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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, ^1H 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 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 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 compound 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 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 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, anew 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 $K_2Cr_2O_7$ 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 (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_2SO_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 ^{0}C. The structure of VANA is shown in the Fig.1.

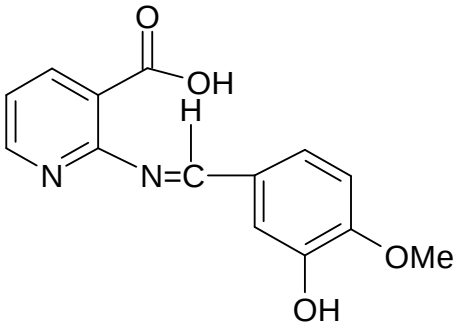

Fig.1. VANA – Structure

Characterization of reagent (VANA)

The reagent has been synthesized and characterized by IR, ^{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 ν (O-H) symmetric stretch, ν (C-H) stretch (pyridine) (sp^2---C-H) , ν (-OCH_3) stretch, ν (C= O) Aromatic stretch , ν (C = N) schiffbase, ν (C =C) Aromatic stretch , ν (HC = N) azomethine stretch , ν (C - C) Aromatic stretch , ν (C - C) stretch (pyridine ring), δ (C - H) (Aromatic ring) , ν (C = N) stretch (pyridine) , ν (C - O) stretch, ν (C - N) stretch and δ (O-H)oop bend(carboxylic acid) . H^{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 $C_{14}H_{12}N_2O_4$ (M.Wt, 273).

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 VANA were 7.0 (pK_1) and 10 (pK_2).

VANA solution

A $1X10^{-2}$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 ($1X10^{-2}$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 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_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^{o}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 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 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=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.

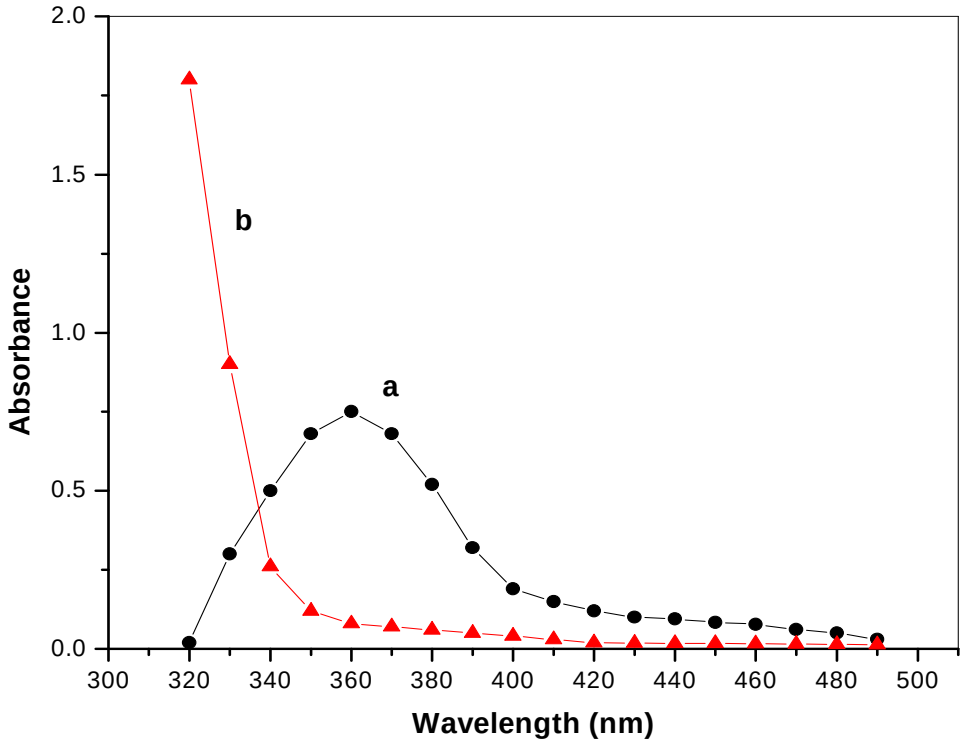

Fig.2. Absorption spectra of (a). Se (IV) – VANA complex (λmax=360 nm) in aqueous solution, (b). VANA Vs Water blank ($1x10^{-3}$ M)

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

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

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/cm^2 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.

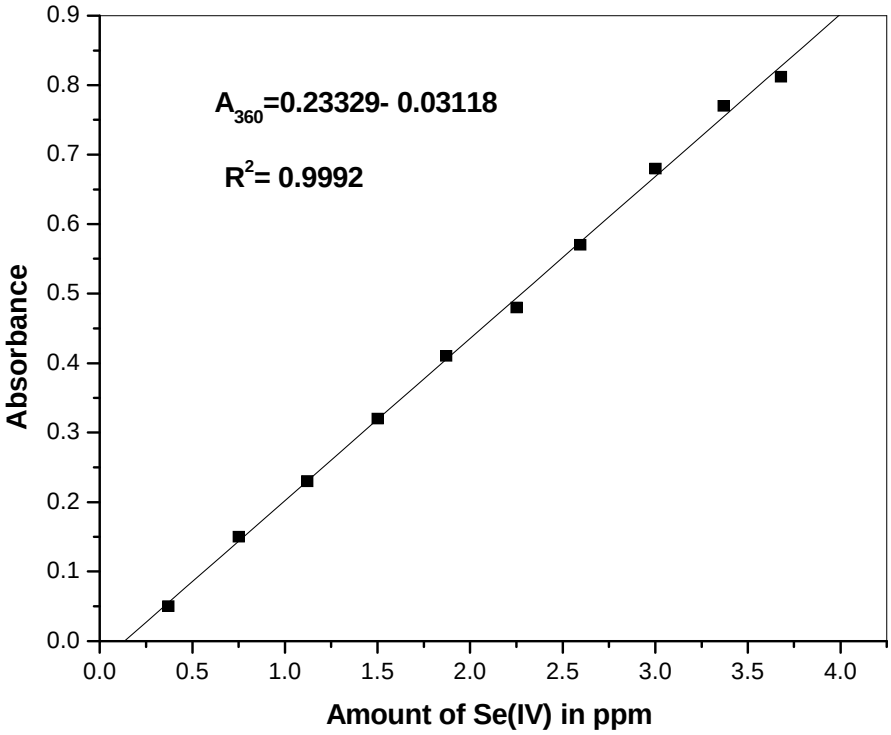

Fig.6. Calibration plot for Se (IV) determination

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 VANA 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 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

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

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-ration 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).

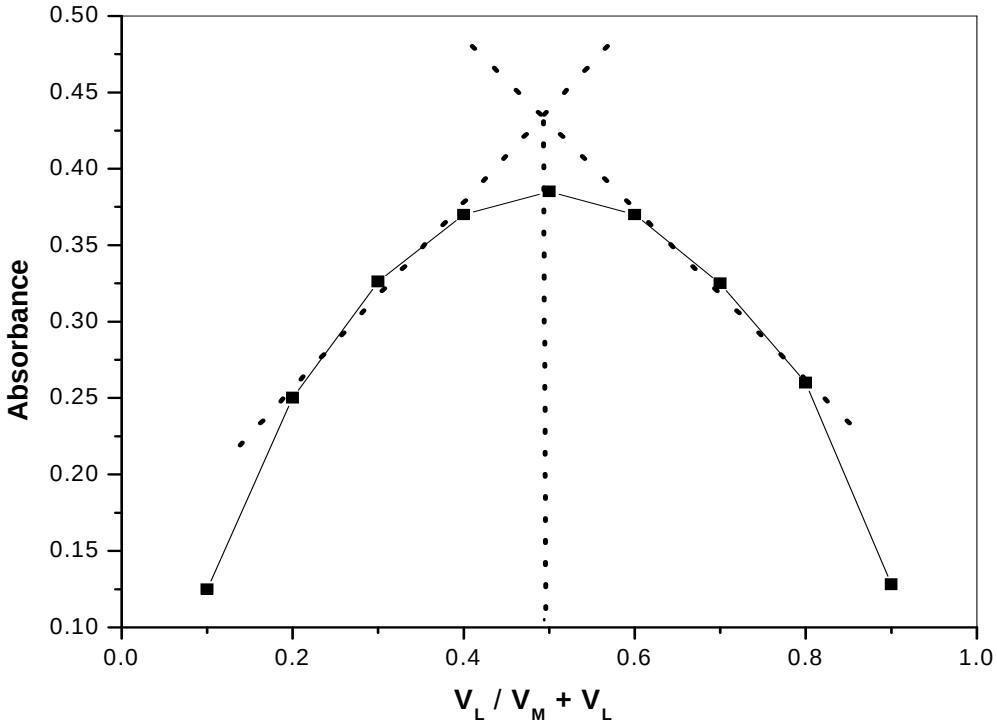

Fig.4. Job's method of continuous variation method Se (IV)-VANA. Se (IV) and VANA 1X10^{-3}M: solvent-Methanol; (pH=5.0)

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]	1.722	0.00013	0.07548	1.713	0.0001	0.00583
Ground water[c]	1.103	0.00011	0.00997	1.210	0.00011	0.00908
Industrial waste water[d]	0.63	0.00158	0.25	0.68	0.00114	0.16730
Ground water[e]	0.28	0.00114	0.40482	0.25	0.00114	0.45310
Ground water (upper)[f]	0.13	0.00114	0.86626	0.16	0.00114	0.70544
Ground water (lower)[g]	0.21	0.0011	0.51935	0.198	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

	Se (IV) found µg/mg					
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.

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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