



Trade Science Inc.

January 2007

Volume 3 Issue 4-6

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 3(4-6), 2007 [133-139]

Sensitive Spectrophotometric Methods For The Determination Selenium By Using Leuco Methylene Blue Reagent In Environmental And Cosmetic Samples



Corresponding Author

P.Chiranjeevi
Environmental Monitoring Laboratory,
Department of Chemistry, S.V.University,
Tirupati-517 502, A.P., (INDIA)
Tel: +91-422-2422222;
Fax: +91-422-2425706/2422387
E-mail: chiranjeevi_sai@yahoo.co.in

Received: 25th November, 2006

Accepted: 10th December, 2006

Web Publication Date : 21th December, 2006



Co-Authors

**P.Reddy Prasad, D.Rekha, J.Dilip Kumar, B.Krishnapriya,
P.Subramanyam**
Environmental Monitoring Laboratory, Department of Chemistry,
S.V.University, Tirupati-517 502, A.P., (INDIA)

ABSTRACT

Simple, sensitive, selective and rapid spectrophotometric method is developed for the determination of selenium (IV) in biological samples. This method is based on the oxidation reaction of leuco methylene blue (LMB) reagent with selenium (IV) to form a blue color derivative at pH-4.0. Which is having maximum absorption peak value at 605 nm. The reaction is instantaneous and color product is stable for 7 days at room temperature. Beer's law is obeyed in the concentration range of 0.4 - 4.2 $\mu\text{g ml}^{-1}$ for selenium (IV). Molar absorptivity and sandell's sensitivity of the system is found to be $3.64 \times 10^5 \text{ ml}^{-1} \text{ cm}^{-1}$, $0.0004 \mu\text{g cm}^{-2}$ respectively. The detailed study of the various interfering ions confirmed the high selectivity of the proposed method. The accuracy of the proposed method is expressed in terms of t-test and variance ratio of f-test. The results obtained were good argument with the reported method at the 95% confidence level. The proposed method is successfully applied for the determination of Se (IV) in environmental and cosmetic samples.

© 2007 Trade Science Inc. - INDIA

KEYWORDS

Leuco methylene blue;
Selenium(IV);
Spectrophotometry;
Environmental samples
and cosmetic samples.

Full Paper

INTRODUCTION

Selenium is widely distributed in nature, but relatively small concentrations in rocks, plants, coal, and other fossil fuels. Selenium enters natural water through seepage from seleniferous soil, 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. In China selenium deficiency in soil is associated with Keshan disease and Kaschin-Beck disease^[1,2]. It's efficiency causes pulmonary edema, abdominal pain, jaundice, chronic gastrointestinal diseases, hair loss and fatigue in human beings. In recent years some increase in the selenium level in human hair has been found^[3]. Selenium (IV) is a paradoxical element reported to have a double character well known as an essential as well as toxic trace element^[3]. Selenium is a by-product of certain industrial and agricultural processes and there are some cases where selenium from such sources has caused environmental damage^[4]. It is widely used in industries as an additive to dyes, lubricants, plastics and rubber^[4]. It also plays a major role in plants (crucifereae family) life-cycle and can be found in concentration as high as 1.5%^[5]. The TLV value for selenium compounds in air^[4] is 0.1-0.2 mg dm⁻³ and in water is 4 ppm^[6].

Several analytical methods have been reported for the measurement of selenium quantitatively in ppm or sub microgram levels^[7-11]. Few reagents are available for the spectrophotometric determination of selenium such as leuco crystal violet^[12], J-acid^[13], 1-aminonaphthalene-7-sulfonic acid^[14], sodium salt of hexamethyleneiminecarbodithioate^[15] variamine blue^[16], resazurin^[17], DPH/4-AAP^[18] etc. The above reported methods suffer from poor sensitivity, selectivity and stability of the color derivative. So, it is desirable to develop simple, highly sensitive, reliable and rapid method for the determination of selenium (IV) in environmental and cosmetic samples.

In the present work a simple, sensitive and selective method using a new reagent leuco methylene blue has been reported for the determination of Selenium in environmental and cosmetic samples. The

proposed method is based on oxidation reaction of leuco methylene blue with Se (IV) shows maximum absorbance at 605 nm. This method has a sensitivity of 0.02 ppm of Selenium (Beer's law range 0.002-0.18 ppm). The method has been successfully applied for the determination of selenium in wide variety of environmental and cosmetic samples.

EXPERIMENTAL

Instrumentation

A Hitachi U 2001 spectrophotometer with 1.0 cm matched quartz cell was used for all absorption measurements. A pH meter Elico Li-129 model glass calomel combined electrode was employed for measuring pH values.

Reagents and solutions

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 ml of deionised - double distilled water. A working solution of selenium (IV) was prepared by further dilution. 1% colourless leuco methylene blue (from B.D.H., Poole, UK) was prepared by dissolving 1.0 g of leucomethylene blue in water and diluted up to the mark in a 100 ml standard flask with deionised - double distilled water and the solution was refrigerated.

Calibration curve

Stock solution containing 1 - 100 µg (0.2 - 2.0 µg ml⁻¹) of selenium (IV) (the volume of the test solution was restricted to 1 ml) were transferred into 25 ml calibrated flasks followed HCl, 3 ml leuco methylene blue. 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 605 nm against the corresponding reagent blank and the calibration graph was constructed and shown in figure 1.

Selenium in water

An aliquot (5 ml) of polluted water sample was taken and selenium was determined by the proposed

Full Paper

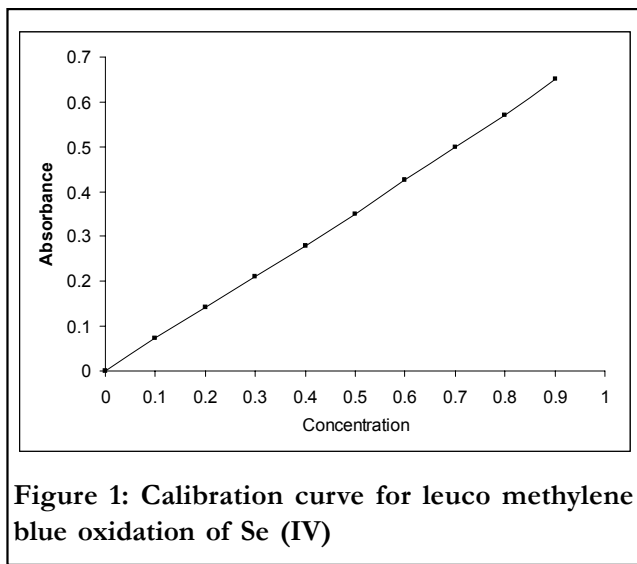


Figure 1: Calibration curve for leuco methylene blue oxidation of Se (IV)

and reported^[13] methods and the results were shown in (TABLE 2). To further check the applicability and recovery of the proposed method, known amounts of selenium was added to water and Se (IV) determined by the proposed method with excellent recoveries in agreement with the reported method.

Selenium in plant materials

The sample of plant material (5 g) was digested with HNO_3 (10 ml) for 20 min. after cooling, perchloric acid (0.5 ml) was added and heating was continued for another 10 min until the evolution of ample fumes of perchloric acid. Water (10 ml) was added to the cooled residue and heated again for 10 min. Then HCl (5 ml) was added and heating continued for 10 min to convert Se^{+6} to Se^{+4} ^[19]. The contents were diluted to solution (5 ml) was taken and Se (IV) determined as described in (TABLE 2).

Selenium in human hair

Human hair sample (0.1 g) was digested with acid mixture ($\text{HCl}:\text{HNO}_3$, 3:2 v/v; 10 ml) for 10 min. The contents were then cooled and made alkaline with 10% NaOH (pH~9.0), and analyzed as in (TABLE 2).

Selenium in cosmetic sample (lipstick)

Selenium is reported to be present in various cosmetic samples^[20]. Samples (0.5)g of several brands of lipstick were dissolved in alcohol to extract the organic material contained in lipsticks. The residue was gently heated with conc. nitric acid (10 ml) for

10 min and the contents were cooled and boiled with HCl (10 ml) for 10 min to convert Se^{+6} to Se^{+4} . Sample residue was cooled and diluted to 50 ml with water. The aliquot (5 ml) was analyzed by the above method as well as by reported method^[13] in (TABLE 2).

RESULT AND DISCUSSION

Absorption spectra

The absorption spectra of the leuco methylene blue with selenium show maximum absorbance at 605 nm. Reagent blank showed negligible absorbance at this wavelength. Beer's law was obeyed over the concentration range $0.4\text{--}4.2\ \mu\text{g ml}^{-1}$ of selenium per 25 ml of the final solution (0.02–0.18 ppm) at 605 nm. The apparent molar absorptivity and Sandell's sensitivity were found to be $3.64 \times 10^5\ \text{dm}^3\ \text{mol}^{-1}\ \text{cm}^{-1}$ and $0.0002\ \mu\text{g cm}^{-2}$ respectively. Repeatability of the method was checked by the replicate analysis of the working standard solution containing $3\ \mu\text{g}$ per 25 ml of selenium over a period of seven days. The standard deviation was found to be ± 0.02 and 0.40 respectively and shown in figure 2.

Effect of pH concentration

The effect of pH on the peak height of selenium (IV) at different concentrations was investigated with a fixed reagent concentration (1.0% leuco methylene blue) in the pH range of 3.0 - 4.5 and the peak height was measured for each concentration level of selenium (IV). At all concentration levels of Se (IV),

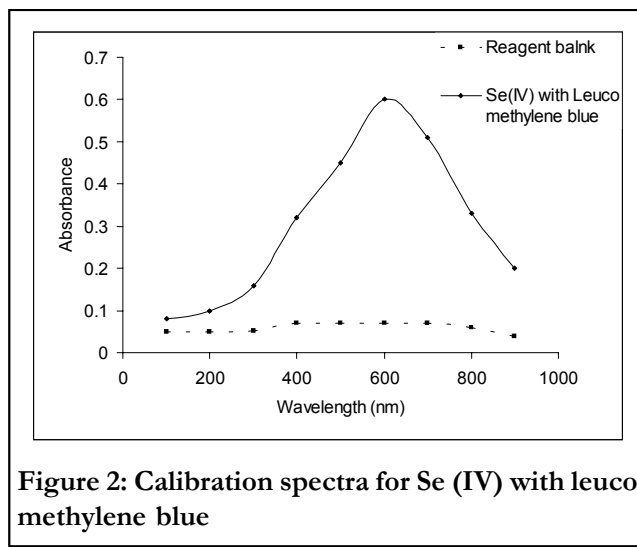


Figure 2: Calibration spectra for Se (IV) with leuco methylene blue

Full Paper

maximum peak heights were found between pH 3.0 - 4.5. Therefore, a pH 4.0 was selected for further studies.

Optimum conditions for color product formation

In order to establish the optimum conditions necessary for a rapid and quantitative formation of the colored 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 605nm.

It was found that a 1 % solution of leuco methylene blue in the range of 2.0 - 4.0 ml, was necessary to achieve the maximum color intensity of the oxidative coupling product figure 1. The color intensity decreased below the lower limit and above the upper limit, and a blue colored product was unstable. Therefore, 3.5 ml of Leuco methylene blue was recommended for all measurements.

Effect of temperature on colored product

The reaction between leuco methylene blue in to selenium (IV) 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 35°C was selected as optimum for maximum color development.

Ringbom plot for blue 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 (0.2 to 0.6) and concentration values (0.2 to 0.6 $\mu\text{g mL}^{-1}$). The slope of Ringbom's plot from figure 3 is 1.11. Hence, the ratio between the relative error in concentration and photometric error is 0.02, for a concentration of 0.2, for 90 % photometric error.

Optical parameters

Beer's law range, molar absorptivity, Sandell's sensitivity, and other parameters of the oxidative cou-

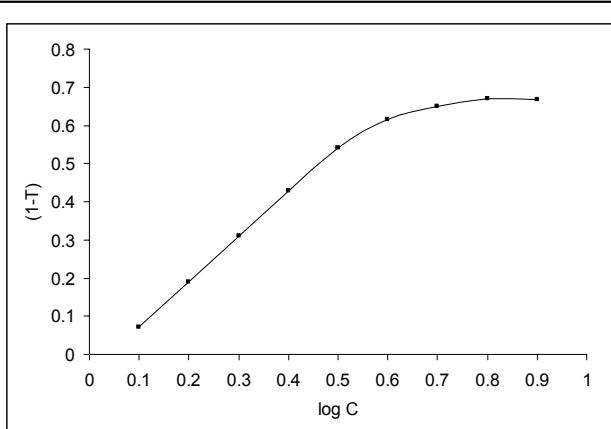


Figure 3: Ringbom plot for Se (IV) with leuco methylene blue

TABLE 1: Optical characteristics of oxidative coupling mixture and precision data

Optical characteristics of present method	
Color	blue
λ_{max} [nm]	605
Stability	More than 15 days
Beer's law range [$\mu\text{g mL}^{-1}$]	0.4 -4.2
Molar absorptivity [$\text{l mol}^{-1} \text{cm}^{-1}$]	3.64×10^5
Sandell's sensitivity [$\mu\text{g cm}^{-2}$]	0.0004
Regression equation (Y) ^b	
Slope ^a	0.253
Intercept ^b	0.00086
Correlation coefficient ^c	1.132
Relative standard deviation [%] ^c	0.609
Range of error (95 % confidence level)	± 0.482
Detection limit [$\mu\text{g mL}^{-1}$]	0.0252
% error (at 95% confidence level) ^c	0.6092

^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 $\mu\text{g mL}^{-1}$, $n=10$ pling mixture were given in (TABLE 1). The precision and accuracy of the 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.

Reaction mechanism

Under the reaction condition, selenium (VI) is reduced to Se (IV) by taking $2e^-$ in leuco methylene

TABLE 2: Applications of the method for determination of selenium in different samples by the present and reported method

	Present Method			Reported Method			
Sample volume or mass	Se added (μg)	Se found (μg)	Recovery (%) ^a	Se found (μg)	Recovery (%) ^a	<i>F</i> -test	<i>t</i> -test
Natural water ^b							
N ₁	2.00	1.97	98.50 ±0.03	1.94	97.00 ±0.03	0.98	1.54
N ₂	3.00	2.96	98.60 ±0.02	2.92	97.33 ±0.03	0.48	1.36
N ₃	4.00	3.96	99.00 ±0.40	3.93	98.25 ±0.03	3.39	1.25
Polluted water ^c							
P ₁	4.00	3.95	98.75 ±0.32	3.48	97.00 ±0.03	2.57	2.07
P ₂	4.00	3.98	99.50 ±0.03	3.85	96.41 ±0.03	0.94	3.09
P ₃	4.50	4.45	98.88 ±0.03	4.12	96.50 ±0.03	1.04	2.42
Plant Materials ^d							
Wheat	2.50	2.42	96.80 ±0.03	2.13	95.00 ±0.04	0.41	1.85
Maize	2.50	2.44	97.60 ±0.03	2.39	94.00 ±0.03	1.01	3.64
Cereals	2.50	2.39	95.60 ±0.04	3.16	95.00 ±0.04	0.41	0.65
Hair sample ^e							
H ₁	3.50	3.44	98.28 ±0.03	3.20	96.00 ±0.03	1.03	2.31
H ₂	3.50	3.41	97.42 ±0.02	3.26	95.50 ±0.02	1.02	1.95
Lipstick ^f							
L ₁	3.00	2.92	97.33 ±0.32	2.84	94.00 ±0.03	1.01	3.35
L ₂	3.00	3.86	95.33 ±0.03	2.78	93.50 ±0.03	1.02	1.86
L ₃	3.50	3.40	97.14 ±0.02	3.10	92.00 ±0.04	0.38	5.19
L ₄	3.50	3.42	97.71 ±0.03	3.10	97.50 ±0.03	1.04	0.74
L ₅	3.00	2.88	96.00 ±0.03	2.62	95.00 ±0.04	0.41	1.04

^an = 4, ^bSwarnamuki river belt, ^c Renigunta Industrial area, Tirupati, ^d Local market; ^e Local Hair saloon; ^b, ^c = 100 ml, ^d = 5 g, ^e = 0.1g, ^f = 0.5g
 blue molecule and changed into methylene blue. The reaction mechanism of oxidative coupling is carried out in SCHEME 1.

Effect of non-targetions

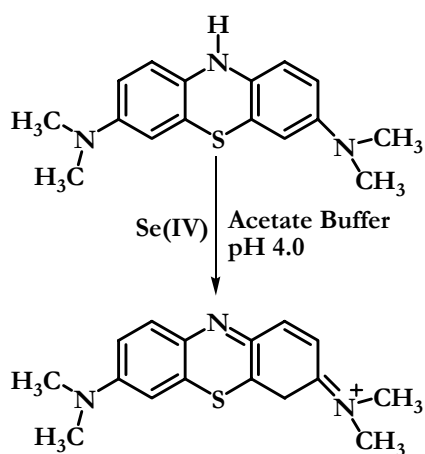
The validity of the method was assessed by investigating the effect of foreign species and other common ions in the analysis of selenium. The tolerance limit value (ppm) of different foreign species in a solution containing 3.0 $\mu\text{g}/25$ ml of selenium

were found to be: Ag^+ , K^+ , Ba^{2+} (4500); Ca^{+2} (4000); Te^{+4} , sulphate, phosphate (2500); Pb^{+2} , Sn^{+4} (1500); Co^{+2} , Cd^{+2} , Fe^{+3} (1200); Cr^{+3} , Al^{+3} , Zn^{+2} , Be^{+2} (850); As^{+5} , Bi^{+2} (800). Hg^{+2} and Mn^{+2} had a positive catalyzing effect on the color reaction. Cu^{+2} , Fe^{+2} and Sn^{+2} which are most common interferents were found not to interfere upto 80 ppm, if masked with EDTA.

Full Paper

TABLE 3: Comparison of spectrophotometric method with present method for the determination of selenium in various environmental Samples

Reagents	Sensitivity ($\mu\text{g mL}^{-1}$)	Remarks	Reference
Leuco crystal violet	-	Less stable, selective and high solvent consumption	[12]
J-acid	5.80	Highly commercial, high reagent consumption and 10 min is needed for color development	-
1-naphthylamine-7-sulphonic acid	5.32	Less selective, more reagent consumption and metal ions are interfered seriously	[14]
Sodium salt of hexamethylene imine carbodithioate	5.50	Needs extraction, less stable, high solvent consumption	[15]
Variamine blue	20	Less sensitive, low detection limit, maintenance of pH conditions	[16]
Resazurin	-	It require some catalytic reactions, high solvent consumption	[17]
DPH/4-AAP	3.5	Less sensitive, stable	[18]
Leuco methylene blue	0.2	More sensitive, rapid, facile, non extractive, economical reagent, highly stable (more than 15 days), metal ions do not interfere.	This work



SCHEME 1: Oxidation leuco methylene blue into methylene blue

Applications and comparison of proposed method with reported method

The proposed method for the analysis of selenium (IV) has been employed for various environmental and cosmetic samples were presented in (TABLE 2). The obtained results were compared with reported method in terms of Student's t-test and variance ratio f-test. The analytical data summarized in (TABLES 2) suggest that the percentage of selenium recovery from environmental and cosmetic samples ranges from 95.33 to 99.50%, 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 method in literature as shown in (TABLE 3).

CONCLUSION

The proposed method is simple, highly sensitive and rapid for the spectrophotometric determination of selenium (IV) in various environmental and analytical 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: oxidative reagent employed in the present method, i.e., leuco methylene blue is fairly soluble in water and very cheap. The color derivatives formed by oxidation is highly stable (more than 7 days) when compared to that of the reported methods (TABLE 2). Less interference, more accuracy, avoidance of lengthy extraction steps and statistical analysis which made present method to be placed among the most sensitive ones for the determination of Se (IV) in various environmental samples.

ACKNOWLEDGEMENT

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

REFERENCES

- [1] J.A.Tan, D.X.Zheg, S.F.Hou, R.B.Li, Z.Y.Zhu, W.Y.Wang; 'Selenium Ecological Chemicro-geography and Endemic Keshan Disease and Kasehin beck Disease in China', (1984).
- [2] G.F.Combs, J.E.Spallhoz, O.A.Levander, J.E.Oldfield; 'Selenium in Biology and Medicine' eds. Van nostrand Reinfold, New York, 859 (1987).
- [3] D.C.J.Adriano; Water, Air, Soil Pollution, **3**, 57/58 (1991).
- [4] F.A.Patty; 'Industrial hygiene and Toxicology', Wiley-Inter-Science, New York, **2**, 886 (1962).
- [5] O.A.Roseneeld Jand Beath; 'Selenium', Academic New York., 279 (1964).
- [6] J.E.Spall Bolz, J.L.Martin, H.E.Ganther; A.V.Publishing, West Pon, (1981).
- [7] Hao Da-Qing, Hong guo-Zie, Zhang Yi-min, Tian Guo-Jcn; Talanta, **43**, 595 (1996).
- [8] F.Macleod, B.A.Mc Gaw, C.A.Shand; Talanta, **43**, 1091 (1996).
- [9] I.I.Stewart A.Chow; Talanta, **40**, 1345 (1993).
- [10] A.H.Jeffrey, G.B.Roger, A.G.Carolys; J.AOAC, **75**, 269 (1992).
- [11] D.B.Porcella, G.L.Bowie, J.G.Sanders, G.A.Cutter; J.Water, Air, Soil Pollution, **3**, 57 (1991).
- [12] D.Agarwal, G.Sunitha, V.K.Gupta; J.Indian Chem. Soc., **73**, 151 (1998).
- [13] K.N.Ramachandran, R.Kaveeshwar, V.K.Gupta; Talanta, **40**, 781 (1993).
- [14] K.Pyrzynsaka; Anal.Sci., **13**, 629 (1997).
- [15] M.N.Pathare, A.D.Sawant; Anal.Lett., **28**, 317 (1995).
- [16] H.D.Revanasiddappa, T.N.Kiran Kumar; Anal.Sci., **17**, 1309 (2001).
- [17] A.Safavi, A.Afkhami; Anal.Lett., **28**, 1095 (1995).
- [18] K.Suresh, K.Suvaradhan, L.Krishnaiah, P.Chiranjeevi; Helvetica Chimica Acta, **88**, 343 (2005).
- [19] Ulf Ornenark and Olin A; Talanta, **41**, 1675 (1994).
- [20] J.R.Shapira; 'Organic Selenium Compounds their Chemistry and Biology', Wiley, Inter-Science, New York, 703 (1971).