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SIMPLE SPECTROPHOTOMETRIC DETERMINATION OF TRACES OF SELENIUM IN ENVIRONMENTAL SAMPLES

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Abstract

A rapid, highly sensitive and selective spectrophotometric method for the determination of traces of selenium(IV) are studied. The methods are based on either the oxidation of 2,4-dinitrophenyl hydrazine hydrochloride (2,4-DNPH) by selenium in hydrochloric acid medium and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride(NEDA) to give pink colored product or the oxidation of 4-aminoresorcinol hydrochloride (4-ARCH) by selenium in sulphuric acid medium and coupling with 4-aminoresorcinol hydrochlorid to yield a orange red colored product. The pink colored product with I_{max} 520 nm is stable for 24 h and the orange red colored product with I_{max} 495 nm for one week. Beer's law is obeyed for selenium in the concentration range 0.03 - 3.5 $\mu\text{g ml}^{-1}$ (pink color product) and 0.07-2.5 $\mu\text{g ml}^{-1}$ (orange red color product), respectively. The optimum reaction conditions and other important analytical parameters are reported. Interference due to various cations and anions have been investigated. The proposed method has been applied to the analysis of polluted water, natural water, plant material, soil samples and synthetic mixture. The results of analysis are compared with the reported method.

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 $\mu\text{g ml}^{-1}$. 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 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, electrothermal atomic absorption spectrometry, hydride generation, polarography, cathode-stripping voltammetry, radiochemical neutron activation analysis, and flow-injection techniques, which are reviewed^[1]. There are many reagents available for the spectrophotometric determination of selenium. The recent spectrophotometric methods for the determination of selenium used J-acid^[2], Leuco crystal violet^[3], resazurin^[4], sodium salt of hexamethyleneimine carbodithioate^[5], 1-

naphthylamine-7-sulphonic acid^[6]. The most recent spectro-photometric method for the determination of selenium used the reagent variamine blue^[7], oxidative coupling with has had several disadvantages.

In the present study, we are reporting rapid, simple, sensitive and selective methods for the determination of traces of selenium(IV). The reactions are oxidative coupling using 2,4-DNPH-NEDA and 4-ARHC-2,4-DNPH and the products are highly stable. The methods have been successfully employed for the determination of selenium in environmental samples and also synthetic mixtures.

EXPERIMENTAL

Apparatus

A HITACHI U 2001 Spectrophotometer with 1.0 cm pathlength was used for electronic spectral measurements.

Reagents and Chemicals

A stock solution containing 100 mg l⁻¹ of selenium dioxide (E-Merk, India) in one liter of double distilled water. Working solutions of selenium (IV) were prepared by further dilution of the stock solution. A 1.5% 2,4-DNPH-NEDA reagent mixture was prepared by dissolving 1 g of NEDA and 0.5 g of 2,4-DNPH (both from Sigma, USA) adding a few drops of concentration HCl in water which made up to 100 ml and was refrigerated. Solutions of 4-aminoresorcinol hydrochloride (SD fine) and 2,4-DNPH were prepared by dissolving 0.5 g of each in 100 ml water. Analar grade hydrochloric acid and sulphuric acid were used for the experiments. All other chemicals and solvents were used of analytical reagents grade. Deionized water was used to prepare all solutions in the experiments.

Procedure

2,4-DNPH – NEDA Method

Stock solution containing 1 to 100 µg (0.03 – 3.5 ml⁻¹) of selenium (the volume of the test solution was restricted to 1 ml) were transferred into 25 ml calibrated flasks, to which 5 ml of concentrated HCl and 2 ml of 2,4-DNPH – NEDA reagent mixture were added. The mixture was allowed to stand for 10 min with occasional shaking for the completion of the reaction. The contents were diluted to the mark with water and the absorbance was measured at 520 nm against the corresponding reagent blank and the calibration graph was constructed.

4-ARHC – 2,4-DNPH Method

Aliquots of stock solutions containing 2 – 60 µg (0.07 – 2.5 µg ml⁻¹) of selenium were transferred into a series of 25 ml standard flasks, to which 4 ml of concentrate H₂SO₄, 2 ml of 4-aminoresorcinol hydrochloride and 4 ml of 2,4-DNPH (0.5%) reagents were added. The mixture was allowed to stand for 10 min with occasional shaking for the completion of reaction. The contents were diluted to the mark with water and absorbance was measured at 495 nm against the corresponding reagent blank and the calibration graph was constructed.

Determination of Selenium in Environmental samples

Determination of Selenium in Polluted water

The distillation procedure for the determination of selenium in polluted water was followed. To an aliquot (100 ml) of known sample contained in a distillation flask, 1 g of KBr and 10 ml of concentrated H₂SO₄ treated with 0.5 ml of saturated bromine water were added and the solution was distilled under vacuum till copious white fumes of SO₃ vapours were evolved, and this process converts all other forms of selenium into selenium (IV). The distillate was collected in 10 ml of 5% hydroxyl ammonium chloride solution, then made up to a fixed volume and analyzed by the standard procedure. For the natural water, any one of the standard procedure is followed.

Determination of Selenium in Plant Material

5 g of finely chopped fresh cabbage sample was placed in a 250 ml beaker and 10 ml of a 1:1 (v/v) mixture of concentrated sulphuric acid and nitric acid were added. This solution was heated, until the mixture was clear. This solution was filtered and concentrated to 5 ml, cooled and diluted up to 50 ml with deionized water. Then the standard procedure was applied to 1 ml of this solution.

Determination of Selenium in Soil Sample

A known weight of selenium was mixed with 20 g of soil sample, fused with 1:1 sodium carbonate and potassium nitrate mixture in a nickel crucible and extracted with water. The filtrate of the extract was treated with 20 ml of 10 mol l⁻¹ hydrochloric acid and then heated to expel chlorine and oxides of nitrogen. The solution was further diluted with water to give a suitable concentration of selenium. An aliquot of the stock solution was passed through the cation exchange resin to remove the iron present in soil. The selenium contents was determined from the eluant following the standard procedure.

Determination of Selenium in Synthetic mixture

Synthetic mixture corresponding to the alloys of Se-Fe (52% Fe), Se-Hg (71% Hg), and Se-Pb (75% Pb) were prepared and selenium content was determined following the standard procedure.

RESULTS AND DISCUSSION

Absorption Spectra

The proposed method involves the formation of the pink colored product with a λ_{\max} of 520 nm and an orange red colored product with λ_{\max} 495 nm. The reagent blanks were negligible absorption at these wave lengths. The absorption spectra of the products are shown in Figure :1

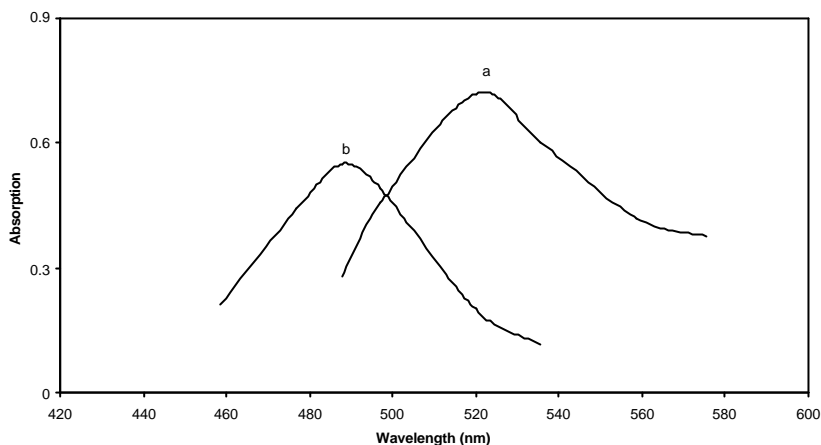


Fig.1 : Absorption Spectra of the (Se⁴⁺ + 2,4-DNPH + NEDA) System^a
Se⁴⁺ + ARHC + 2,4-DNPH System^b

Time, Temperature and Color Stability:

Under the optimized conditions, although the color developed instantaneously, 5 min were allowed to obtain the maximum and constant absorbance in both the methods. The pink colored product was stable for 24 h and the orange red colored product for one week. The absorbance varied by not more than 2% over a period of two days for both the methods and color development was independent of temperature in the range of 20 °C – 45 °C .

Effect of reagent and acid concentrations

The effect of NEDA + 2,4-DNPH reagent mixture was studied in the range of 1-8 ml . A range of 2-4 ml of 1.5 % NEDA-2,4-DNPH reagent mixture was necessary to achieve the maximum color intensity. Hence 3 ml of the reagent mixture was selected for further studies, as it gave excellent results. The maximum intensity of the pink color was achieved in the range of 3-7 ml of concentrated hydrochloric acid. Therefore, 5 ml of concentrated hydrochloric acid was used.

The maximum intensity of the orange red color was achieved in the range of 3-5 ml concentrated sulphuric acid. Therefore, 4 ml of concentrated sulphuric acid was used for the better results. A range of 2-4 ml of 0.5 % 4-ARCH and 3-5 ml of 0.5% 2,4-DNPH reagents were necessary to achieve the maximum color intensity. Hence, 2 ml of 4-aminoresorcinol hydrochloride and 4 ml of 2,4-DNPH were employed for the experiments.

Optical parameters

Beer's law range, molar absorptivity, Sandell's sensitivity and other parameters are given in Table-1. The precision and accuracy of the method was studied by analysing the solution containing known amounts cited reagents within the Beer's law limit. The lower values of relative standard deviation (%) and percentages of error indicated the high accuracy of the methods.

Table 1: Optical characteristics and precision data

Parameters/Characteristics	2,4-DNPH-NEDA	4-ARHC – 2,4-DNPH
Color	Pink	Orange red
λ_{max} (nm)	520	495
Beer's law range ($\mu\text{g ml}^{-1}$)	0.03 – 3.5	0.07 – 2.5
Molar absorptivity ($1 \text{ mol}^{-1} \text{ cm}^{-1}$)	3.10×10^4	2.85×10^4
Sandell's sensitivity ($\mu\text{g cm}^{-2}$)	0.00259	0.00268
Regression equation (Y) ⁺		
Slope (a)	0.18734	0.34865
Intercept (b)	0.0148	-0.0124
Correlation coefficient (r)	0.99971	0.99975
Relative standard deviation (%) ⁺	0.8957	0.3035
Range of error (95 % confidence level)	± 1.2425	± 0.4213
Detection limit ($\mu\text{g ml}^{-1}$)	0.0165	0.0335
% error	0.1427	0.0745

* $Y = ax + b$, where x is the concentration of selenium in $\mu\text{g ml}^{-1}$

⁺ n=5

Reaction mechanism

Under the reaction condition, 2,4-DNPH loses $2 e^-$ and a proton on oxidation with Se (IV) in acidic medium forming the electrophilic intermediate (active coupling species) , which couples with NEDA to give a pink colored product as shown in Scheme -1.

Similarly, 4-ARHC get oxidized first to form ketone with selenium (IV) , which is mild oxidizing in concentrated sulphuric acid medium. Then the keto group of 4-ARHC condensed with 2,4-DNPH forming orange red color product and the tentative mechanism is shown in Scheme -2. Since, phenolic groups are prone to oxidation 4-ARHC gets oxidized first and not 2,4-DNPH.

Interference of Foreign Ions

The effect of various species on the determination of selenium (IV) was investigated. The tolerance limit was taken as the amount that caused $\pm 2\%$ absorbance error in determination of $2 \mu\text{g ml}^{-1}$ (2,4-DNPH + NEDA method) and $1.2 \mu\text{g ml}^{-1}$ (4-ARHC+ 2,4-DNPH method) of selenium. The results are given Table 2 and 3.

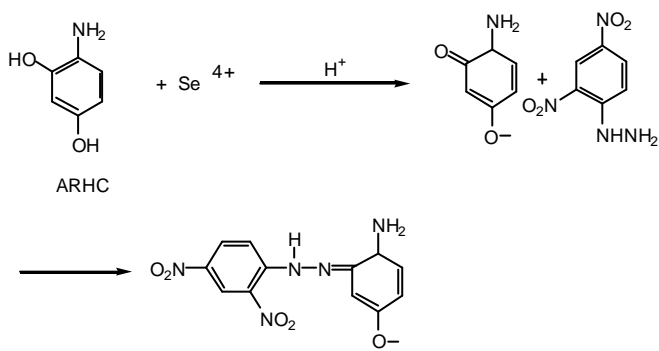
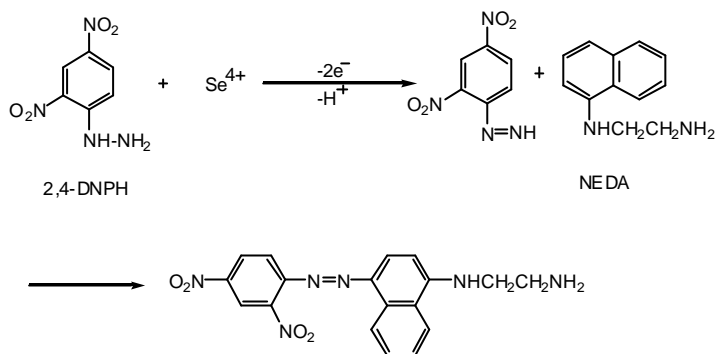


Table -2 Effect of foreign species on the determination of $2 \mu\text{g ml}^{-1}$ selenium in 2,4-DNPH – NEDA method.

Species	Tolerance limit ($\mu\text{g ml}^{-1}$)
EDTA	10500
Na^+ , Mg^{2+} , Cl^- , NO_3^- , F^- , CHCOO^- , CO_3^{2-} , K^+	2080
Ba^{2+} , SO_4^{2-} , CN^- , SCN^- , Tartarate	1090
PO_4^{3-} , Al^{3+} , Cd^{2+} , NO_2^-	810
Cu^{2+} , Ni^{2+} , Co^{2+} , Ca^{2+}	86 ^b
Zn^{2+} , Pb^{2+} , SO_3^{2-} , NO_3^- , Cr^{3+} , As^{3+}	55
Fe^{2+} , S^{2-}	40

^a Can be masked upto $810 \mu\text{g ml}^{-1}$ by the addition of 3 ml of 2 % sulphamic acid.

^b Can be masked upto $86 \mu\text{g ml}^{-1}$ by the addition of 3 ml of 5% EDTA.

Table 3 Effect of foreign species on the determination of $1.2 \mu\text{g ml}^{-1}$ selenium in the 4-ARHC – 2,4-DNPH.

Species	Tolerance limit ($\mu\text{g ml}^{-1}$)
EDTA	4070
Na^+ , Mg^{2+} , Tartarate, Oxalate, Mn^{2+}	1030
F^- , PO_4^{3-} , Hg^{2+} , SO_4^{2-} , CO_3^{2-} , K^+ , CH_3COO^- , S^{2-} , Al^{3+} , Fe^{2+} , SO_3^{2-} , Cd^{2+} , CN^- , SCN^- , $^a\text{Fe}^{3+}$	100
$^a\text{NO}_2^-$, $^b\text{Cu}^{2+}$, Zn^{2+} , Ca^{2+} , Cl^-	86
Pb^{2+} , Co^{2+} , Te^{2+} , Ni^{2+} , $^c\text{I}^-$, NO_3^-	29

^a Can be masked upto $100\mu\text{g ml}^{-1}$ by the addition of 2 ml of orthophosphoric acid.

^b Can be masked upto $86\mu\text{g ml}^{-1}$ by the addition of 2 ml of 5% EDTA.

^c Can be masked upto $29\mu\text{g ml}^{-1}$ by the addition of 1 ml of 1% HgCl_2 .

Applications

The proposed methods are applied to the determination of selenium (IV) in natural water, polluted water, plant material, soil sample and synthetic mixture. The results of analysis in compared with the reported method^[21] are give in Table 4. Recovery experiments were performed using the method of addition. These results are shown in Table-5.

Conclusions

The proposed methods for the spectrophotometric determination of selenium in various environmental samples are simple, rapid and sensitive. The coupled products formed are fairly soluble, very cheap and readily available in market. The oxidative coupled products formed are quite stable when compare to reported in the literature. The methods do not require heating or extraction and time interval for formation of coupled products and do not interfere with various cations and anions. Statistical analysis of the results indicates that the methods have good precision and accuracy.

Table 4 Determination selenium in environmental samples

Sample	Selenium added (μg)	Proposed method		Reported method[2]	
		Selenium Found [†] (μg)	Mean Recovery(%)	Selenium found(μg)	Mean Recovery(%)
Polluted water*	-	0.28	-	0.24	-
	-	0.37	-	0.37	-
	-	0.42	-	0.40	-
	-	0.51	-	0.50	-
Natural water*	0.4	0.39	97.5	0.38	95.0
	1.0	0.98	98.0	0.98	98.0
	1.6	1.59	99.3	1.58	98.8
Fresh cabbage*	-	1.0	---	0.98	---
	-	1.2	--	1.2	--
Soil**	0.4	0.39	97.5	0.38	95.0
	1.0	0.99	99	0.97	97.0
	1.4	1.39	99.2	1.36	97.1
	1.6	1.58	98.8	1.58	98.8

*2,4-DNPH – NEDA method ** 4-ARHC– 2,4-DNPH method [†] n=5

Table 5 Determination* of selenium (IV) in synthetic mixture corresponding to Se -Fe, Se-Hg and Se-Pb alloys.

Se taken ($\mu\text{g ml}^{-1}$)	Fe added ($\mu\text{g ml}^{-1}$)	Hg added ($\mu\text{g ml}^{-1}$)	Pb added ($\mu\text{g ml}^{-1}$)	Se found [†] ($\mu\text{g ml}^{-1}$)
1.0	1.1	--	--	1.0
2.0	2.2	--	--	1.99
1.0	--	3.0	--	0.98
2.0	--	6.0	--	1.99
1.0	--	--	3.0	0.99
2.0	--	--	6.0	2.08

* 4-ARHC – 2,4-DNPH method [†] n=3

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