

An easy spectrophotometric determination of selenium using azure B as a chromogenic reagent

Mendalin Mathew & Badiadka Narayana*

Department of Studies in Chemistry, Mangalore University, Mangalagangothri 574 199, India
Email: nbadiadka@yahoo.co.uk

Received 20 September 2005; revised received 3 March 2006; accepted 2 June 2006

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of trace amounts of selenium in water samples, soil, plant materials and human hair. The proposed method is based on the reaction of selenium with potassium iodide in acidic medium to liberate iodine. The liberated iodine bleaches the violet colour of azure B, which is measured at 644 nm. This decrease in absorbance is directly proportional to selenium concentration. Beer's Law was obeyed in the range 2.0-10.0 $\mu\text{g mL}^{-1}$ of selenium. The molar absorptivity, Sandell's sensitivity, quantitation limit and detection limit of the method were found to be $0.9473 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$, $8.33 \times 10^{-4} \mu\text{g cm}^{-2}$, $0.070 \mu\text{g mL}^{-1}$ and $0.023 \mu\text{g mL}^{-1}$, respectively. The proposed method has been successfully applied to the determination of selenium in various samples.

Keywords: Selenium determination, Spectrophotometry, Azure B

IPC Code: C01B19/00, G01J3/00

Selenium compounds are extensively used in paints, dyes, glass, electricals, rubber, insecticides, industries¹ and photocell devices² in which variations in the intensity of the incident light cause a corresponding variation of an electric current. Grey crystalline selenium is the only allotrope suitable for this purpose, its conductivity increasing approximately thousand-fold when illuminated. Grey selenium is used to greater extent for a.c. rectification, utilizing the property of asymmetric conduction exhibited by thin layers of this allotrope³. Less important uses of selenium are in the manufacture of coloured (red or reddish-yellow) glasses or ceramic and enamel pigments. Both selenium and tellurium have been used as secondary vulcanizing agents for natural rubber and, in the form of organo-compounds, as oxidation inhibitors in lubricating oils⁴.

Selenium is both highly toxic and an essential trace element for all living beings^{5,6} because selenium acts as a cofactor in cell membrane glutathione peroxidase and is important in cellular detoxification of peroxidase⁷. It is widely distributed in nature in relatively small concentrations in rocks, plants, coal and other fossil fuels. In China, selenium deficiency in the soil is associated with Keshan disease Kaschin Beck disease^{8,9}. The toxicity, availability and

environmental mobility of selenium are very much dependent on its chemical form. Selenium plays a major role in the life cycle of plants (*Cruceferae* family), which absorb organoselenium compounds accumulated in the oil of semiarid areas, and may poison livestock that graze on them. Selenium enters into natural water through seepage from seleniferous soil and industrial waste. Water drained from such soil may cause severe environmental pollution and wild life toxicity. Selenium is also reported to be present in cigarette paper, tobacco¹⁰, and various cosmetic samples¹¹.

Because of its significance, several analytical techniques have been reported concerning the determination of selenium¹²⁻¹⁵. Many spectrophotometric methods for the determination of selenium have been reported with some chromogenic reagents, such as 3,3-diaminobenzidine¹⁶, 1,1-dianthrimide^{17,18}, 2,2-dianthrimide¹⁹, 4-methyl-*o*-phenylenediamine²⁰, diaminochryszazine²¹, N-methyl-*o*-phenylenediamine²², 1,10-phenanthroline (with eosin)²³, dithiozone²⁴, 4,5-diamino-2,6-dimercaptopyrimidine²⁵, 8-hydroquinoline²⁶, 4,5,6-triaminopyrimidine²⁷, chromotropic acid²⁸, 1-naphthylamine-7-sulphonic acid²⁹, leuco crystal violet³⁰, variamine blue³¹, thionine³², maxilon blue-SG³³, rhodamineB-thiocyanate-gelatine-OP system³⁴

and potassium iodide and starch³⁵. Of these reagents, some have been reported to be less selective and sensitive. There is thus a need to develop an entirely new method, which would overcome the existing inadequacies in the determination of trace amounts of selenium.

In the present investigation, a rapid, sensitive and selective method has been reported for the determination of selenium with azure B as a reagent. The developed method has been successfully employed for the determination of selenium in water samples, soil, plant material and human hair.

Experimental Procedure

A Secoman Anthelie NUA022 UV-visible spectrophotometer with 1 cm quartz cell was used for all absorbance measurements. A WTW 330 pH meter was used. All the reagents used were of analytical reagent grade and double distilled water was used throughout the study. A standard stock solution (1000 $\mu\text{g mL}^{-1}$) of selenium was prepared by dissolving 1.910 g of NaHSeO_3 in 1000 mL of water. The hydrochloric acid 2 M, and potassium iodide, 2% solutions were used. A (0.1%) solution of azure B in methanol-water mixture was used.

An aliquot of the solution containing 2.0-10.0 $\mu\text{g mL}^{-1}$ of selenium was transferred into a series of 10 mL calibrated flasks. A volume of 1 mL of 2% potassium iodide solution followed by 1 mL of 2 M hydrochloric acid was added to it. The mixture was gently shaken until the appearance of yellow colour, indicating the liberation of iodine. Azure B (0.1%, 0.5 mL) was added to the solution and the reaction mixture was shaken for 2 min. The contents were diluted to 10 mL in a standard flask with distilled water. The absorbance of the resulting solution was measured at 644 nm against a reagent blank. A blank was prepared by replacing the selenium solution with distilled water. The absorbance corresponding to the bleached colour, which in turn corresponds to the selenium concentration, was obtained by subtracting the absorbance of the blank solution from that of the test solution. The amount of the selenium present in the volume taken, was computed from the calibration graph.

Determination of selenium in water and soil

An aliquot (≤ 4 mL) of water sample containing not more than 10 $\mu\text{g mL}^{-1}$ of selenium was treated with 0.5 mL of 1 M NaOH and 0.5 mL of 0.2 M EDTA. The solution was mixed and centrifuged to remove

any precipitate formed. The centrifugate was transferred to a 10 mL calibrated flask and its selenium content was determined directly, according to the general procedure for the determination of selenium. Similarly, the soil sludge sample (50 g) was treated with concentrated HCl to convert Se(VI) to Se(IV), followed by 10% NaOH and 5% EDTA solution was added. The made up solution was analyzed for selenium according to the general procedure for selenium(IV).

Determination of selenium in plant material and human hair

A sample of plant material (5 g- cabbage) was digested with 10 mL of HNO_3 for 20 min. After cooling, the digested sample was treated by the above procedure and analyzed for selenium content according to the general procedure. Similarly, a known amount of human hair sample was digested with a 10 mL mixture of HCl and HNO_3 (3:2 v/v) for 10 min. The solution was cooled and neutralized ($\text{pH} \approx 7$) with 10% NaOH and analyzed for selenium content according to the general procedure.

Results and Discussion

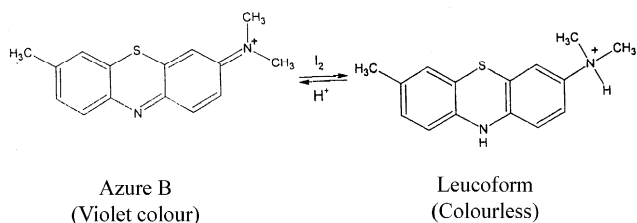
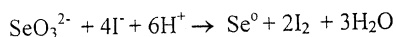
The reaction of selenium with potassium iodide in acidic medium liberate iodine. The liberated iodine bleaches the violet colour of azure B to colourless leucoform (Scheme 1) and the absorbance is measured at 644 nm (Fig. 1).

Effect of the iodide concentration and acidity

The oxidation of iodide to iodine by selenium was effective in the pH range 1.0-1.5. It was found that 1 mL each of 2 % potassium iodide and 2 M HCl were sufficient for the liberation of iodine from iodide by selenium.

Analytical data

The adherence to Beer's law was studied by measuring the absorbance values of solutions, varying



Scheme I

selenium concentration. A straight line graph was obtained by plotting absorbance against concentration of selenium. From the graph it can be seen that at 644 nm, Beer's Law was obeyed up to 10 µg mL⁻¹ of selenium. The molar absorptivity and Sandell's sensitivity for the coloured system were found to be 0.9473 × 10⁵ L mol⁻¹ cm⁻¹ and 8.33 × 10⁻⁴ µg cm⁻², respectively. The quantitation limit ($Q_L = 10 \sigma/S$) and detection limit ($D_L = 3.3 \sigma/S$) (where σ is the standard deviation of the reagent blank ($n= 5$) and S is the slope of the calibration curve) for the selenium determination were found to be 0.070 µg mL⁻¹ and 0.023 µg mL⁻¹, respectively.

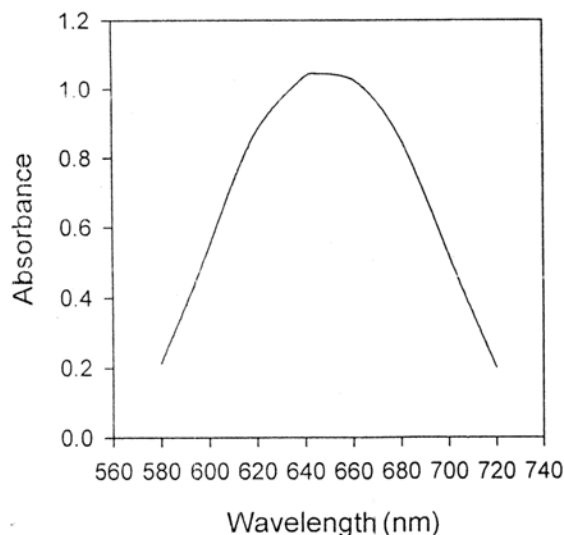


Fig. 1—Absorption spectra of coloured azure B

Effect of diverse ions

The effect of some ions that often accompany selenium was studied by adding their different amounts to selenium solutions. An error of ± 2% in absorbance reading was considered tolerable. The tolerance limits of various foreign ions are given in Table 1.

Application

The developed method was applied to the quantitative determination of traces of selenium in different samples such as water, soil, plant material and human hair. The result of analysis of the above samples (Table 2) compared favourably with those from a reference method³¹.

Table 1—Effect of diverse ions on the determination of selenium (10 µg mL⁻¹)

Foreign ion	Tolerance limit (µg mL ⁻¹)	Foreign ion	Tolerance limit (µg mL ⁻¹)
Hg(II)	75	Mo(VI)	50
Cd(II)	75	W(VI)	50
Zn(II)	75	U(VI)	50
Ni(II)	50	Chloride	150
Co(II)	50	Phosphate	200
Mg(II)	100	Bromide	200
Ba(II)	100	Nitrite	100
Cu(II)*	25	Acetate	150
Fe(III)*	100	Sulphate	100
In(III)	25	Borate	100
Pt(IV)	10	Chromate	150
V(IV)	100		
Ti(IV)	50		
Al(III)	50		

* Masked by secondary masking agents

Table 2—Determination of selenium in various samples using azure B as reagent

Sample	Proposed method			Reference method ³¹			
	Selenium Added (µg mL ⁻¹)	Selenium found (µg mL ⁻¹) ^a	Recovery (%)	Selenium found (µg mL ⁻¹) ^a	Recovery (%)	F-test ^b	t-test ^c
Natural water	4.0	4.01±0.02	100.25	3.98±0.03	99.5	2.25	1.86
	6.0	6.01±0.03	100.2	6.02±0.02	100.3	2.25	0.62
	10.0	9.99±0.01	99.9	10.02±0.02	100.2	4.0	2.99
Polluted water	4.0	4.03±0.01	100.75	4.01±0.02	100.25	4.0	1.99
	6.0	6.04±0.03	100.6	6.02±0.03	100.3	2.25	1.24
	10.0	10.05±0.03	100.5	10.02±0.03	100.2	1.0	1.58
Soil sludge	4.0	4.01±0.01	100.25	4.03±0.02	100.75	4.0	1.99
	6.0	6.03±0.02	100.5	6.06±0.03	101.0	2.25	1.58
	10.0	10.03±0.03	100.3	10.07±0.04	100.3	1.77	1.77
Plant material Cabbage(5 g)	4.0	4.03±0.02	100.75	4.02±0.01	100.5	4.0	0.99
	6.0	6.02±0.03	100.3	6.04±0.02	100.6	2.25	1.24
	10.0	10.03±0.02	100.3	9.99±0.03	99.9	2.25	2.48
Human hair(0.5 g)	4.0	4.06±0.01	101.5	4.03±0.02	100.75	4.0	2.99
	6.0	6.01±0.03	100.16	5.98±0.03	99.66	1.0	1.58
	10.0	10.04±0.02	100.4	10.03±0.03	100.3	2.25	0.62

^aMean standard deviation(n=5); ^bTabulated F-value for (4,4) degree of freedom at 95% probability level is 6.39; ^cTabulated t-value for 8 degree of freedom at 95% probability level is 2.776.

Conclusion

The reagent provides a simple, rapid, sensitive and accurate method for the spectrophotometric determination of selenium. The method needs neither heating for the complete colour development nor extraction into any organic phase. The proposed method has been successfully applied to the determination of traces of selenium in water, soil, plant materials and human hair.

Acknowledgement

Authors thank the Microtron Centre of Mangalore University for technical help.

References

- 1 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 19th edn (Washington, D.C), 1995, 3.
- 2 Lange B, *Photoelements and their Applications* (Reinhold Publishing Co. Inc., New York, N.Y), 1938.
- 3 Henisch H K, *Metal Rectifiers* (Clarendon Press, Oxford), 1945.
- 4 Bagnall K W, *The Chemistry of Selenium, Tellurium and Polonium* (Elsevier, Amsterdam), 1966, 14.
- 5 Adriano D C, *J Water Air Soil Pollut*, 3 (1991) 58.
- 6 Wang H C, Peng H W & Kuo M S, *Anal Chem*, 17 (2001) 532.
- 7 Ahrens L H & Taylor S R, *Spectrochem Anal*, 2 (1961) 93.
- 8 McDowell L R, *Minerals in Animals and Human Nutrition* (Academic Press, New York), 1992, 294.
- 9 Tan J & Huang Y, *J Water Air Soil Pollut*, 57 (1991) 59.
- 10 West P W & Shendrikar A D, *Anal Chim Acta*, 82 (1977) 403.
- 11 Shapira J R, *Organic Selenium Compounds their Chemistry and Biology* (Wiley Interscience, New York), 1971, 703.
- 12 Jeffrey A H, Roger G B & Carolyn A G, *J AOAC Int*, 75 (1992) 269.
- 13 Stewart & Chow A, *Talanta*, 40 (1993) 1345.
- 14 Hao D Q, Hang G Z, Zhang Y M & Tian G J, *Talanta*, 43 (1996) 595.
- 15 Macleod B A & Shand C A, *Talanta*, 43 (1996) 1091.
- 16 Russel B G, *Talanta*, 14 (1967) 957.
- 17 Langmyhr F J & Omang S H, *Anal Chim Acta*, 23 (1960) 565.
- 18 Langmyhr F J & Myhrstad J A, *Anal Chim Acta*, 35 (1966) 212.
- 19 Langmyhr F J & Dahl, *Anal Chim Acta*, 29 (1963) 377.
- 20 Kawashima T & Ueno A, *Anal Chim Acta*, 58 (1972) 219.
- 21 Brown R S, *Anal Chim Acta*, 74 (1975) 441.
- 22 Kasterka B, *Chem Anal (Warsaw)*, 25 (1980) 215.
- 23 Idriss K A, Seleim M M & Abu-Bakr M S, *Mikrochim Acta*, 39 (1980) 179.
- 24 Campbell A D & Yahaya A H, *Anal Chim Acta*, 119 (1980) 171.
- 25 Izquierdo A, Part M D & Aragonés L, *Talanta*, 106 (1981) 720.
- 26 Bhatt A & Gupta V K, *J Indian Chem Soc*, 59 (1982) 888.
- 27 Bodini M E & Alzamora O, *Talanta*, 30 (1983) 409.
- 28 Lavale S C & Dave M, *J Indian Chem Soc*, 66 (1989) 974.
- 29 Pyrzyńska K, *Anal Sci*, 13 (1997) 629.
- 30 Agarwal O, Sunitha G & Gupta V K, *J Indian Chem Soc*, 75 (1998) 151.
- 31 Revanasiddappa H D & Kiran kumar T N, *Anal Sci*, 17 (2001) 1309.
- 32 Revanasiddappa H D & Kiran kumar T N, *Anal Bioanal Chem*, 374 (2002) 1124.
- 33 Gurkan R & Akcay M, *Microchem J*, 75 (2003) 39.
- 34 Liang D, Jin-huan S, Xiu-mei W, Zi-hua Y & Yu W, *Guangpuxue Yu Guangpu Fenxi*, 24 (2004) 1419.
- 35 Narayana B, Mathew M, Bhat N G & Sreekumar N V, *Mikrochim Acta*, 141 (2003) 178.