Since MTCED method is more sensitive, it was applied for the determination of lead in medicinal leaf and environmental samples.

Key words: Lead determination, spectrophotometry, 5-methylthiophene –2-carboxaldehyde ethylenediamine (MTCED), medicinal leaf and environmental samples.

(Under Review)
List of papers presented in seminars

1. 'Spectrophotometric determination of arsenic, selenium, lead, and mercury pollutants of environmental importance by using 2,4-Dihydroxy-benzophenone-2-aminothiophenol’’
Presented at National Seminar NSCME-2011, Department of chemistry, S.V.University, Tirupati, A.P.,India

2. ‘‘Spectrophotometric determination of arsenic, selenium, lead, and mercury pollutants of environmental importance by using 2,4-Dihydroxy-benzophenone-2-aminothiophenol, Vanillin-2-amino nicotinic acid and 5-methyl-2-thiophene carboxaldehyde ethylenediamine’’
Presented at National Seminar RRTCS-2011, Department of chemistry, S.V.University, Tirupati, A.P.,India

3. 'Spectrophotometric determination of arsenic pollutant of environmental importance by using benzil-5-amino-1,3,4-thiadiazole-2-thiol’’
Presented at National Seminar NSPPMC-2013, Department of chemistry, S.V.University, Tirupati, A.P.,India
SPECTROPHOTOMETRIC DETERMINATION OF ARSENIC, SELENIUM, LEAD, AND MERCURY POLLUTANTS OF ENVIRONMENTAL IMPORTANCE

K. DEEPA, K. DAKSHAYANI and Y. LINGAPPA*. Dept. of Chemistry, S.V. University, Tirupati.

ABSTRACT

Analytical applications of 2,4-Dihydroxy-benzophenone-2-aminothiophenol are reported for the first time. The reagent has been synthesized and characterized using IR, NMR, and Mass spectral data. 2,4-Dihydroxy-benzophenone-2-aminothiophenol has been used for the spectrophotometric determination of AS (III), Se (IV), Pb (II) and Hg (II). The reagents react with AS (III), Se (IV), Pb (II), and Hg (II) in both acidic and basic mediums to form yellow colored complexes 1:1(M:L) complexes. The color reactions are instantaneous and absorbance values remain constant for 24 hours. The molar absorptivity and sandell’s sensitivity of 2, 4-Dihydroxy-benzophenone-2-aminothiophenol methods are found to be $1.45 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$ and 0.0040 $\mu$g cm$^{-2}$ of AS (III), $1.002 \times 10^{-4} \text{ L mol}^{-1}\text{cm}^{-1}$ $8.66 \times 10^{-4} \mu $g cm$^{-2}$, 0.075 $\mu$gmL$^{-1}$ and 0.0250 $\mu$gmL$^{-1}$ of Se (IV), $1.89 \times 10^{4} \text{ L mol}^{-1}\text{cm}^{-1}$ and 0.0019 $\mu$g cm$^{-2}$ of Pb (II) and 2.5$x10^4$dm$^{-3}$ mol$^{-1}$cm$^{-1}$ and 0.0084 $\mu$g cm$^{-2}$ of Hg (II) respectively. The method is successfully applied to a number of water (ground, polluted) and other environmental samples. The results of the proposed method in the analysis of samples are comparable with those obtained by spectrophotometric method. The method has high accuracy and precision.

Key words: Arsenic, Selenium, Lead, Mercury determination, non-extractive spectrophotometry, environmental water (ground, polluted) and other samples, 2,4-Dihydroxy-benzophenone-2-aminothiophenol.
SPECTROPHOTOMETRIC DETERMINATION OF ARSENIC, SELENIUM, LEAD, AND MERCURY POLLUTANTS OF ENVIRONMENTAL IMPORTANCE

K. DEEPA, K. DAKSHAYANI and Y. LINGAPPA*. Dept. of Chemistry, S.V. University, Tirupati.

ABSTRACT

A new analytical reagents namely 2,4-Dihydroxy-benzophenone-2-aminothiophenol, Vanillin-2-amino nicotinic acid, 5-methyl-2-thiophene carboxaldehyde ethylenediamine are proposed as a sensitive and selective analytical reagents for the non-extractive spectrophotometric determination of Arsenic (III), Selenium (IV), Lead (II), Mercury (II) in aqueous medium. The reagents have been synthesized and characterized using IR, NMR, and Mass spectral data. The reagents react with Arsenic (III), Selenium (III), Lead (II), Mercury (II) in acidic medium and basic mediums to form yellow colored complexes 1:1 (M:L) complexes. The color reactions are instantaneous and absorbance values remain constant for 24 hours. The molar absorptivity and sandell’s sensitivity of 2, 4-Dihydroxy-benzophenone-2-aminothiophenol, Vanillin-2-amino nicotinic acid 5-methyl-2-thiophene carboxaldehyde ethylenediamine methods are found to be $1.45 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$ and $0.0040 \mu g \text{ cm}^{-2}$ of AS (III), $1.002 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$, $8.66 \times 10^4 \mu g \text{ cm}^{-2}$, $0.075\mu g\text{mL}^{-1}$ and $0.0250\mu g\text{mL}^{-1}$ of Se (IV), $1.89 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$ and $0.0019 \mu g \text{ cm}^{-2}$ of Pb (II) and $2.5 \times 10^4 \text{ dm}^{-3} \text{ mol}^{-1}\text{cm}^{-1}$ and $0.0084 \mu g \text{ cm}^{-2}$ of Hg (II) respectively. The method is successfully applied to a number of water (ground, polluted) and other environmental samples. The results of the proposed method in the analysis of samples are comparable with those obtained by spectrophotometric method. The method has high accuracy and precision.

Key words: Arsenic, Selenium, Lead, Mercury determination, non-extractive spectrophotometry, environmental samples, and 2,4-Dihydroxy-benzophenone-2-aminothiophenol, Vanillin-2-amino nicotinic acid, 5-methyl-2-thiophene carboxaldehyde ethylenediamine.
SPECTROPHOTOMETRIC DETERMINATION OF ARSENIC, POLLUTANT OF ENVIRONMENTAL IMPORTANCE

K. DEEPA, K.DAKSHAYANI and Y.LINGAPPA*. Dept. of Chemistry, S.V.University, Tirupati.

ABSTRACT

Analytical applications of benzil-5-amino-1,3,4-thiadiazole-2-thiol are reported for the first time. The reagent has been synthesized and characterized using IR, NMR and Mass spectral data. Benzil-5-amino-1,3,4-thiadiazole-2-thiol has been used for the spectrophotometric determination of AS (III), Se (IV), Pb (II) and Hg (II). The reagents react with AS (III), Se (IV), Pb (II) and Hg (II) in both acidic and basic mediums to form yellow colored complexes. The color reactions are instantaneous and absorbance values remain constant for 24 hours. The molar absorptivity and sandell’s sensitivity of benzil-5-amino-1,3,4-thiadiazole-2-thiol methods are found to be 1.45x10^4 L mol^{-1}cm^{-1} and 0.0040 \mu g cm^{-2} of AS (III), 1.002 \times 10^{-4} L mol^{-1}cm^{-1} 8.66x10^{-4} \mu g cm^{-2}, 0.075 \mu gmL^{-1} and 0.0250 \mu gmL^{-1} of Se (IV), 1.89x10^4 L mol^{-1}cm^{-1} and 0.0019 \mu g cm^{-2} of Pb (II) and 2.5x10^4 dm^{-3} mol^{-1}cm^{-1} and 0.0084 \mu g cm^{-2} of Hg (II) respectively. The method is successfully applied to a number of water (ground, polluted) and other environmental samples. The results of the proposed method in the analysis of samples are comparable with those obtained by spectrophotometric method. The method has high accuracy and precision.

Key words: Arsenic, Selenium, Lead, Mercury determination, non-extractive spectrophotometry, environmental water (ground, polluted) and other samples, benzil-5-amino-1,3,4-thiadiazole-2-thiol.
LIST OF CONFERENCES


