

Analytical, Nutritional and Clinical Methods

Selenium determination in various vegetable samples by spectrophotometry

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Received 28 April 2006; received in revised form 15 July 2006; accepted 19 July 2006

Abstract

A rapid, highly sensitive and selective spectrophotometric method for the determination of traces of selenium (IV) is described. The method is based on oxidation of 4-aminopyridine by selenium (IV) followed by coupling reaction with *N*-(1-naphthalene-1-yl)ethane-1,2-diamine dihydrochloride (NEDA) in acid medium to give purple color derivative with λ_{\max} 560 nm and is stable for more than 15 days at 35 °C. Beer's law is obeyed for selenium (IV) in the concentration range of 0.05–21 $\mu\text{g ml}^{-1}$ at the wavelength of maximum absorption. The optimum reaction conditions and other analytical parameters were investigated to enhance the sensitivity of the present method. The detailed study of various interferences made the method more selective. The proposed method was successfully applied to the analysis of selenium in various vegetable samples. The results obtained were agreed with the reported methods at the 95% confidence level. The performance of proposed method was evaluated in terms of *F*-test and Variance ratio *f*-test which indicates the significance of proposed method over reported method.

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Keywords: Selenium (IV); Oxidation; 4-Aminopyridine; *N*-(1-naphthalene-1-yl) ethane-1; 2-Diamine dihydrochloride (NEDA); Spectrophotometry; Vegetable samples

1. Introduction

Selenium is widely distributed in nature, but relatively small concentration in rocks, plants, coal, and other fossil fuels. Selenium enters natural water through seepage from seleniferous rocks, chemical weathering and industrial wastes. Trace levels ingestion of selenium is not only toxic to animals but also toxic to human beings. Selenium is an essential trace nutrient to living system. Diseases due to selenium deficiency are well known in veterinary medicine. The selenium concentration of most drinking waters and natural waters is less than 10 $\mu\text{g ml}^{-1}$ (Hemanth Kumar, Nagaraja, Yathirajan, & Prakash, 2002). Several analytical

techniques have been reported for the determination of selenium, which includes spectrofluorometry, electrothermal atomic absorption spectrometry, polarography, flow-injection technique, radiochemical neutron activation analysis and differential pulse cathode-stripping voltametry were reviewed (Holok & Speechio, 1994). Few reagents were available for the spectrophotometric determination of selenium in various environmental matrices such as leuco crystal violet (Agarwal, Sunitha, & Gupta, 1998), *J*-acid (Manish, Ramachandran, & Gupta, 1994), sodium salt of hexamethyleneiminecarbodithioate (Pathare & Sawant, 1995), 1-aminonaphthalene-7-sulfonic acid (Pyrzysaka, 1997), variamine blue (Revanasiddappa & Kumar, 2001) and resazurin (Safavi & Afkhami, 1997). The above reported methods suffer from poor sensitivity, selectivity and stability of the color derivative. So, it is

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desirable to develop simple, highly sensitive, reliable and rapid method for the determination of selenium (IV) in environmental samples.

In the present study, we reported simple, sensitive, selective and rapid method for the determination of traces of selenium (IV). The implied reaction was oxidative coupling in the presence of Se (IV), the 4-aminopyridine was oxidized and coupled with *N*-(naphthalene-1-yl) ethane-1, 2-diamine dihydrochloride (NEDA) in acid medium. The method was successfully employed for the determination of selenium in various vegetable samples.

2. Materials and method

2.1. Instrumentation

A Hitachi U 2001 spectrophotometer with 1.0 cm matched quartz cell was used for all absorption measurements. A Perkin-Elmer® Model 2380 atomic absorption spectrometry (AAS) was used for the analysis of selenium. All instrumental settings were those recommended in the manufacturer's manual book. An Elico Model Li-129 pH meter was used for pH measurements.

2.2. Reagents

All chemicals used were of analytical reagent grade and deionised – double distilled water was used to prepare all solutions through out experiments.

A stock solution containing 100 mg l⁻¹ of selenium was prepared by dissolving 0.14 g of selenium dioxide (Merck, Mumbai, India) in 1 l of deionised – double distilled water. A working solution of selenium (IV) was prepared by further dilution. 1.5% 4-aminopyridine solution was prepared by dissolving 1.5 g of 4-aminopyridine (from Sigma, USA) in 2 ml of 5 M HCl and made up to the mark in 100 ml standard flask with deionised – double distilled water, 0.5% solution of *N*-(naphthalene-1-yl)ethane-1,2-diamine dihydrochloride (NEDA) (from S.D. Fine Chemicals, India) was prepared by dissolving 0.5 g of *N*-(1-naphthalene-1-yl)ethane-1,2-diamine dihydrochloride (NEDA) in 10 ml of 1 M HCl and diluted up to the mark in a 100 ml standard flask with deionised – double distilled water and the solution was refrigerated.

2.3. Collection of samples

Tirupati is one of the famous pilgrim centers in India and is widely known all over the world. Pollutants are mainly responsible for destroying the natural beauty of the holy town. Ultimately this rich pilgrim center was chosen as the collection area. Several small and medium scale industries are situated in the suburban fringes of Tirupati. The samples were collected from different locations of the study areas. All possible precautions are taken at various stages starting from sample containers, sample collection and storage, processing and analyzing the samples.

2.4. General procedure for determination of selenium (IV)

Stock solution containing 1–100 μg (0.03–3.5 μg ml⁻¹) of selenium (IV) (the volume of the test solution was restricted to 1 ml) were transferred into 25 ml calibrated flasks, 3 ml of 4-aminopyridine – NEDA reagent mixture was added. The mixture was allowed to stand for 2 min to complete the reaction. The content was diluted to the mark with deionised – double distilled water and absorbance was measured at 560 nm against the corresponding reagent blank and the calibration graph was constructed.

2.5. Determination of selenium (IV) in vegetable materials

Finely chopped fresh vegetable samples (5 g each) were placed in a 250 ml beaker, and a solution of concentrated H₂SO₄/HNO₃ (1/1 v/v) (10 mL) was added. This mixture was heated until the solution is clear. The solution was filtered off and concentrated to 5 ml then cooled and diluted to 50 ml with deionised double – distilled water as recommended in reported methods (Tuzen, Soylyak, & Elci, 2005a; Tuzen, Parlar, & Soylyak, 2005b). Then the general procedure was applied to 1 ml of this solution.

The Standard Reference Material (SRM) 1573a was analyzed by the proposed procedure to determine the accuracy of the present method which was distributed by National Institute of Standard and Technology.

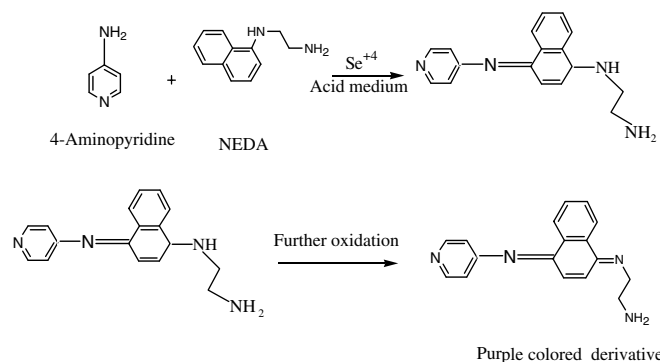
3. Results and discussion

3.1. Absorption spectra of oxidative-coupling mixture

The proposed method involves the formation of the purple colored derivative in presence of Se (IV) as shown in Scheme 1 with λ_{max} 560 nm against the corresponding reagent blank and the calibration graph was constructed as shown in Fig. 1.

3.2. Optimum conditions for color product formation

In order to establish the optimum conditions necessary for a rapid and quantitative formation of the colored



Scheme 1. Oxidative coupling reaction of 4-aminopyridine with NEDA by selenium (IV).

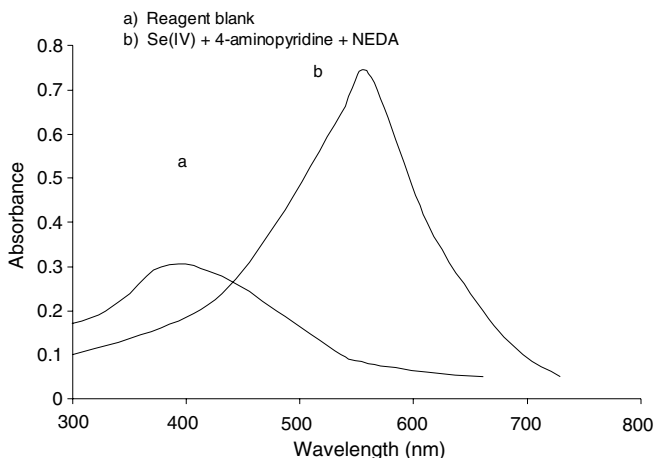


Fig. 1. Absorption spectra of the oxidation of 4-aminopyridine and coupled with NEDA by selenium (IV).

product with maximum stability and sensitivity, the investigators measured the absorbance of a series of solutions by varying one and fixing the other parameters at 560 nm.

It was found that a 1.5% solution of 4-aminopyridine in the range of 2.0–4.0 ml, 0.5% solution of NEDA in the range of 5.0–10.0 ml were necessary to achieve the maximum color intensity of the oxidative coupling product (Fig. 1). The color intensity decreased below the lower limit and above the upper limit, and a red colored product was unstable. Therefore, 3.5 ml of 4-aminopyridine and 7.0 ml of NEDA solution were recommended for all measurements.

3.3. Effect of temperature on colored product

The reaction between oxidized 4-aminopyridine and NEDA was found to be instantaneous. The effect of the temperature on the product was studied at different temperatures; it was found that the colored product was stable for more than 15 days in the temperature range of 25–40 °C. So, the temperature of 25 °C was selected as optimum for maximum color development.

3.4. Effect of pH on absorption maximum

The effect of pH on the peak height of selenium (IV) at different concentrations was investigated with a fixed reagent concentration (1.5% 4-aminopyridine and 0.5% NEDA) in the pH range of 3.0–8.0 and the peak height was measured for each concentration level of selenium (IV). At all concentration levels of Se (IV), maximum peak heights were found between pH 3.0 and 6.0. Therefore, a pH 4.0 was selected for further studies.

3.5. Effect of volume of acid concentration on absorption maximum

The effect of $H_2SO_4/HNO_3/HCl$ concentration on absorption maximum of colored product was investigated. The maximum intensity of the red color was achieved in the range of 10–12 ml of concentrated H_2SO_4/HNO_3 . Therefore, 10 ml of concentrated H_2SO_4/HNO_3 was used.

3.6. Ringbom plot for red colored derivative

Ringbom's plot is the established standard adopted to know the optimum range of concentration for a system that obeys Beer's law. The plot is drawn between $\log C$ of Selenium (IV) and $(1-T)$ (where T is the transmittance). The plot has a sigmoid shape with a linear segment at intermediate absorbance values (0.50–2.25) and concentration values (8.0–20 $\mu g ml^{-1}$). The slope of Ringbom's plot from Fig. 2 is 0.10. Hence, the ratio between the relative error in concentration and photometric error is 8.0, for a concentration of 0.80, for 0.1% photometric error.

3.7. Optical parameters

Beer's law range, molar absorptivity, Sandell's sensitivity, and other parameters of the oxidative coupling mixture were given in Table 1. The precision and accuracy of the

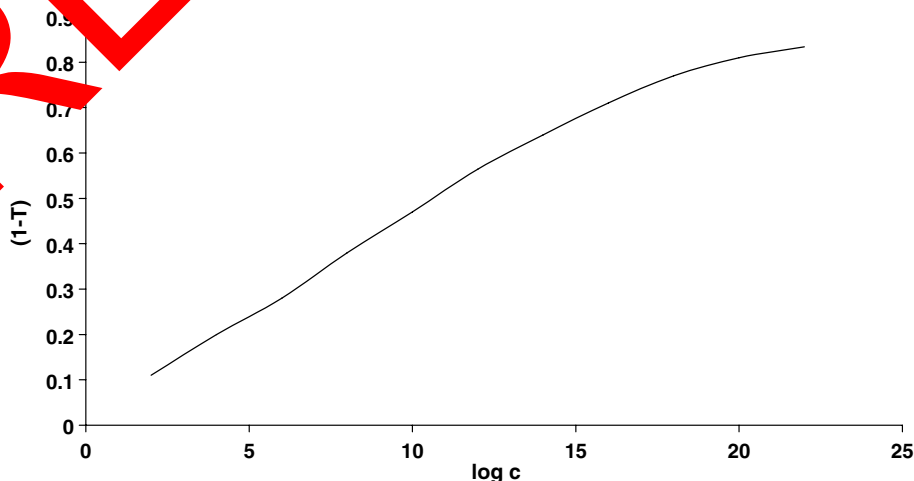


Fig. 2. Ringbom plot of oxidation of 4-aminopyridine and coupled with NEDA by selenium (IV).

Table 1
Optical characteristics of oxidative coupling mixture and precision data

| Optical characteristics of present method | |
|---|-------------------|
| Color | Purple |
| λ_{\max} [nm] | 560 |
| Stability | More than 15 days |
| Beer's law range [$\mu\text{g ml}^{-1}$] | 1.0 - 21 |
| Molar absorptivity [$l \text{ mol}^{-1} \text{ cm}^{-1}$] | 5.0×10^4 |
| Sandell's sensitivity [$\mu\text{g cm}^{-2}$] | 0.00380 |
| Regression equation (Y) ^b | |
| Slope ^a | 0.238 |
| Intercept ^b | 0.00069 |
| Correlation coefficient ^r | 1.110 |
| Relative standard deviation [%] ^c | 0.602 |
| Range of error (95% confidence level) | ± 0.590 |
| Detection limit [$\mu\text{g ml}^{-1}$] | 0.0192 |
| % error (at 95% confidence level) ^c | 0.8032 |

^a Experiments performed under optimized conditions (see text) with 1 ml of 0.014% aqueous SeO_2 solution.

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

^c $n = 4$.

method was studied by analyzing the coupling solution containing known amounts of the cited reagents within Beer's law limit. The low values of the standard deviation (%) and the percentages of error indicated the high accuracy of the present investigation.

Table 2
Effect of non-target ions on the determination of $3.0 \mu\text{g ml}^{-1}$ Selenium (IV)

| Species | Tolerance limit [$\mu\text{g ml}^{-1}$] |
|--|---|
| EDTA | 10600 |
| Na^+ , Mg^{2+} , Cl^- , NO_3^- , F^- , CHCOO^- , CO_3^{2-} , K^+ | 2090 |
| Ba^{2+} , SO_4^{2-} , CN^- , SCN^- , Tartarate | 1100 |
| PO_4^{3-} , Al^{3+} , Cd^{2+} , NO_2^- ^a | 800 |
| Cu^{2+} ^b , Ni^{2+} , Co^{2+} , Ca^{2+} | 84 |
| Zn^{2+} , Pb^{2+} , SO_3^{2-} , NO_3^- , Cr^{3+} , As^{5+} | 50 |
| Fe^{2+} , S^{2-} | 36 |

^a Can be masked up to $810 \mu\text{g ml}^{-1}$ by the addition of 0.5 ml of 2% sulfamic acid.

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

3.8. Reaction mechanism

Under the reaction condition, 4-aminopyridine losses probably $2e^-$ and a proton on oxidation with Se (IV) in acidic medium to form an electrophilic intermediate (active coupling species), which couples with *N*-(1-naphthalene-1-yl) ethane-1,2-diamine dihydrochloride (NEDA) to give purple derivative.

Table 3
Determination of selenium in various vegetable samples with spectrophotometer

| Vegetable samples | Proposed method | | | | | | FAAS method | |
|---|-----------------|----------------------------------|----------------------------------|---------------------------|----------------|----------------|----------------------------------|---------------------------|
| | Local name | Selenium added (μg) | Selenium found (μg) | Recovery (%) ^a | <i>t</i> -test | <i>f</i> -test | Selenium found (μg) | Recovery (%) ^a |
| <i>Solanum tuberosum</i> ^b | Ginger | 4.0 | 0.12 | – | – | – | 0.14 | – |
| | | 4.0 | 4.57 | 98.80 ± 0.05 | a | b | 4.60 | 99.10 ± 0.06 |
| <i>Brassica oleracea</i> var. <i>gangyloides</i> ^c | Cholera | – | 0.18 | – | – | – | 0.20 | – |
| | | 5.0 | 5.1 | 98.40 ± 0.08 | a | b | 5.13 | 98.60 ± 0.09 |
| <i>Allium cepa</i> ^d | Onion | – | 0.43 | – | – | – | 0.45 | – |
| | | 7.50 | 7.89 | 99.40 ± 0.18 | a | b | 7.92 | 99.60 ± 0.16 |
| <i>Allium sativum</i> ^e | Garlic | – | 0.38 | – | – | – | 0.40 | – |
| | | 10.0 | 10.30 | 99.20 ± 0.030 | a | b | 10.33 | 99.30 ± 0.07 |
| <i>Raphanus sativus</i> ^f | Raddish | – | 0.21 | – | – | – | 0.22 | – |
| | | 5.0 | 5.12 | 98.20 ± 0.080 | a | b | 5.14 | 98.40 ± 0.06 |
| <i>Daucus carota</i> ^g | Carrot | – | 0.55 | – | – | – | 0.54 | – |
| | | 4.50 | 4.98 | 98.40 ± 0.16 | a | b | 4.98 | 98.60 ± 0.32 |
| <i>Beta vulgaris</i> ^h | Beet root | – | 0.90 | – | – | – | 0.93 | – |
| | | 2.50 | 3.37 | 98.80 ± 0.07 | a | b | 3.41 | 99.20 ± 0.08 |
| <i>Solanum melongena</i> ⁱ | Brinjal | – | 0.29 | – | – | – | 0.30 | – |
| | | 4.0 | 4.21 | 98.0 ± 0.42 | a | b | 4.24 | 98.50 ± 0.07 |
| <i>Lycopersicon esculentum</i> ^j | Tomato | – | 0.09 | – | – | – | 0.11 | – |
| | | 6.0 | 6.04 | 99.10 ± 0.03 | a | b | 6.07 | 99.30 ± 0.08 |
| <i>Cucumis sativa</i> ^k | Cucumber | – | 0.26 | – | – | – | 0.25 | – |
| | | 8.0 | 8.21 | 99.30 ± 0.20 | a | b | 8.21 | 99.50 ± 0.06 |
| <i>Dolichos lab lab</i> ^l | Pea's | – | 0.70 | – | – | – | 0.72 | – |
| | | 10.0 | 10.59 | 98.90 ± 0.18 | a | b | 10.71 | 99.0 ± 0.07 |
| <i>Pisum sativum</i> ^m | Bean's | – | 0.22 | – | – | – | 0.25 | – |
| | | 2.50 | 2.67 | 98.0 ± 0.15 | a | b | 2.71 | 98.40 ± 0.16 |
| <i>Lycopersicon esculentum</i> Leaves ⁿ | Tomato leaves | 0.055 | 0.054 | 98.08 ± 0.12 | a | b | 0.054 | 98.08 ± 0.09 |

^a Mean \pm standard deviation ($n = 4$), ^{b,c,d,e} Collected from Chandragiri area, ^{f,g,h,i} Collected from Renigunta area, ^{j,k,l,m} Collected from local market, Tirupati. ⁿ Standard reference material (mg kg^{-1}).

^a $P = 0.059$.

^b $P > 0.000284$.

Table 4
Comparison of spectrophotometric method with present method for the determination of selenium in various vegetable samples

| Reagents | Sensitivity ($\mu\text{g ml}^{-1}$) | Remarks | References |
|---|---------------------------------------|---|---------------------------------------|
| MBTH/dopamine | 4.0 | Less sensitive, stable | Hemanth Kumar et al. (2002) |
| J- acid | 5.80 | Highly commercial, high reagent consumption and 10 min is needed for color development | Manish et al. (1994) |
| 1-Naphthylamine-7-sulphonic acid | 5.32 | Less selective, more reagent consumption and metal ions are interfered seriously | Pyrzynsaka (1997) |
| Sodium salt of hexamethylene imine carbodithioate | 5.50 | Needs extraction, less stable, high solvent consumption | Pathare and Sawant (1995) |
| Varamine blue | 20 | Less sensitive, low detection limit, maintenance of pH conditions | Revanasiddappa and Kiran Kumar (2001) |
| 4-Aminopyridine | 21 | More sensitive, rapid, facile, non-extractive, economical reagent, highly stable (more than 15 days), metal ions do not interfere | This work |

3.9. Interference of non-target ions

The effect of various species on the determination of Se (IV) was investigated. The tolerance limit was taken as the amount that caused $\pm 2\%$ absorbance error in determination of $3.0 \mu\text{g ml}^{-1}$ of Se (IV). The results are shown in Table 2.

4. Applications and comparison of proposed method with reported method

The proposed method for the analysis of selenium (IV) has been employed for real and SRM samples of vegetables. The obtained results were compared with FAAS in terms of Student's *t*-test and Variance ratio *f*-test. The analytical data summarized in Tables 3 suggest that the percentage of selenium recovery from vegetable samples ranges from 98.0% to 99.40%, which is more reliable and sensitive than the other methods.

It is evident from the above data that the proposed method is simple, highly sensitive and rapid than the reported methods in literature as shown in Table 4.

5. Conclusions

The proposed method is simple, highly sensitive and rapid for the spectrophotometric determination of selenium (IV) in various vegetable samples. The limit of detection of the proposed method is superior and compared with previously reported methods. The method has additional advantages over reported methods owing to its: Coupling reagent employed in the present method, *i.e.*, *N*-(1-naphthalene-1-yl) ethane-1, 2-diamine dihydrochloride (NEDA) is fairly soluble in water and very cheap. The color derivatives (Scheme 1) formed by oxidative coupling is highly stable (more than 15 days) when compared to that of the reported methods (Table 4). Less interference, more accuracy, avoidance of lengthy extraction steps and statistical

analysis when make present method to be placed among the most sensitive ones for the determination of Se(IV) in various vegetable samples.

Acknowledgements

The authors are grateful to Head, Department of Biotechnology, S. V. University, Tirupati, for providing infrastructural facility.

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