CHAPTER - II

Selenium

Colorimetric Determination of Selenium in Various Environmental Samples.
COLORIMETRIC DETERMINATION OF SELENIUM IN VARIOUS ENVIRONMENTAL SAMPLES

SUMMARY

A new reagent system using rhodamine-B dye for the determination of selenium is described. The method is based on the reaction of selenium with acidified potassium iodide to liberate iodine. The liberated iodine bleaches the pink colour rhodamine-B, which is measured at 555nm. Beer's law is obeyed over the concentration range of 1-10 µg of selenium in final solution volume of 25ml (0.04 - 0.4ppm) and the apparent molar absorptivity and Sandell's sensitivity were found to be $1.96 \times 10^5$ l mol$^{-1}$ cm$^{-1}$ and 0.0004 µg cm$^{-2}$ respectively. The method is simple, sensitive, selective and is satisfactorily applied to micro-level determination of selenium in various environmental and cosmetic samples.
INTRODUCTION

Selenium (IV) is reported to be toxic as well as an essential trace element for all living beings (1,2). It is found in nature in relatively small concentrations in rocks, coal, plants and fossil fuels. In large concentrations it is associated with sulphide minerals of lead, iron, copper and other metals (3,4). The major sources of selenium in the environment are volcanic eruption, manufacture of insecticides, fertilizers, smelting ceramics, metallurgical operation, glass rubber accelerators and electronic goods, etc. Certain industrial and agricultural process release selenium as a byproduct and their cases where selenium from such sources has caused environmental disaster (5,6). It is also used as a catalyst in chemical industry and additives to dyes, plastic and lubricants, in the rubber industry to increase tensile strength, abrasion resistance and life span and in the ceramic and glass industry as a colouring and decolouring agent (7).

Selenium enters into natural water through seepage from seleniferous soils, irrigation, drainage and industrial waste (8,9). The liberated selenium accumulates in soil and undergoes complex biogeochemical reaction leading to form organoselenium compounds which are much more toxic than inorganic selenium compounds. It plays a major role in crucifereae family life cycle and can be found in concentration as high as 1.5% (10).

With the development of science and technology the selenium content in serum has become an important monitoring index in clinical medicine. Selenium is not only a nutritional anti-cancer element but also as a cancer chemoprevention agent (11). It can suppress the effect of chemical carcinogenic substances. Selenium plays a part in the study of tumours, cancer, cardiology, liver diseases and endemic disease (12). It is now recognized that deficiencies of selenium are associated with heart diseases, muscular dystrophy, reproductive disorder and some cancer (13). In China selenium deficiency in soil and diets is associated with Keshan disease and Kaschin Beck disease (14,15).
In recent years due to increase in the selenium level the loss of hair and nails, abdominal pain, jaundice, chronic gastrointestinal diseases and fatigue in human beings has been noted (16). Mild inhalation of selenium dust fumes or vapours irritate the membranes of the eyes, nose, throat and respiratory tracts, causing lacrimation, sneezing, nasal congestion, coughing, etc in human beings. Prolonged exposure through inhalation can cause marked pallor, coated tongue, gastrointestinal disorder, nervousness and garlicky odour of breath and sweat (17). The Threshold Limit Value (TLV) of selenium compounds in air is 0.1-0.2 mg dm\(^{-3}\) and in water is 4.0 ppm (6). It has been already been reported that selenium acts as an antidote or scavenger for necessary cadmium, arsenic, mercury and other elements (18). Selenium appears to be a natural protective agent against heavy metals toxicity to biological samples. Selenium has been detected in human milk, serum and urine at concentrations 0.8 - 498 \(\mu g/ml\) (19).

There are many reported methods for the determination of micro amounts of selenium in various samples. Some of these are graphite furnace atomic absorption spectrometry (20), cathodic stripping voltammetric analysis (21,22), hydride generated AAS (23,24), inductively coupled plasma atomic emission spectrometry, isotope dilution - MS (26), ICP - OES (27), ICP-MS (28), polarographic (29), flow injection analysis (30), etc. No doubt some of these techniques are highly sensitive but these high tech instruments are cost effective, required time and high maintenance.

Various colorimetric methods using reagents 3,3-diaminobenzidine(31), 4-nitrophenylhydrazine and 8-quinoleneo(32), 2-3 diaminonaphthalene(33), J-acid (34,35) xylenol orange(36), 1-2-diamino-4nitrobenzene(37) and leucocrystalviolet (LCV)(38) etc have been reported in the literature for the determination of traces of selenium in various environmental samples. Reagents such as benzidine and o-diamine are reported to be carcinogenic (39) while many others are less sensitive, less selective, require long waiting time and have less stability of colour. Some recent spectrophotometric methods use different reagents for the determination of selenium in various samples (40-44).
In the present communication a simple, sensitive and selective method using a new reagent rhodamine-B has been reported for determination of selenium in various samples. The proposed method is based on the liberation of iodine by the reaction of selenium with potassium iodide in acidic medium. The liberated iodine bleaches rhodamine-B, which is measured at 555nm. Beer's law range is 1-10μg (0.04-0.4ppm). The method has been successfully applied for determination of selenium in various environmental and cosmetic samples.

**EXPERIMENTAL**

**Apparatus**

A Systronics 106 digital spectrophotometer and a Systronics 335 digital pH meter were used.

**Reagents**

All chemicals used were of A.R. grade or the best quality available. Double distilled deionised water was used throughout experiment.

**Standard selenium solution**

A 1 mg/ml stock solution of selenium was prepared by dissolving selenium (10mg) in concentrated nitric acid (0.5ml) by gentle heating and evaporating to dryness. The dried mass was then dissolved in distilled water (10ml) and working standard solution was prepared by appropriate dilution of the stock solution.

**Potassium iodide (E. Merck, Mumbai)**

0.5% aqueous solution was prepared.

**Hydrochloric acid**

1M-aqueous solution was used.
Rhodamine-B (Stuttgart, W. Germany)

0.05% aqueous solution was prepared.

PROCEDURE

Preparation of calibration curve

To an aliquot of a working standard containing 1-10 µg selenium was added potassium iodide solution (2ml) followed by hydrochloric acid (1ml). The mixture was shaken gently till the appearance of a yellow colour indicating the liberation of iodine. The rhodamine-B solution (1ml) was added to it and shaken for two minutes. The volume made up to 25 ml by adding deionized water. After allowed 15-20 min. for completion of the reaction, the absorbency of the rhodamine-B dye was measured at 555 nm against the reagent blank.

Determination of selenium in water

An aliquot (5ml) of the polluted water sample was taken and selenium was determined by the proposed and reported methods (34). To further check the applicability and recovery of the proposed method, a known amount of selenium was added to water and selenium determined by the proposed method with excellent recoveries 97-98.5% (Table-1)

Determination of selenium in plant material

Selenium is reported to be present in the plant material i.e., wheat, maize, cereals, and cabbage and crucifereac plants. Hence plant materials were checked for the presence of selenium. The spiked samples of plant material (5g.) were digested with nitric acid (10 ml) for 20 min. After cooling, per chloric acid (0.5 ml) was
added and heating was continued for another 10 min. until the evolution of ample fumes of perchloric acid. Water (10ml) was added to the cooled residue and heated again for 10 min. Then hydrochloric acid (5 ml) was added and heating continued for 10 min. to convert selenium (VI) to selenium (IV) (46).

The contents were diluted to 50 ml after adding EDTA solution (10 ml). An aliquot of this solution (5 ml) was taken and selenium determined as described above (Table -1).

**Determination of selenium in steel plant dust**

Several samples of steel plant dust were collected from the different sites of steel plant. The samples were weighed and digested with acid mixture (10 ml) (8 ml concentrated nitric acid + 2 ml of 60% perchloric acid) and then evaporated to 2 ml. The content were cooled and boiled with hydrochloric acid (10 ml) for 10 min. The process was repeated again so as to convert all the selenium (VI) to selenium (IV) and the samples was diluted to 25 ml with water. An aliquot (5 ml) was taken and analysed for selenium by the proposed and reported methods (34) (Table -1).

**Determination of selenium in hair samples**

Selenium has been reported to be present in human hair (2). The hair sample (0.1 g) was digested with acid mixture (hydrochloric acid: nitric acid, 3: 2 v/v 10 ml) for 10 min. The contents were cooled and made alkaline with 10% sodium hydroxide (pH ~ 9.0) and the analysed as described above (Table -1).

**Determination of selenium in cosmetic samples (Lipstick)**

Selenium is reported to be present in various cosmetic samples like lipstick, shampoo, hair cream, etc. (47-48). Hence these cosmetic samples were analysed to check the applicability of the method. The sample (0.5 g) of several
brands of lipsticks were dissolved in alcohol to extract the organic material contained in lipsticks. The residue was gently heated with concentrated nitric acid (10 ml) for 10 min and cooled then boiled with hydrochloric acid (10 ml) for 10 min. Sample residue was cooled and diluted to 50 ml with water. The aliquot (5 ml) was analysed by the proposed and reported method (34) (Table - 1).

**Determination of selenium in shampoo**

Selenium is reported to be present in various antidandruff shampoos (49). 0.5 g of various brands of shampoos, which contain selenium as selenium sulphide were taken and treated with 10 ml alcohol, and then filtered with Whatman filter paper. The residue was made up to 50 ml after treatment with concentrated nitric acid and hydrochloric acid to convert selenium (VI) to selenium (IV) and the analysed by the proposed and reported method (34) (Table - 1).

**RESULTS AND DISCUSSION**

**Spectral characteristics**

The absorption spectra of rhodamine-B dye showed maximum absorbance at 555nm. Reagent blank showed maximum absorbance at this wavelength (Fig-1).

**Adherence to Beer’s law, Molar absorptivity and Sandell’s sensitivity**

Beer’s Law is obeyed over the concentration range 1-10µg selenium per 25ml of the final solution (0.04-0.4 ppm) (Fig-2). The molar absorptivity and Sandell’s sensitivity was found to be $1.96 \times 10^5$ mol$^{-1}$ cm$^{-1}$ and 0.0004µg cm$^{-2}$ respectively.

**Effect of reagent concentration**

Constant and maximum absorbance values were obtained when 2 ml of
0.5% potassium iodide, 1 ml of 1 M hydrochloric acid, 1 ml of 0.05% rhodamine-B were used (Fig-3). By increasing the amount of potassium iodide and rhodamine-B, the absorbance remains constant.

**Effect of pH, time and temperature**

Constant absorbance values were obtained within the pH range 1-2. The room temperature (30°C) and a time of 15-20 minutes were necessary for the completion of the colour reaction.

**Reproducibility**

The repeatability of the method was checked by the analysis of the working standard solution containing 5 μg per 25 ml of selenium over a period of seven days. The standard deviation and relative standard deviation were found to be ±0.0097 and 1.96% respectively.

**Effect of co-pollutants**

Effect of various co-pollutants on the reaction were studied by adding known amount of these compounds to a solution containing 5 μg per 25 ml of selenium. The tolerance limit given in table-2 show that the method is free from interference of various ions.

**Colour reaction**

The colour reaction involves two steps (Scheme-A):

1. Liberation of iodine with the reaction of selenium in acidic potassium iodide.
2. Liberated iodine bleaches the pinkish red coloured rhodamine-B dye.

**APPLICATION**

The method has been successfully applied for the determination of selenium in several samples of water, polluted water, plant materials, steel plant dust, human hair and cosmetic samples (Lipstick and shampoo). The results obtained are
shown in Table 1, which is in good agreement with those obtained by the J-acid method (34).

CONCLUSION

The proposed method has been compared with other colorimetric methods and found to be simple, sensitive and selective. This method is a good alternative to some reported methods (Table 3) and its advantages is mainly due to cheaper cost, easier availability and higher stability of colour for rhodamine-B dye.

Scheme - A  Colour reaction of selenium

1) \( \text{Se}^{+4} + 2\text{KI} + 2\text{HCl} \xrightarrow{\text{Acidic Medium}} \text{I}_2 + 2\text{KCl} + \text{H}_2\text{O} + \text{Se}^{+6} \)

Selenium  Iodine

2) \( \text{Et}_2\text{N} \cdot \text{N'Et}_2\text{Cl} \cdot \text{COOH} + \text{I}_2 \xrightarrow{\text{Oxidation} + e^-} \text{Et}_2\text{N} \cdot \text{NET}_2 \cdot \text{CO} \)

Rhodamine - B dye  Bleached dye
(Quinonoid form)  (Benzenoid form)
Pinkish red colour  \( \lambda_{\text{max}} 555 \text{ nm} \)
Fig. 1  *Absorption spectra of rhodamine - B*

A - Concentration of Selenium is 5 µg / 25 ml
B - Reagent Blank

![Absorption spectra of rhodamine - B](image)

Fig. 2  *Calibration curve for the determination of selenium*

![Calibration curve for the determination of selenium](image)
Fig - 3  
Effect of reagent concentration
Concentration of Selenium is 5µ g / 25ml

Amount of rhodamine - B in ml - B

Amount of potassium iodide in ml - A
<table>
<thead>
<tr>
<th>S.N.</th>
<th>Sample volume or mass</th>
<th>Selenium originally found (µg)</th>
<th>Selenium added (µg)</th>
<th>Total selenium(µg)</th>
<th>% of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Natural water (100 ml)</td>
<td></td>
<td>3</td>
<td>2.91 (2.88)</td>
<td>97.0 (96.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>4.91 (4.85)</td>
<td>98.3 (97.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>6.69 (6.82)</td>
<td>98.5 (97.5)</td>
</tr>
<tr>
<td>02</td>
<td>Polluted water (100 ml)</td>
<td>3.21 (3.17)</td>
<td>5</td>
<td>8.0 (7.88)</td>
<td>97.5 (96.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.79 (3.73)</td>
<td>5</td>
<td>8.63 (8.46)</td>
<td>98.2 (97.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.29 (4.22)</td>
<td>5</td>
<td>9.15 (8.97)</td>
<td>98.5 (97.3)</td>
</tr>
<tr>
<td>03</td>
<td>Plant material (5g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A-Wheat</td>
<td>1.13 (1.08)</td>
<td>6</td>
<td>6.91 (6.79)</td>
<td>97.0 (96.0)</td>
</tr>
<tr>
<td></td>
<td>B-Maize</td>
<td>1.46 (1.40)</td>
<td>6</td>
<td>7.31 (7.03)</td>
<td>98.0 (95.0)</td>
</tr>
<tr>
<td></td>
<td>C-Cereals</td>
<td>2.13 (2.07)</td>
<td>6</td>
<td>8.02 (7.78)</td>
<td>97.5 (96.5)</td>
</tr>
<tr>
<td></td>
<td>D-Cabbage</td>
<td>3.96 (3.85)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E-Cruciferace Plant</td>
<td>3.46 (3.36)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>Steel plant dust (0.027 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
<td>3</td>
<td>2.92 (2.88)</td>
<td>97.5 (96.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>4.91 (4.92)</td>
<td>98.3 (98.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>6.69 (6.75)</td>
<td>98.5 (96.5)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.16 (1.09)</td>
<td>6</td>
<td>7.00 (6.84)</td>
<td>97.8 (96.5)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.99 (0.91)</td>
<td>6</td>
<td>6.88 (6.72)</td>
<td>98.6 (97.3)</td>
</tr>
<tr>
<td>05</td>
<td>Hair sample (0.1 g)</td>
<td>1.23 (1.19)</td>
<td>6</td>
<td>7.04 (6.90)</td>
<td>97.5 (96.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.49 (1.27)</td>
<td>6</td>
<td>7.35 (7.21)</td>
<td>98.2 (96.5)</td>
</tr>
<tr>
<td>06</td>
<td>Lipstick (0.5g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.99 (0.94)</td>
<td>5</td>
<td>5.88 (5.80)</td>
<td>96.5 (95.2)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.23 (1.16)</td>
<td>5</td>
<td>6.15 (6.03)</td>
<td>97.6 (95.5)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.36 (1.30)</td>
<td>5</td>
<td>6.23 (6.11)</td>
<td>96.2 (94.3)</td>
</tr>
<tr>
<td>07</td>
<td>Shampoo (0.5 g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>2.13 (2.10)</td>
<td>5</td>
<td>6.93 (6.85)</td>
<td>97.2 (96.5)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.26 (1.22)</td>
<td>5</td>
<td>6.15 (6.07)</td>
<td>98.4 (97.6)</td>
</tr>
</tbody>
</table>

Figure in parentheses are those by reported method (34).
Table - 2  Effect of various co-pollutants  
Concentration of selenium 5 μg / 25 ml = 0.2 ppm

<table>
<thead>
<tr>
<th>Co-pollutants</th>
<th>Tolerance limit (ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺, Ba⁺⁺, Ca⁺⁺</td>
<td>4500</td>
</tr>
<tr>
<td>Te⁺⁺, SO₄²⁻, PO₄³⁻</td>
<td>2600</td>
</tr>
<tr>
<td>Pb⁺⁺, Sn⁺⁺</td>
<td>1600</td>
</tr>
<tr>
<td>Co⁺⁺, Cd⁺⁺, Fe⁺⁺</td>
<td>1400</td>
</tr>
<tr>
<td>Cr⁺⁺⁺, Al⁺⁺⁺, Zn⁺⁺</td>
<td>950</td>
</tr>
<tr>
<td>As⁺⁺, Br⁺⁺⁺, Ni⁺⁺</td>
<td>900</td>
</tr>
</tbody>
</table>

* Tolerance limit is the amount of interference that causes an error of ± 2% in absorbance values.

Table - 3  Comparison with other colorimetric methods.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagents / Ref.</th>
<th>Medium / pH</th>
<th>λ Max (nm)</th>
<th>Beer's law range (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>3,3-Diamino benzidine (31)</td>
<td>Aqueous</td>
<td>420</td>
<td>0.1-10</td>
<td>Coloured salts and oxidants like Bi⁺³, Cr⁺³, Mo⁴⁺, V⁺⁵⁺, etc.</td>
</tr>
<tr>
<td>02.</td>
<td>4-Nitrophenyl hydrazine and 8-quinolenol (32)</td>
<td>Aqueous / 9-11</td>
<td>550</td>
<td>0.28 - 1.8</td>
<td>Fe⁺³, Cu⁺² and V⁺⁵⁺ interfere</td>
</tr>
<tr>
<td>03.</td>
<td>2,3-Diaminonaphtharene (33)</td>
<td>Acidic / 2.0</td>
<td>378</td>
<td>0.5 - 12</td>
<td>Less sensitive and most of the oxidant and reductant interfere</td>
</tr>
<tr>
<td>04.</td>
<td>J-acid (34)</td>
<td>Acidic / 1.0-2.5</td>
<td>520</td>
<td>0.03 - 0.3</td>
<td>Extractive and sensitive</td>
</tr>
<tr>
<td>05.</td>
<td>LCV (38)</td>
<td>Aqueous / 5.5 - 6.7</td>
<td>593</td>
<td>0.02 - 0.18</td>
<td>Non-extractive, simple, sensitive but costly chemical used.</td>
</tr>
<tr>
<td>06.</td>
<td>Rhodamine - 3 (proposed method)</td>
<td>Aqueous / 1-2</td>
<td>555</td>
<td>0.04 - 0.4</td>
<td>Non-extractive but sensitive, cheaper and easily available reagent used.</td>
</tr>
</tbody>
</table>
REFERENCES


(2) Adriano D.C., J. Water Air and Soil pollution, Kulwar Academic Publisher London, 57-58, 1991, 3

(3) Shapira J.E. "Inorganic Selenium Compounds, their Chemistry and Biology", Wiley Interscience, New York, 1971, 703


