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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 5methylthiophene–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 380nm. The reagent reacts with lead in acidic medium to form light yellow colored 1:1. The color reactions are 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⁻¹of Pb (II). The molar absorptivity and sandall's sensitivity of the method were 5.86 x 10⁴ Lmol⁻¹ cm⁻¹ and 0.0017 µg/ cm⁻² respectively. Since MTCED method is more sensitive it was applied for the determination of lead in medicinal leaves and environmental samples.

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

INTRODUCTION

Lead is accumulative poison that enters the body from lead water pipes, lead-based paints and leaded petrol (Renner, R. 1995) [1]. Presence of even traces of Pb (II) in environmental samples leads to environmental pollution and many fatal diseases including dysfunction of renal blood and neurological systems. Pb (II) easily deposits in blood, kidney, reproductive system, nervous system and brain, and acute lead poisoning can result in colic shock, severe anemia and irreversible brain damage. Lead compounds as antiknocking agents in automobile fuels cause air pollution

The determination of trace amounts of lead is very important in the context of environmental monitoring however a large number of spectrometric methods for determination of lead are reported to face interference due to the presence of several metal ions, (Du, B. et. al 2002 [2], Fargussion, J.1990 [3], Ferreira, S. L. C. et.al 1991 [4], Dutta, S. & Das, A. K. 2007 [5] Kiehei, U. et. al 2000 [6], Mondal [7] B. C., Das, A. K. 2002 [5], Dutta, S. & Das, A. K. 2007 [8]. The present study was planned to determine the prevalence of selected Trace elements in medicinal leaves and environmental samples.

Medicinal plants are starting material for any herbal preparation such as herbal medicines, herbal teas, herbal oil etc. These preparations are being used worldwide due to their therapeutic potential and as they are considered to be safe as compared to allopathic medicines. Lead is the most common toxic metal that has become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients[9-14]. Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration [15-16].

Ocimum sanctum, Linn (English: Holy Basil, Sanskrit: tulsi; Family: Labiatae) is a well-known traditional plant used in Ayurvedic system of medicine [17]. Amongst the indigenous herbals of India, several therapeutic properties have been attributed to O. sanctum-a Medhya rasayanas.

The essential oils from Ocimum contain many terpenes (linalool, citral, 1, 8-cineole) and phenylpropanoids (e.g. methyl chavicol, eugenol) produced in specialized glandular trichomes [18]. Presence of eugenol attributes to its anti-oxidative property and is also thought to be responsible for inhibition of lipid per oxidation [19]. This property helps in maintaining good health and in preventing the chances occurrence of heart diseases as well as most of the other biochemical diseases because oxidative stress is the hallmark of such diseases [20]. In addition, available literature data indicates that there is a great deal of diversity in the composition of essential oil of O. sanctum cultivated in different localities [21]. 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 lead in various water samples and medicinal leaves.

MATERIALS AND METHODS

Apparatus

Schimadzu 160A UV-Visible spectrophotometer equipped with 10cm quartz cell and an ELICO model L1-610 pH meter were used in the present study. ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry Model-7000) methods were used for the quantitative analysis of heavy metals in all samples. To determine the heavy metals levels, 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 heavy metals were analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (Varian Vista-MPX CCD Simultaneous Spectrophotometer, Mugrave-Victoria, Australia) [22 & 23].(Mandal & Suzuki 2002).

REAGENT AND SOLUTIONS

Preparation of reagent (MTCED)

5-methylthiophene-2-carboxaldehyde (1ml, 0.0092 mol) in 50ml of methanol, ethylenediamine (0.55g, 0.0092mol) dissolved in 50ml of methanol were taken in 250ml round bottom flask. Suitable quantity (10ml) of 1M 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. Percent of a Yield is 4.32g; m.p.82-83 °C. The structure of MTCED as shown in the (Fig.1).



Fig.1. MTCED - Structure

Characterization of reagent (MTCED)

The reagent has been synthesized and characterized by IR, ¹HNMR and Mass spectral data. Infrared spectrum of MTCED shows bands at 3087, 2915, 2830, 1634, 1476, 1375, 1305, 706 and 593 respectively corresponding to ν (C-H) stretch (sp²---C-H) (thiophene), ν (C-H) stretch (methyl and methylene), ν (C-H) stretch (aldehyde), ν (C = N) schiffbase, δ (-CH₂) symmetrical stretch, δ (-CH₃) symmetrical stretch, ν (C - N) stretch, ν (C - S) stretch and Hetero cyclic ring C - C oop bend . H¹NMR spectrum of MTCED (CDCl₃+DMSO-d₆) 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 C₁₄ H₁₆N₂S₂ (M.Wt, 276).

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 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 (pK₁= pK₂).

MTCED solution (1X10⁻²M)

A 0.01M solution was prepared by dissolving 0.069 gm of MTCED in 100 ml of methanol. The reagent solution is stable for at least 24 h.

Lead (II) solution

A $(1x10^{-2}M)$ stock solution of Lead was prepared by dissolving 0.3312 g of Lead nitrate (PbNO₃) (Merck Darmstadt) in double distilled water containing few drops of Conc. HNO₃ and made up to the mark in a 100ml volumetric flask.

1000 ppm stock solution of Lead was prepared by dissolving 0.1598 gm of Lead nitrate in 100 ml distilled water or 1.5985 gm of Lead nitrate 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 NH_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 in 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 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 10ml con HNO₃ and 5ml of con H_2SO_4 in a fume cupboard and then cooled to room temperature. The sample was digested in the presence of an excess potassium permanganate solution according 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.

Preparation of medicinal leaf samples:

Sample preparation of the leaf samples were carried out by microwave digestion. Approximately 1g (dry mass) of leaf sample materials were weighed directly in to the PTFE vessels, to which 10mlof Conc.HNO₃ was added and the vessels were capped immediately. The digestion programme consisted of a ramp time of 10min tom reach 150° C. The power was 800W. After the completion of program vessels were cooled ,vented and opened and then 2ml of 30% H₂O₂ as added and filtered the solutions in to 25ml volumetric flasks and made up with double distilled water. Blanks were prepared by following similar digestion procedure without plant material.

The reactions of some impact metal ions are tested with (MTCED) at different pH values. The samples are prepared in 10.0 ml of the solution containing constant volume of 2.0 ml of buffer solution (pH 3.0), 1.0 ml of 1×10^{-2} M MTCED and 2.0 ml lithium chloride solution and different metal ions. Each solution is then shaken with 10.0 ml portions of methanol for two minutes and the allowed to stand, each time, the combined organic phase of aliquot are

taken in 10.0 ml standard flasks and made up to the mark with methanol. The absorbance is measured in 200-800 nm range against reagent blank.

Recommended procedure

An aliquot of the metal solution was taken in 25ml standard flask containing 10ml of buffer solution of pH-3.0 and 1ml of MTCED reagent solution and made up to the mark with distilled water. The absorbance of the complex was measured against the reagent blank at 380 nm.

General procedure:

A known aliquot of the sample solution was taken in a 25-ml standard flask containing constant volume of 10ml of buffer solution(pH 3), 1.0 ml of 1×10^{-4} M MTCED and 1ml of Pb (II) 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 lead.

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

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

RESULTS AND DISCUSSION

Lead reacts with 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) in sodium acetate-hydrochloric acid buffer solution of pH 3.0 and gives 1:1 light Yellow colored complex. The complex has a maximum absorbance at 380 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 Lead (II) at microgram levels.



Fig. 2. Absorption spectra of (b). Pb (II) –MTCED complex (λ max=380 nm) in aqueous solution, (a). MTCED Vs Water blank (1x10⁻⁴ M)

Absorption spectra of the reagent and Pb (II)-MTCED complex:

Absorption spectra of Pb (II)-MTCED complex and reagent show maximum absorbance at 380 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 380 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 2.0-4.0, the complex has maximum absorbance in buffer solution of (pH = 3.0). The analytical studies were therefore, carried out at (pH 3.0).

Different molar excess of MTCED were added to fixed metal ion concentration and the absorbances are 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 Pb (II) complex of MTCED are summarized in Table -1.

Table- 1. Physico - chemical and analytical characteristics of Pb (II) - MTCED complex

S.No.	Characteristic Property	Results
1	Absorbance Maximum λ_{max} (nm)	380
2	pH – range (optimum)	2.0 - 14.0
3	Mole of reagent required mole of metal ion for full color developed	10 Fold
4	Beer's law validity range (µg/ml)	0.27-2.4
5	Molar absorptivity (L mol ⁻¹ cm ⁻¹)	5.86 x 10 ⁴
6	Specific absorptivity (ml g ⁻¹ cm ⁻¹)	0.1212
7	Sandell's sensitivity (µg/cm ⁻²)	0.0017
8	Composition of complex as obtained in Job's and molar ratio methods (M:L)	1:1
9	Stability constant of the complex	1.23×10^{6}
10	Relative standard deviation (RSD)%	1.46360
11	Y-intercept	+0.00135
12	Angular coefficient (m)	0.32862
13	Correlation coefficient(v)	0.9988



Fig.3. Calibration plot for Pb (II) determination

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Adherence of the Pb (II) – MTCED Complex System to Beers law:

For the possible determination of Pb (II) at micro level, the absorbance of the solution containing different amounts of the metal iron is measured at 380 nm. The linear plot between the absorbance and the amount of Pb (II) is drawn and the straight line obtained the equation $A_{380} = 0.32862C+0.00135$ (Fig.3). Further Beers law is obeyed in the range of 0.27-2.4 µg/ml. the molar absorptivity and sandell's sensitivity are found to be 5.86 x 10⁴ L.mol⁻¹cm⁻¹ and 0.0017 µg/cm² respectively the standard deviation of the method for ten determinations of 1.68 µg/ml is ± 0.01316.

To assess the precision and accuracy of the method, estimations are carried out for a set of five determinations of Pb (II), under optimum conditions. The results showed that standard deviation of the method was not more than 0.01316 and relative standard deviation was less than 1.4636. 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 Pb (II) was studied by measuring the absorbance of Lead. The complex contains 1.68 μ g/ml of Lead (II) in solution. The colour reaction is developed as described in the standard procedure. An error of $\pm 2\%$ in the absorbance reading was considered tolerable. The tolerance limit (TL) values in ppm for various anions and cations in the MTCED methods respectively are presented in Table.2. Higher amounts of Fe²⁺ do not interfere in the presence of 70ppm of fluoride. Larger amounts of Hg²⁺ do not interfere in the presence of 600ppm of iodide.

The present method (MTCED) was applied for the determination of Lead when present alone and present in water samples and medicinal leaves.

The present ligands containing aromatic ring are found to be potential and cost effective for the determination of Lead (II) 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 Lead (II) in aqueous medium.

Effect of foreign ions on the extraction of the Pb (II) –MTCED complex: The effect of foreign iron is studied by measuring the absorbance of the reaction mixture containing 1.68 μ g/ml of Pb (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.

Ion Added	Tolerance limit µg/ml	Ion Added	Tolerance limit µg/ml
Tartrate	594	W(v)	365
Iodate	509	Mn (II)	2.3
Urea	288	Pb (II)	8.3
Citrate	386	Cr (VI)	1.2
Bicarbonate	245	TI (III)	0.41
Thiocyanate	234	Cd (II)	0.23
Sulphate	386	Hg(II)	0.40
Oxalate	351	Ni (II)	0.23
Thiourea	303	Fe (II)	0.21
Nitrate	249	Au (III)	0.42
Acetate	236	Pt (IV)	0.38
Phosphate	38	Pd(II)	0.24
Bromide	34	Ag (I)	0.21
Chloride	15	V (V)	0.11
Fluoride	7.9	Cu (II)	0.13

Table-2. Effect of foreign ions

Composition and stability constant of the complex:

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



Fig.4. Job's method of continuous variation method Pb (II) - MTCED. Pb (II) and MTCED 1x10⁴M: solvent-Methanol; pH-3.0

Application

The proposed extractive spectrophotometric method is applied for the determination of Pb (II) in medicinal leaves and 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 are checked with parallel determinations by direct ICP-OES. The data obtained in the analyses of medicinal leaves and water samples are given in Table 3,4 and 5.

	Pb(II) found µg/ml							
Sample Name	ICP-OES	S.D	R.S.D(%)	Proposed Method ^a	S.D	R.S.D (%)		
Ground water ^b	0.915	0.00016	0.01748	0.921	0.00018	0.01954		
Ground water ^c	0.832	0.00009	0.01081	0.828	0.00011	0.01328		
Industrial waste water ^d	1.348	0.00011	0.00815	1.342	0.00012	0.00894		
Ground water ^e	0.38	0.00114	0.29889	0.39	0.00017	0.04343		
Ground water (upper) ^f	0.11	0.00122	1.08928	0.14	0.00114	0.80508		
Ground water (lower) ^g	0.106	0.00011	0.10361	0.103	0.00017	0.16482		

Table - 3. Determination of trace amount of Pb (II) in water samples

a. Average of the five determination; b. Collected at Pollur (Palamaneru-chittoor), A.P, India.; c. Collected at Ranipet, A.P, India. d. Collected at (Karakam baadi), 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	of Se	(IV)	in soil	sample
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			Pb(II) fo	und µg/g			
Sample Name	ICP-OES	S.D	R.S.D (%)	Proposed Method ^a	S.D	R.S.D (%)	
Polluted soil ^b	1.36	0.00114	0.0807	1.32	0.00114	0.0862	

a. Average of the five determination

b. Collected at Pollur (Palamaneru-chittoor), A.P, India.

	Pb(II) found µg/g							
Sample Name	ICP-OES	S.D	R.S.D (%)	Proposed Method ^a	S.D	R.S.D (%)		
Azadirachta indicab	2.777	0.00013	0.00468	2.681	0.00011	0.00410		
Dathura ^b	6.649	0.00011	0.00165	6.580	0.00008	0.00121		
Ocimum sanctum b	2.501	0.00011	0.00439	2.520	0.00008	0.00317		
Grass ^b	1.537	0.0001	0.00650	1.541	0.00013	0.00843		

a. Average of the five determination b. Collected at Karakam baadi. A.P. India.

CONCLUSION

The proposed spectrophotometric method is simple, highly sensitive and selective for the determination of Pb (II) in Water samples and medicinal leaves. The proposed method is simple, rapid and common metal ions such as Fe^{3+} , Pb^{2+} , Co^{2+} , Ni^{2+} , Mn^{2+} , Zn^{2+} , Cr^{3+} do not interfere. 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 lead in various medicinal and environmental samples.

In this paper, a new, simple, sensitive, selective, and inexpensive method with the Pb (II)–MTCED complex was developed for the determination of lead in industrial, environmental, biological, and soil samples, for continuous monitoring to establish the trace levels of lead 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 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 lead in real samples down to ng g-1 levels in aqueous medium at room temperature ($25\pm5^{\circ}C$).

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