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SPECTROPHOTOMETRIC DETERMINATION OF SELENIUM IN INDUSTRIAL AND ENVIRONMENTAL SAMPLES USING 5-METHYLTHIOPHENE–2-CARBOXALDEHYDE ETHYLENEDIAMINE (MTCED)

K. Deepa*, T. Vasantha, Cheera Prasad, N. V. V. Jyothi and Y. Lingappa

Department of Chemistry, S.V. University, Tirupati-517501, Andhra Pradesh, India.

***Corresponding Author: K. Deepa**
Department of Chemistry, S.V. University, Tirupati-517501, Andhra Pradesh, India.

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ABSTRACT
A simple, rapid and sensitive spectrophotometric method was developed for the determination of Se (IV) using 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) 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 MTCED at (pH= 4.0) (sodium acetate- acetic acid buffer solution) showing maximum absorbance at 390 nm. Hence, analytical studies were further carried out at 390 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 MTCED was studied by the method of job's continuous variation and molar ratio method. Beer's law was obeyed in the range 0.007-3.47 μg $ml^{-1}$of Se (IV). The molar absorptivity and Sandell's sensitivity of the method were found to be 6.67 $x10^4$ $Lmol^{-1}cm^{-1}$ and 0.0015 μg/cm^{-2} respectively. Since MTCED method is more sensitive, it was applied for the determination of selenium in environmental samples.

KEYWORDS: Selenium determination, Non-extractive spectrophotometry, 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED), 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 μgml^{-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 seleniferous 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 compound are widely used in paints, dyes, glass electrical, rubber, insecticides and many other industries. Some industrial and agricultural processes release selenium as aby-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 inoraganic compounds. In many environmental matrixes, 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 sample.[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 anew reagent MTCED.

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 selenium in various water and soil samples.

MATERIALS AND METHODS

Experimental

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 $K_2Cr_2O_7$ followed by washing with con. 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 (MTCED)

5-methylthiophene-2-carboxaldehyde (1 ml, 0.0092 mol) dissolved in 50 ml of methanol, ethylenediamine (0.55 g, 0.0092 mol) dissolved in 50 ml of methanol were taken in 250 ml round bottom flask. Suitable quantity (10 ml) 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 and 50 percent methanol and n-hexane. This compound was recrystallised from methanol and dried in vacuum. Percent of a Yield is 93; m.p.82-83^{0}C. The structure of VANA is shown in the Fig.1.

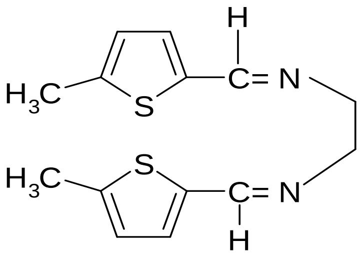

Fig. 1: MTCED – Structure.

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 (s), 2915 (m), 2830 (s), 1634 (s), 1476 (m), 1375 (m), 1305 (m), 706 (δ) and 593 respectively corresponding to ν (C-H) symmetric stretch (sp^2---C-H), ν (C-H) stretch (methyl and methylene) , ν (C-H) stretch (aldehyde), ν (C = N) stretch (Schiff base), δ (-CH_2) symmetrical stretch, δ (-CH_3) symmetrical stretch, υ (C- N) stretch, υ (C- S) stretch and Hetero cyclic ring C - C oop bend. H^{1}NMR spectrum of MTCED ($CDCl_3$+DMSO-d_6) showed signals at 6.74-7.16 (3H), 2.45 (2H), 3.30 (2H) due to thiophene, methyl and 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 $C_{14}H_{16}N_2S_2$ (M.Wt, 276).

pKa values of reagents

The pKa values were determined by recording the UV-Visible spectra of 1X10^{-3}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.

Se (IV) solution

A ($1X10^{-2}M$) stock solution of selenium was prepared by dissolving 0.1890 g of sodium selenate (Na_2Seo4) (Merck Darmstadt) in double distilled water containing few drops of conc. H_2SO_4 and made up to the mark in a100 ml volumetric flask. Aliquots of this solution were standardized with EDTA titration using xylenol 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 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 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 HNO_3 and 5 ml 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 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 NH_4OH. 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= 4.0) 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 390 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= 4), 1.0 ml of $1x10^{-3}M$ MTCED 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 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) in sodium acetate-hydrochloric acid buffer solution of pH 4.0 and gives 1:1 light greenish yellow colored complex. The complex has a maximum absorbance at 390 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)-MTCED complex

Absorption spectra of Se (IV)-MTCED complex and reagent show maximum absorbance at 390 nm and 360 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 390 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 3.0-5.0, the complex has maximum absorbance in buffer solution of (pH = 4.0). The analytical studies were therefore, carried out at (pH= 4.0).

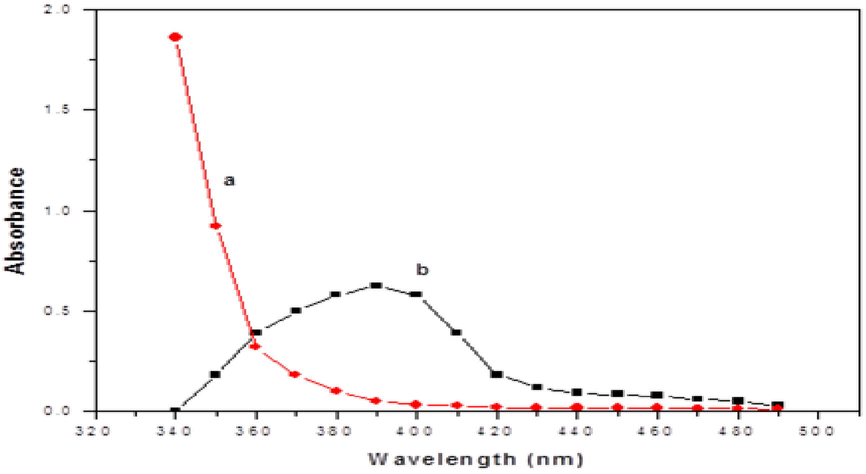

Fig. 2: Absorption spectra of (a). Se (IV) – MTCED complex (λmax =390 nm) in aqueous solution, (b). MTCED Vs Water blank ($1x10^{-3}$ M).

Different volumes of molar excess of MTCED were 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 MTCED were summarized in Table 1.

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

S. No.	Characteristics	Results
1	λ max (nm)	390
2	pH range (optimum)	3.0 – 5.0
3	Mean absorbance	0.2316±0.001
4	Mole of reagent required per mole of metal ion for full color developed	10 Fold
5	Time stability of the complex (in Hrs)	24
6	Beer's law validity range (μg/ml)	0.007-3.47
7	Molar absorptivity (L mol-1 cm-1)	6.67×10^4
8	Specific absorptivity (ml g-1 cm-1)	0.1840
9	Sandell's sensitivity (μg/ml)	0.0015
10	Composition of complex as obtained in Jobs and molar ratio methods (M:L)	1:1
11	Stability constant of the complex	1.02×10^4
12	Standard deviation	0.0011
13	Relative standard deviation (RSD)%	1.1864
14	Y-intercept	-0.02278
15	Angular coefficient (m)	0.23009
16	Correlation coefficient (v)	0.9991
17	Detection limit (μg ml-1)	0.07
18	Determination limit (μg ml-1)	0.21

Adherence of the Se (IV) – MTCED 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 390 nm. The linear plot between the absorbance and the amount of Se (IV) is drawn and the straight line obtained with the equation A_{390} = 0.23009-0.02278 (Fig.3). Further Beers law is obeyed in the range of 0.007-3.47μg/ml, the molar absorptivity and sandell's sensitivity were found to be 6.67×10^4 $L.mol^{-1}cm^{-1}$ and 0.0015μg/cm^2 respectively. The standard deviation of the method for ten determinations of 2.12 μg/ml is ± 0.0011. The results showed that standard deviation of the method was not more than 0.0011 and relative standard deviation was less than 1.1864.

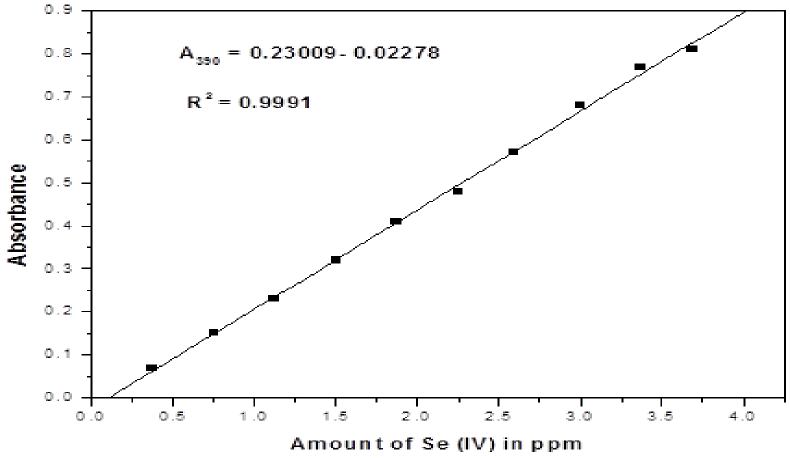

Fig. 6: Calibration plot for Se (IV) determination.

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 MTCED methods respectively were presented in Table 2. Higher amounts of Fe^{2+} 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 5-methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) 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.

Effect of foreign ions on the extraction of the Se (IV) – MTCED 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.

Table 2: Effect of foreign ions.

Ion Added	**Tolerance limit µg/ml**	**Ion Added**	**Tolerance limit µg/ml**
Citrate	384	W(v)	368
Tartarate	296	Mn (II)	22
Urea	288	Pb (II)	0.82
Iodate	254	Cr (VI)	1.0
Bicarbonate	244	Zn (II)	0.13
Thiocyanate	232	Cd (II)	0.22
Sulphate	192	Hg(II)	0.40
Oxalate	176	Ni (II)	0.23
Thiourea	152	Fe (II)	0.22
Nitrate	124	Au (III)	0.40
Acetate	118	Pt (IV)	0.39
Phosphate	20	Tl (III)	0.25
Bromide	16	Ag (I)	0.22
Chloride	7.1	V (V)	0.20
Fluoride	4.0	Cu (II)	1.2

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 Se (IV) as shown in the (Fig.4).

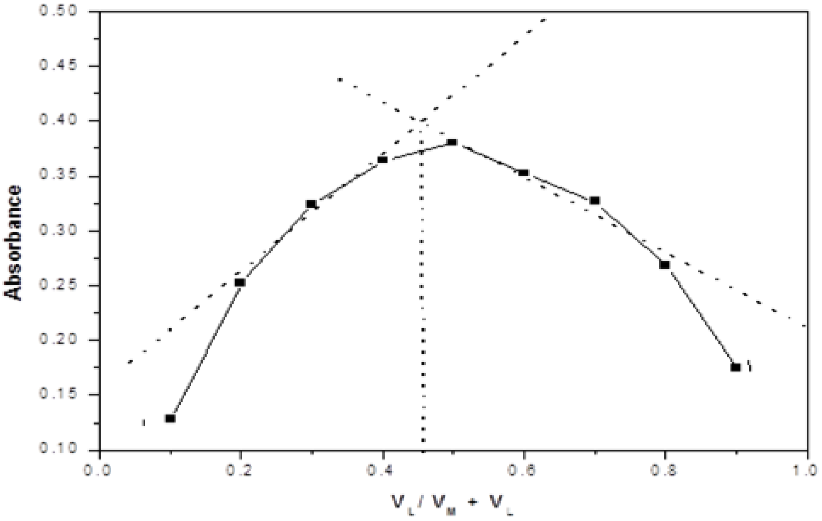

Fig. 4: Job's method of continuous variation method Se (IV)-MTCED. Se (IV) and MTCED, 1X10^{-3} M: solvent-Methanol; (pH = 4.0).

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.

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

Sample Name	Se (IV) found µg/ml					
	ICP-OES	S.D	R.S.D (%)	Proposed Method[a]	S.D	R.S.D (%)
Ground water[b]	1.704	0.000114	0.0066	1.698	0.0001	0.00583
Ground water[c]	1.09	0.00083	0.0760	1.06	0.00011	0.00908
Industrial waste water[d]	0.613	0.000158	0.25	0.64	0.00114	0.16730
Ground water[e]	0.218	0.000114	0.40482	0.212	0.00114	0.45310
Ground water (upper)[f]	0.124	0.000114	0.86626	0.132	0.00114	0.70544
Ground water (lower)[g]	0.24	0.00011	0.51935	0.178	0.00011	0.05551

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 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.

Sample Name	**Se (IV) found µg/mg**					
	ICP-OES	**S.D**	**R.S.D (%)**	**Proposed Method[a]**	**S.D**	**R.S.D (%)**
Polluted soil[b]	1.286	0.00015	0.0116	1.265	0.00015	0.0118

a. Average of the five determination

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

CONCLUSION

The author has introduced a new sensitive reagent MTCED 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)–MTCED 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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