

Spectrophotometric Determination of Vanadium Using Variamine Blue and its Application to Synthetic, Environmental and Biological Samples

T.N. Kiran Kumar and H.D. Revanasiddappa*

Department of Chemistry, University of Mysore, Manasagangothri, Mysore-570 006 India

(Received 9 May 2004, Accepted 27 April 2005)

A simple, rapid and accurate spectrophotometric method is described for the determination of trace amounts of vanadium using variamine blue (VB) as a chromogenic reagent. The method is based on the oxidation of variamine blue to form a violet-colored species on reaction with vanadium(V), having an absorption maximum at 570 nm. Beer's law is obeyed in the range of 0.1-2.0 $\mu\text{g ml}^{-1}$. The molar absorptivity and Sandell's sensitivity were found to be $1.65 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.003 \mu\text{g cm}^{-2}$, respectively. Optimum reaction conditions were evaluated in order to delimit the linear range. The effect of interfering ions on the determination is described. The proposed method has been successfully applied to the determination of vanadium in steel, pharmaceutical, environmental, and biological samples.

Keywords: Spectrophotometry, Vanadium determination, Variamine blue

INTRODUCTION

Vanadium is a trace element of highly critical role in biochemical processes and of significant importance in environmental, biological and industrial analysis due to its toxicity. The toxicity of vanadium is dependent on its oxidation state, with vanadium(V) being more toxic than vanadium(IV) [1]. Vanadium in trace amounts is an essential element for cell growth at $\mu\text{g l}^{-1}$ levels, also has been shown to inhibit cholesterol synthesis and to increase the oxidation of fatty acids of higher concentrations. It is excreted through urine. The amount of vanadium in blood and urine depends upon intensity and duration of its exposure. The Threshold Limit Values (TLV) reported are 0.5 mg/cubic meter of air and 0.1 mg/cubic meter of fume [2]. The amount of vanadium in excess of the TLV value is reported to cause anemia, cough, emaciation, irritation of mucous membrane, gastrointestinal

disturbances and bronchopneumonia [3]. Industrial applications of vanadium include dyeing, ceramics, ink and catalyst manufacture. Discharge from such sources can contribute to its presence in a water supply [4]. A substantial amount of vanadium is released burning crude petroleum, coal and lignite and it would then settle on the soil. Some plants accumulate vanadium up to $80 \mu\text{g ml}^{-1}$ by dry weight [5], and most of it will accumulate in leaves and roots [6]. Vanadium acts as a growth-promoting factor and participates in fixation and accumulation of nitrogen in plants [7], whereas high concentration of vanadium reduces the productivity of the plants. Therefore, the determination of vanadium in environmental, and biological samples is highly desirable.

Earlier, many spectrophotometric methods for the determination of vanadium based on the complex formation, redox reaction, ion-association, catalytic-kinetic reactions and solvent extraction have been reported in reviews [7,8]. Some of the recently proposed organic reagents for the spectrophotometric determination of vanadium include 5,7-

* Corresponding author.

E-mail: hdrevasiddappa@yahoo.com

dichloroxine-Rhodamine 6G [9], 6-chlor-3-hydroxy-7-methyl-2-(2-thienyl)-4H-chromen-4-one [10], 1,5-diphenylcarbazine [11]. In addition, several kinetic methods based on its the catalytic action of vanadium(V) on the oxidation of organic reagents have been reported [12,13]. However, most of these methods suffer from a number of limitations such as interference by large number of ions specially Zn(II), requiring extraction [9], standing for a long time for color development followed by extraction [10], lack of sensitivity, stringent reaction conditions and high blank values. The catalytic methods [12,13] have serious interference from the oxidants and reductants and also required expensive experimental set up. Although the highly sensitive spectrophotometric determination of vanadium with pyridylazoresorcinol (PAR) is often used as a standard method [14], it suffers from poor selectivity and interfering effect of some metal ions. Other methods for the determination of vanadium including ICP-AES [15], voltammetry [16] and AAS [17] have also some limitations in terms of high cost of instruments used in routine analysis and matrix effects. These deficiencies have encouraged the authors to develop a facile, sensitive, accurate and reliable method for the determination of trace amounts of vanadium using variamine blue (VB) as a chromogenic reagent. The utility of variamine blue as an analytical reagent has been well reviewed by Bishop [18]. The method has been successfully applied to the determination of vanadium in steel, pharmaceutical preparation, environmental, and biological samples.

EXPERIMENTAL

Apparatus

Jasco (model UVIDEK-610) and Elico (model SL-171) spectrophotometers with 1 cm matched quartz cells were used for all absorbance measurements. An Elico (model IL-610) digital pH meter was used for all pH measurements.

Reagents

All of the chemicals used were of analytical-reagent grade, and distilled water was used throughout the study.

Standard Solutions

Vanadium(V). A standard 1000 $\mu\text{g ml}^{-1}$ solution of

vanadium(V) was prepared by dissolving 0.2393 g of NaVO_3 in a 100-ml volumetric flask and diluting to the mark with water. Working solutions were prepared by appropriate dilution of the standard solution.

Variamine blue. As stock 0.05% solution of variamine blue was prepared by dissolving 50 mg of the reagent (Merck) in 25 ml methanol and made up to 100 ml with distilled water, and stored in an amber bottle.

Buffer solution. A buffer of solution was prepared by dissolving 13.6 g of sodium acetate trihydrate in 80 ml water and adjusting the pH to 3 with hydrochloric acid, and the mixture was diluted to 100 ml with water.

Procedures

General procedure for the determination of vanadium(V). An aliquot of a sample solution containing 0 to 20 μg of vanadium was transferred into a series of 10 ml calibrated flasks, followed by addition of 1 ml of 0.05% VB and 3 ml of the buffer solution of pH 3. The mixture was gently shaken until the appearance of a violet color, indicating the oxidation of VB. The contents were diluted to the mark with the buffer solution and mixed well. The absorbance of the colored solution was finally measured at 570 nm against a reagent blank.

Determination of vanadium in alloy steels. A 0.1 g amount of a steel sample containing 0.13% of vanadium was weighed accurately and placed in a 50-ml beaker. To it, was added 10 ml of 20% (v/v) sulfuric acid and carefully covered with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after addition of 5 ml of concentrated HNO_3 until all carbides were decomposed. Then, 2 ml of a 1:1 (v/v) H_2SO_4 solution was added and the mixture was evaporated carefully until the dense white fumes driven off the oxides of nitrogen, and then cooled to room temperature. After appropriate dilution with water, the contents of the beaker were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH solution in the presence of 1-2 ml of 0.01% (w/v) tartrate. The resulting solution was filtered, if necessary, through a Whatman No. 40 filter paper into a calibrated flask of known volume. The residue (silica) was washed with a small volume of hot 1% H_2SO_4 followed by water and the volume was made up to the mark with water.

Spectrophotometric Determination of Vanadium Using Variamine Blue

A suitable aliquot of the above solution was taken into a 10-ml calibrated flask and the vanadium content was determined by the general procedure using 1-2 ml of saturated thiocyanate or fluoride solution as masking agent. Iron(III) can be effectively removed from the solution by precipitation with saturated fluoride solution. The precipitates were filtered off before the addition of VB. Higher concentrations of iron(III) were removed by adding 5-10 ml of saturated ammonium thiocyanate solution to the test solution, and the resulting Fe(III) and Fe(II) complexes with thiocyanate were extracted into methyl isobutyl ketone (MIBK) in an aqueous acidic medium prior to the determination of vanadium.

Determination of vanadium in pharmaceutical sample.

A volume of 15 ml of elixir sample was treated with 10 ml of concentrated nitric acid; the mixture was then evaporated to dryness. The residue was leached with 5 ml of 0.5 M H₂SO₄. The solution was diluted to a known volume with water, after neutralizing with dilute ammonia. An aliquot of the made-up solution was analyzed for vanadium according to the general procedure for vanadium determination.

Determination of vanadium in soil. An air-dried homogenized soil sample (1 g) was weighed accurately and placed in a 100-ml Kjeldahl flask. The sample was digested in the presence of an oxidizing agent following the method recommended by Jackson [19]. The content of flask was filtered through a Whatman No. 40 filter paper, into a 25 ml calibrated flask and neutralized with dilute ammonia in the presence of 1-2 ml of 0.01% (w/v) tartrate solution. It was then diluted to the mark with water. Appropriate aliquots of 1-2 ml of the solution was transferred into a 10-ml calibrated flask and analyzed for vanadium content according to the general procedure, after adding 1-2 ml of 0.01% (w/v) thiocyanate or fluoride solution as masking agent [11].

Determination of vanadium in water. Each filtered environmental water sample (100 ml) was analyzed for vanadium. They tested negative. To these samples known amounts of vanadium(V) were added and analyzed by the proposed procedure for vanadium.

Determination of vanadium in urine. 50 ml of the urine sample was concentrated to 5 ml, by evaporation. To this solution was spiked a known amount of vanadium and mixed with 5 ml of concentrated HNO₃ and 5 g of potassium sulfate, and heated to dryness. The process was repeated 2-3 times.

Then HNO₃ (1:3, 25 ml) was added to the residue and digested on a water bath for 30 min [11]. The contents were again evaporated to dryness, cooled, and the residue was dissolved in water, filtered, and neutralized with dilute ammonia. The mixture was diluted to a known volume with water. Appropriate aliquots of this solution were taken and the proposed general procedure was followed for the vanadium determination.

Determination of vanadium in biological samples. The samples of plants and animal tissues were washed with distilled water to get them free from adhering soil or blood. They were carefully wiped with filter paper before taking their wet weight. The samples were then dried, ashed and brought into solution by acid treatment as per standard procedures [20,21], and neutralized with dilute NH₄OH and then diluted to a known volume with water. An appropriate aliquot of this solution was finally analyzed according to the general procedure for vanadium. Since the vanadium content in samples used was negligible, synthetic samples were prepared by the addition of known amounts of vanadium to each sample prior to digestion.

RESULTS AND DISCUSSION

The method involves the oxidation of variamine blue by vanadium(V) in the presence of a buffer medium of pH 3 to form a violet-colored solution, which shows a maximum absorbance at 570 nm. The reagent blank has a negligible absorbance at this wavelength. The absorption spectra of the colored solution are depicted in Fig. 1, and the reaction system is represented in Scheme 1. Constant and maximum absorbance values were obtained in the pH range 2.0-4.0. This could be achieved by the addition of 3 ml of buffer solution of pH 3 in a total volume of 10 ml. A change in the pH range will markedly affect the stability of the colored complexes, whereas the color development did not take place below pH 2.

The optimum concentration of VB leading to maximum color stability was found to be 1.0 ml of 0.05% reagent per 10 ml of the reaction mixture. A 3 ml portion of a buffer solution of pH 3 was found to be sufficient for complete and maximum color development. Good results are obtained when the reaction mixture is diluted with a buffer solution of pH 3. The maximum absorbance was obtained instantaneously and

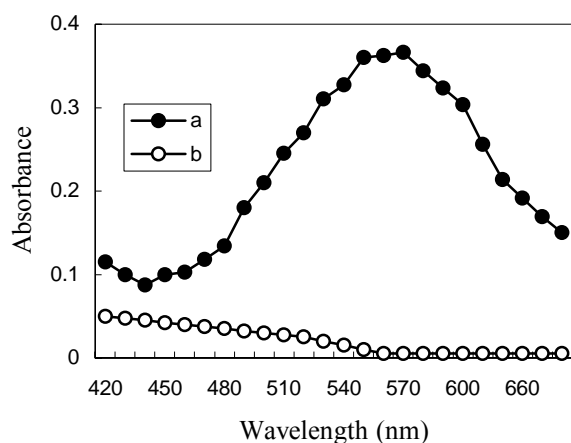


Fig. 1. Absorption spectra of colored V(V)-VB adduct (V(V), $1 \mu\text{g ml}^{-1}$) vs. a reagent blank (a) and reagent blank vs. distilled water (b).

required no heating or time gap under the experimental conditions. Under the optimum conditions, the color system was stable for a period of 30 min.

Analytical Data

A linear calibration graph was obtained for 1 to 20 μg of vanadium in a final volume of 10 ml. The detection limit and quantitation limit of vanadium determination were found to be 0.01 and 0.04 $\mu\text{g ml}^{-1}$, respectively. The calibration graph has a correlation coefficient of 0.999. The molar absorptivity, specific absorptivity and Sandell's sensitivity of the colored system were found to be $1.7 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, $0.03 \text{ ml g}^{-1} \text{ cm}^{-1}$ and $0.003 \mu\text{g cm}^{-2}$, respectively. The reproducibility of the method was established by the analysis of standard solutions of 3, 6 and 8 μg of vanadium in a final volume of 10 ml. Ten replicate determinations of each concentration resulted in relative standard deviations of 0.008, 0.006 and

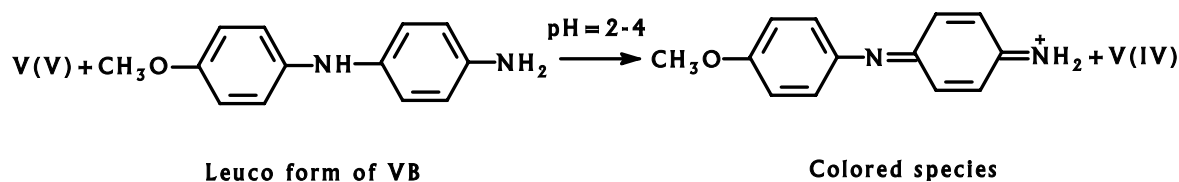
Table 1. Effect of Interfering Ions on the Determination of Vanadium(V) [$1 \mu\text{g ml}^{-1}$]

Interferents	Tolerance Limit ($\mu\text{g ml}^{-1}$)
Na^+ , K^+ , Ba^{2+} , Pb^{2+} , citrate, Hg^{2+} , Ni^{2+} , NO_3^- , I^- , F^- , Ca^{2+} , Cl^- , SO_4^{2-} , tartrate, oxalate, CH_3COO^- , Zn^{2+} , Br^- , Mn^{2+} , SCN^-	≤ 3000
Al^{3+} , SeO_3^{2-} , SbO_7^{2-} , NO_2^- , EDTA	≤ 500
CrO_4^{2-} , Fe^{3+} , Ce^{4+} , WO_4^{2-}	≤ 1
Fe^{2+} , IO_3^- , $\text{S}_2\text{O}_3^{2-}$, Cu^{2+} , MoO_4^{2-}	≤ 40

0.003%, for 3, 6 and 8 μg of vanadium, respectively.

Effect of Interfering Ions

The influence of various potential interferents on the determination of vanadium by the proposed procedure was examined. The results of these experiments are shown in Table 1. The tolerance limits of the interfering species were established at those concentrations that do not cause more than $\pm 2\%$ error in the absorbance values of vanadium at a $1.0 \mu\text{g ml}^{-1}$ level. The developed method is based on the oxidation of VB with vanadium. Therefore, strong oxidizing or reducing species are expected to interfere by oxidation of VB or the reduction of V(V). Chromium(VI), iron(III), cerium(IV) and



Scheme 1. Reaction between V(V) and VB

Spectrophotometric Determination of Vanadium Using Variamine Blue

tungsten(VI) at a 10 µg level caused low recovery of vanadium. Iron(II), copper(II), iodate, molybdenum(VI), and thiosulphate up to 400 µg level caused positive interferences. Masking agents like citrate, tartrate, EDTA and sodium fluoride are not interfere in the recovery of vanadium. Therefore, these masking agents were used to obviate interferences such as iron(III), cerium(IV) and tungsten(VI) up to a 10 µg level in the determination of vanadium. Small concentrations of As(III) at temperature ≥ 40 °C can effectively reduce chromium(VI) and quantitatively eliminate its effects on the coloring reagent. Therefore, As(III) ion was adopted as an effective reducing agent for Cr(VI) in the

presence of vanadium(V) [22]. If a precipitate was formed during the interference studies, it was removed by centrifugation.

Advantages of the Proposed Method

The rapidity of color development with VB is an advantage in analyzing various samples, in which vanadium can vary over a wide range. When compared to other existing methods, the developed method retains the specific interaction of vanadium(V) with VB to form a colored adduct and a good sensitivity is achieved at room temperature without the need for extraction; hence, the use of organic solvents, which are

Table 2. Determination of Vanadium in Various Samples

Sample	Vanadium added (ppm)	Proposed method			Reference method ¹⁴			F- test ^b	t-test ^c
		Vanadium found (ppm) ^a	RSD (%)	Recovery (%)	Vanadium found, (ppm) ^a	RSD (%)	Recovery (%)		
Steel ^d	-	3.88 ± 0.04	1.0	99.5	3.87 ± 0.05	1.3	99.2	1.6	0.3
(0.1 g/100 ml)	6.0	9.87 ± 0.03	0.3	99.9	9.85 ± 0.04	0.4	99.8	1.8	0.9
Pharmaceutical preparation ^e	-	7.86 ± 0.05	0.6	99.6	7.84 ± 0.06	0.8	99.3	1.4	0.6
(15 ml/150 ml)	4	11.85 ± 0.03	0.3	99.9	11.82 ± 0.04	0.3	99.8	1.8	1.3
Soil ^f (1 g)	5.0	4.99 ± 0.03	0.6	99.8	4.98 ± 0.04	0.8	99.6	1.8	0.5
	8.0	7.98 ± 0.02	0.3	99.8	7.97 ± 0.03	0.4	99.6	2.3	0.6
Natural water ^f	6.0	5.98 ± 0.04	0.7	99.7	5.97 ± 0.05	0.8	99.5	1.6	0.3
	9.0	8.99 ± 0.02	0.2	99.9	8.98 ± 0.03	0.3	99.7	2.3	0.6
Urine ^f	7.0	6.99 ± 0.05	0.7	99.9	6.97 ± 0.06	0.9	99.6	1.4	0.6
	10.0	9.98 ± 0.04	0.4	99.8	9.96 ± 0.04	0.4	99.6	1.0	0.8
Plant material ^f	8.0	7.98 ± 0.04	0.5	99.8	7.96 ± 0.05	0.6	99.5	1.6	0.7
(cabbage 5 g)	12.0	11.97 ± 0.02	0.2	99.8	11.95 ± 0.03	0.3	99.6	2.3	1.3
Goat liver ^f	7.0	6.98 ± 0.04	0.6	99.7	6.96 ± 0.05	0.7	99.4	1.6	0.7
(3 g)	9.0	8.98 ± 0.03	0.3	99.8	8.96 ± 0.02	0.2	99.6	2.3	1.3

^a Mean ± standard deviation (n = 5). ^b Tabulated F-value for (4,4) degrees of freedom at *P* (0.95) is 6.39. ^c Tabulated t-value for 8 degrees of freedom at *P* (0.95) is 2.306. ^d GKW Steel Ltd., India [C, 0.54%; Mn, 0.89%; S, 0.018%; P, 0.034%; Si, 0.33%; Cr, 1.02%; V, 0.13%], vanadium taken 3.9 ppm. ^e Neogadine Elixir[®], Raptakos Brett & Co. Ltd. India [Each 15 ml contains Iodised peptone 29 mg, magnesium chloride 20 mg, magnesium sulfate 4 mg, sodium metavanadate 0.66 mg, zinc sulfate 6 mg, pyridomine HCl 0.75 mg, cyanocobalamin 0.5 µg, nicotinamide 10 mg, alcohol (95%) 0.95 ml, Total alcohol 6% (v/v)], vanadium taken 7.89 ppm. ^f Gave no test for vanadium.

generally toxic or carcinogens, are avoided. The proposed method has significant advantages over other spectrophotometric methods in terms of its simplicity and free from most interfering substances. Accurate and reproducible results are obtained with permissible standard deviations.

Applications

The vanadium contents of alloy steels, pharmaceutical preparations, soil, water, urine, plant material and animal tissue samples were analyzed by the proposed method, and the results are summarized in Table 2. In all cases, the results were compared with the pyridylazoresorcinol (PAR) method [14] and the performance of the proposed method was judged based on the Student's t- and F-tests. At a 95% confidence level, the calculated t- and F-values do not exceed the theoretical values (Table 2). Therefore, there is no significant difference between the proposed and the reference method, indicating that the developed method is as accurate and precise as the reference method [14] and the certified values of the samples.

CONCLUSIONS

In comparison with most instrumental techniques (*e.g.*, ICP-MS or chromatographic methods) that usually require prior separation and/or pre-concentration processes, the present method describes a new, simple, sensitive, cost-effective, precise, reliable and reproducible spectrophotometric method for vanadium determination in real matrices as well as in synthetic samples. The developed method does not involve any troublesome reaction conditions and it can be used as an alternative method for the determination of vanadium in different samples.

ACKNOWLEDGEMENT

One of the authors (T.N. Kiran Kumar) is grateful to the University Grants Commission, New Delhi, and Department of Collegiate Education, Government of Karnataka, for awarding a Teacher Fellowship.

REFERENCES

- [1] B. Patel, G.E. Henderson, S.J. Haswell, R. Grzeskowiak, *Analyst* 115 (1990) 1063.
- [2] I.M. Kolthoff, P.I. Elving, F.H. Stross, *Treatise on Analytical Chemistry, Part III, Vol. 2*, Wiley Interscience, New York, 1971, p. 89.
- [3] F.A. Patty, *Industrial Hygiene and Toxicology; Vol. II*, Interscience Publishers, New York, 1963, p. 1171.
- [4] APHA, *Standard Methods for the Examination of Water and Wastewater, 19th Edn.*, American Public Health Association, Washington DC, 1995, p. 3-101.
- [5] H. Helmut, *Metal Ions in Biological Systems*, Marcel Dekker Inc, Vol. 6, New York, 1976.
- [6] K.R. Paul, V.K. Gupta, *Am. Ind. Hyg. Assoc. J.* 43 (1982) 529.
- [7] M.J.C. Taylor, J.F. van Staden, *Analyst* 119 (1994) 1263.
- [8] H.A. Mottola, D. Perez-Bendito, *Anal. Chem.* 66 (1994) 131R.
- [9] K. Luxmi Varma, M.P.L. Reddy, T. Prasad Rao, *Chem. Anal. (Warsaw)* 45 (2000) 745.
- [10] N. Agnihotri, R. Dass, J.R. Mehta, *Anal. Sci.* 15 (1999) 1261.
- [11] M.J. Ahmed, S. Banoo, *Talanta* 48 (1999) 1085.
- [12] T.S. Sikalos, Y.M. Arabatzis, M.I. Prodromidis, P.G. Veltsistas, M.I. Karayannis, *Mikrochim. Acta* 135 (2000) 197.
- [13] A.A. Mohamed, K.F. Fawy, *Anal. Sci.* 17 (2001) 769.
- [14] Z. Marczenko, *Separation and Spectrophotometric Determination of Elements, 2nd Edn.*, Ellis Harwood Ltd., Chichester, England, 1986, p 627.
- [15] R.G. Wuilloud, J.A. Salomia, R.A. Olsima, L.D. Martinez, *Spectrochim Acta Part B: Atom Spectr.* 55 (2000) 671.
- [16] A.A. Ensafi, B. Naderi, *Fresenius' J. Anal. Chem.* 358 (1997) 480.
- [17] T. Yanashige, M. Yanamoto, H. Sunahara, *Analyst* 114 (1989) 1071.
- [18] Edmund Bishop (Ed.), *Indicators*, Pergamon Press Ltd.,

Spectrophotometric Determination of Vanadium Using Variamine Blue

- Oxford, Inc., New York, 1972, p. 395.
- [19] M.L. Jackson, Soil Chemical Analysis, Prentice-Hall, Englewood Cliffs, 1987, p. 326.
- [20] S.A. Abbasi, Anal. Lett. 20 (1987) 1347.
- [21] D. Glick (Ed.), Methods of Biochemical Analysis, John Wiley, Vol. 21, 1973, p. 39.
- [22] A.A. Mohamed, M.F. El-Shahat, Anal. Sci. 16 (2000) 151.