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A Highly Sensitive and Selective Spectrofluorimetric Method for the Determination of Vanadium at Pico-Trace Levels in Some Real, Environmental, Biological, Soil and Food Samples Using 2-(α-Pyridyl)-Thioquinaldinamide

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Abstract

A new spectrofluorimetric reagent 2-(α-pyridyl)-thioquinaldinamide (PTQA) has been synthesized and characterized through novel reaction techniques. A very simple, ultra-sensitive and highly selective non-extractive new spectrofluorimetric method for the determination of vanadium at Pico-trace levels using 2-(α-pyridyl)-thioquinaldinamide (PTQA) has been developed. PTQA has been proposed as a new analytical reagent for the direct non-extractive spectrofluorimetric determination of vanadium (V). This novel fluorimetric reagent, PTQA becomes oxidized in a slightly acidic (0.0035 - 0.0085 M H_2SO_4) solution within vanadium (V) in 20% ethanol to produce highly fluorescent oxidized product (λ_{ex} = 319 nm; λ_{em} = 371 nm). Constant and maximum fluorescence intensities were observed over a wide range of acidity (0.0035 - 0.0085 M H_2SO_4) for the period between 5 min and 24 h. Linear calibration graphs were obtained for 0.001 - 600-μg·L^{-1} of V, having a detection limit of 0.3-ng·L^{-1}; the quantification limit of the reaction system was found to be 3-ng·L^{-1} and the RSD was 0% - 2%. A large excess of over 60 cations, anions and complexing agents (like, chloride, phosphate, azide, tartrate, oxalate, SCN^- etc.) do not interfere in the determination. The developed method was successfully used in the determination of vanadium in several Certified Reference Materials (alloys, steels, serum, bovine liver, drinking water, soil and sediments) as well as in some environmental waters (potable and pol-

luted), biological fluids (human blood, urine, hair and milk), soil samples and food samples (vegetables, rice and wheat) solutions containing both vanadium (IV) and vanadium (V) speciation and complex synthetic mixtures. The results of the proposed method for assessing biological, food and vegetable samples were comparable with inductively coupled plasma optical emission spectroscopy (ICP-OES) and atomic-absorption spectrophotometer (AAS) was found to be in excellent agreement.

Keywords

Spectrofluorimetry, Vanadium-Determination,
2-(*α*-Pyridyl)-Thioquinaldinamide, Environmental, Biological, Soil, Food-Samples

1. Introduction

Vanadium is an essential mineral [1]. Vanadium is used for treating diabetes, low blood sugar, high cholesterol, heart disease, tuberculosis, syphilis, a form of "tired blood" (anemia), and water retention (edema); for improving athletic performance in weight training; and for preventing heart disease and cancer. Over the last several years, a diverse range of bio-logical actions of various vanadium compounds has been documented [1] [2]. Among these, the cardio-protective effects of vanadium have been studied in great detail, and several studies have demonstrated that the vanadyl (IV) form of vanadium possesses cardio-protective properties. Treatment with inorganic and organic compounds of vanadium has been shown to exert a wide range of cardio-protective effects in myocardial ischemia/reperfusion-induced injury, myocardial hypertrophy, hypertension, and vascular disease [1] [2] [3]. Vanadium in trace amounts is important industrially [4], as a: biological nutrient [5], epidemiological preventive [6] and occupational health hazard [7] and it has been said that vanadium can serve as an inhibitor and/or cofactor in various enzyme systems [8] and plays an important role in prevention of heart disease [8]. Human studies suggest that vanadium may lower blood sugar levels and improve insulin sensitivity in patients with type 2 diabetes, according to the University of Maryland Medical Center [9]. Vanadium has become subject of interest among nutritionists since the discovery that various marine species have this metal as an essential element [10] [11]. Foods are the major source of exposure to vanadium for the general population because most of foods contain allow amount of vanadium (<1 ng/g) [12]. Deficiency problems of vanadium have not been clearly shown in humans, though there is a suspicion that low vanadium can increase susceptibility to heart disease and cancer or lead to higher cholesterol and triglyceride levels [13]. Vanadium is not commonly supplemented or contained in many vitamin-mineral combinations. Some newer formulae may contain small amounts. Eating fish and using vegetable oils in the diet will usually supply sufficient vanadium. On the other hand, it is one of the most important heavy metal pollutants [14]. Its compounds

can be highly toxic to humans and animals and cause serious diseases [14]. The literature [13] reports that the toxicity of vanadium depends on its oxidation state, V (V) being more toxic than other species. Therefore, all these facts make it a prime necessity for an accurate determination vanadium at trace levels using simple and rapid methods is of paramount importance.

Many methods have been proposed for the determination of vanadium which include spectrophotometry [15]-[20] and catalytic kinetic methods [21]. Those methods, although sensitive enough, faced several problems with selectivity, especially in food matrixes, where the abundant presence of other metallic ions such as iron, copper, zinc, and molybdenum presented significant interferences. Recently a non-aqueous catalytic method was proposed, after cloud point extraction [22], yielding remarkable results. Electrochemical approaches also produced good results for the determination of vanadium in water samples [23] [24], but in large, the use of element-specific techniques is usually recommended for the analysis of vanadium in food samples [25] [26] [27] [28]. Sophisticated techniques, such as Inductively coupled plasma mass spectrometry (ICP-MS) [29], Inductively coupled plasma atomic emission spectroscopy (ICP-AES) [30] and Inductively coupled plasma optical emission spectroscopy (ICP-OES) [31] [32] as well as flame atomic absorption spectrophotometry (AAS) [33] have been used for the determination of total vanadium. ICP-MS and ICP-OES are highly expensive and are not used for routine analysis in the developing country like Bangladesh. AAS is sensitive but suffer from interferences of matrix condition of samples, such as salinity [34]. However, the spectrofluorimetry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. This method still has the advantages of being simple and without requiring expensive or complicated test equipment. For this reason, a wide variety of spectrofluorimetric methods for determination of vanadium has been developed [35]-[60]. Several authors have reported on the extractive spectrofluorimetric determination of vanadium using complexes formed variety of reagents [35]-[60]. In most of the methods [35] [36] [37] [38] cited in literature as shown in **Table 1**, vanadium forms soluble or insoluble complexes with reagents with various organic solvents for spectrofluorimetric determination of vanadium. Most of these reagents are expensive and non-recoverable. Most of the organic solvents which were used are themselves carcinogenic according to EPA [61]. However, interference from coexist ions, especially for some rare earth ions, Fe^{3+}, Mo^{VI} and PO_4^{3-} are always faced in the existing methods.

The goal of the present work was to develop a simpler direct spectrofluorimetric method for the pico-trace determination of vanadium. In the search for a more sensitive reagent, in this work a new reagent was synthesized according to the method of Porter [62] and a oxidation reaction of 2-(*α*-pyridyl)-thioquinaldinamide (PTQA); with V (V) and forms an intensely fluorescent oxidized product. Although PTQA has been reported to be spectrofluorimetric reagent for Cr (VI) [63], Se (IV) [64] and Mn (VII) [65] but has not previously been used for the

Table 1. Comparison of present and existing spectrofluorimetric methods for vanadium.

Reagent	Nature of reaction	Solvent	Medium Aqueous/Surfactant/ Organic	Acidity/pH	λ_{ex}:λ_{em} (nm)	Beer's Law ($\mu g \cdot L^{-1}$)	Detection Limit ($\mu g \cdot L^{-1}$)	RSD (%)	Interference	Remarks
o-phenylenediamine[35]	Catalytic oxidation	Bromate	Gallic acid	4.0	415:555	0 - 8	6.0	5.0	Many, humic acid and Fe^{3+}	i) Less selective due to much interference ii) Less sensitive iii) Lengthy and time consuming
Azomethine-H[36]	Catalytic oxidation	potassium bromate	Aqueous	4.2	382:422	5.0×10^{-4} - 0.022	7.0	5	Fe (III), Ce (IV)	i) Less sensitive ii) Time consuming iii) Less selective due to much interference
l-amino-4-hgdroxyanthra auinone[37]	Oxidation	Ethanol	Sulfuric acid	4.2	480:575	100 - 530	50	5	Mo (VI), Fe (III), Ce (IV)	i) Less selective due to much interference ii) Lengthy and time consuming iii)pH dependent
rhodamine 6G (R6G)[38]	Catalytic Oxidation	EDTA	Sulfuric Acid	2.5	525:555	20 - 300	2.0	4.3	Many	i) Less sensitive ii) Time consuming iii) Less selective due to much interference iv)pH dependent
2-(*a*-Pyridyl)-thioquinaldi namide **(Present Method)**	Oxidation reaction	Ethanol	Acidic medium	0.05 M	319:371	0.001 - 600	0.3 $ng \cdot L^{-1}$	0 - 2	Nil, using suitable masking agents	i) Ultra sensitive ii) Highly selective iii) Pico-trace levels determination ($pg \cdot g^{-1}$) iv) Fluorescence is stable for 24 h. v) Very simple, rapid and non-extractive. vi) No organic toxic and carcinogenic solvents were used.
1,8-diaminonaphthalene[39]	Catalytic oxidation	KBr with Tiron	weakly acidic medium	3.6 - 4.0	356:439	0.05 - 50	8	3	Cu(II) and Fe(III) interfere seriously	i) Time consuming & hence lengthy ii) Less selective due to much interference ii) Less sensitive iii) pH dependent

spectrofluorimetric determination of vanadium. The method possesses distinct advantages over existing methods [35]-[60] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision and ease of operation. The method is based on the oxidative reaction of non-fluorescent PTQA in a slightly acidic (0.0035 - 0.0085 M H_2SO_4) solution with V(V) in presence of 20% ethanol to produce a highly fluorescent oxidized product, followed by a direct measurement of the fluorescence intensity in an aqueous solution at room temperature. Oxidation is very rapid and no extraction is required. With suitable masking, the reaction can be made to be highly selective and the reagent blank solutions do not show any fluorescence.

2. Materials and Methods

2.1. Apparatus

A Shimadzu (Kyoto, Japan) (Model-RF-5301PC) Spectrofluorophotometer with 1-cm quartz cells were used and a Jenway (England, UK) (Model-3010) pH meter with combination of electrodes were used for measurements of the fluorescence intensity and pH, respectively. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate (10-mg·L^{-1}). A Shimadzu (Kyoto, Japan) (Model-9800) Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), [λ = 418 nm, plasma gas flow rate (L·min^{-1}) = 15, LOD: 1-μg·L^{-1} of V, RF Power (W) = 1400, Nebulizer gas flow rate (L·min^{-1}) = 1 - 10 and a Shimadzu (Kyoto, Japan) (Model: AA7000) atomic absorption spectrophotometer equipped with a microcomputer-controlled air-acetylene flames were used to compare of the results. The Elemental Analyzer (Exeter Analytical Inc. Model: CE 440) equipped with supersensitive thermal conductivity detector for simultaneous determination of CHN was used. Infrared spectrum was recorded with a FTIR Spectrophotometer, Shimadzu (Kyoto, Japan) (Model-IR Prestige 21, Detector DTGS KBr) in the range 7500 - 350 cm^{-1} and Model: JEOL 500SS, magnetic field strength: 500 MHz, solvent used: DMSO D6, standard: TMS, four channel NMR spectrometer with signal-to-noise ratio of 5000:1 for proton were used for characterization of the ligand.

2.2. Synthesis and Characterization of the Reagent

2.2.1. Synthesis of the Reagent

2-(*α*-pyridyl)thioquinaldinamide (PTQA, $C_{15}H_{11}N_3S$) (Molecular wt. = 265.18) was synthesized according to the method of Porter [62]. The mixture containing 2-aminopyridin, quinaldine and sulphur powder in the molar ratio of 2:1:1.5 were mixed and refluxed for 6 hours in 250-mL round bottom flask fitted with bulb condenser under controlled temperature (140 - 150)˚C at 1 atm. pressure over oil bath. The reaction mixture was kept overnight. The thio-compound was filtered and crystallized using petroleum ether (60 - 80)˚C to give a bright yellow crystalline (needle shaped) solid. The compound recrystallized from lime-distilled ethanol and was kept under vacuum (0.1 mm of Hg) for 24 hours. Yield of the product was 70%. The structure of the reagent is shown in **Figure 1**.

2.2.2. Characterization of the Reagent

The reagent (PTQA) was characterized by taking the melting point, elemental analysis and an FTIR spectrum (**Figure 2**) and ^{1}HNMR spectrum (**Figure 3**) and thermogravimetric analysis (**Figure 4**). The melting point of the synthesized compound (PTQA) was 155˚C ± 2˚C (lit. 155˚C ± 1˚C) [65] which indicated the purity of PTQA.

The results elemental analysis (C = 72.25%, N = 13.35% and H = 4.25%) of the reagent are very in good agreement with the calculated values (C = 72.43%, N = 13.55% and H = 4.55%). The FTIR spectrum of prepared reagent (PTQA) is

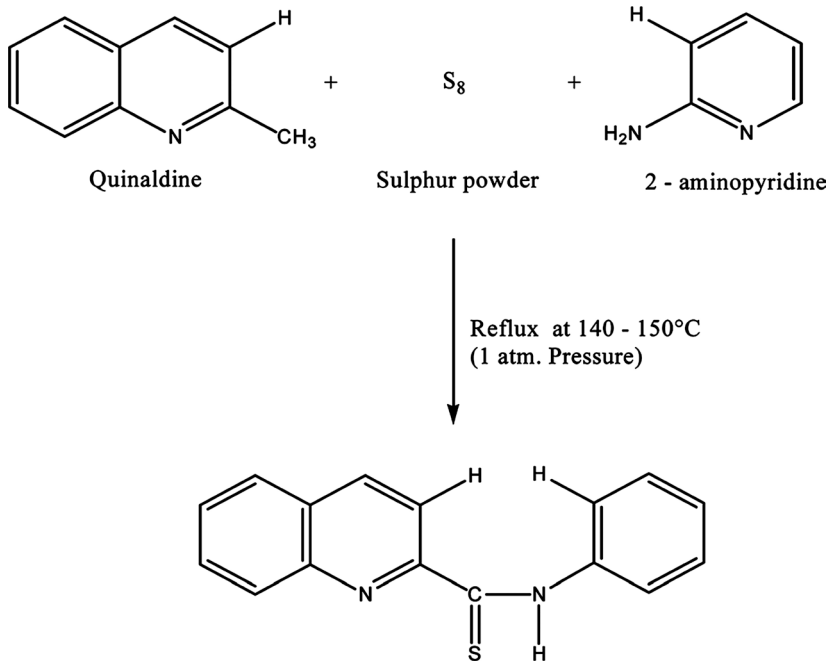

Figure 1. Reaction scheme of 2-(*α*-pyridyl)-thioquinaldinamide (PTQA).

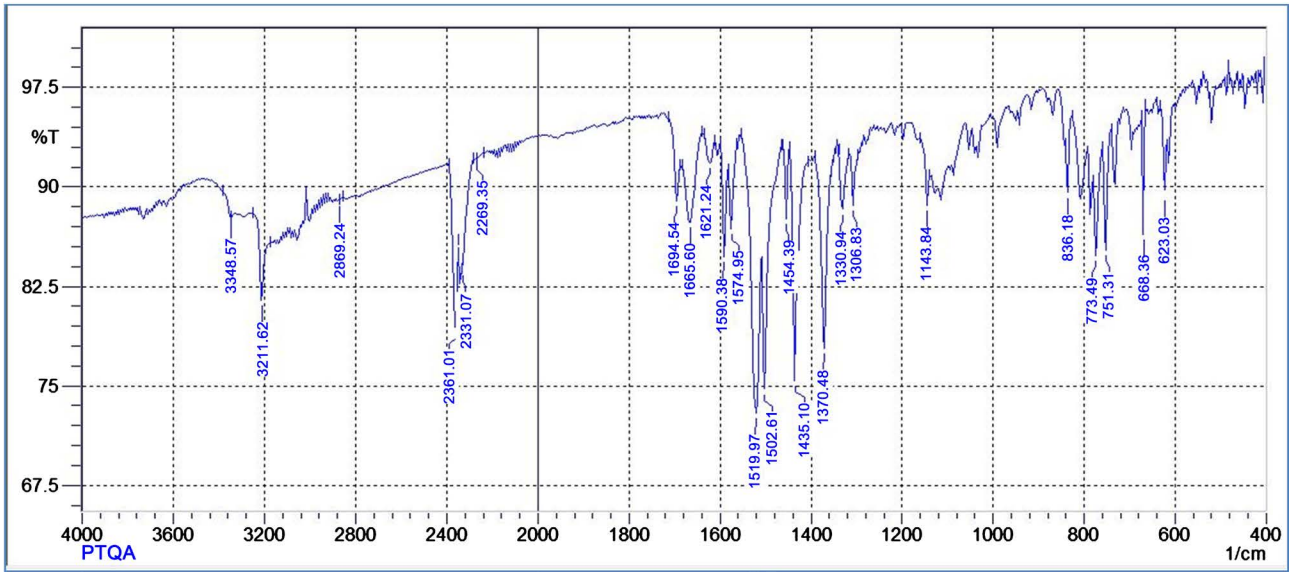

Figure 2. FTIR spectrum of 2-(*α*-pyridyl)-thioquinaldinamide (PTQA).

shown in **Figure 2**. The presence of FTIR peak at 1126.43 cm^{-1} in **Figure 2** was due to the characteristic C=S double bond peak ($\nu^{C=S}$, 1050 - 1200 cm^{-1}) [65] of the reagent indicating the formation of PTQA. Both FTIR spectral and elemental analysis data indicated the formation of the reagent PTQA. The formation of the reagent also tested by ^{1}HNMR spectrum is shown in **Figure 3**. The steadiness of the thermogravimetric curve (**Figure 4**) obtained for about 1 g of the reagent at 80˚C - 90˚C indicated that the reagent did not contain any moisture.

The elemental analysis was performed by the National Center of Excellence in Analytical Chemistry, University of Sindh, Pakistan and FTIR spectra was recorded

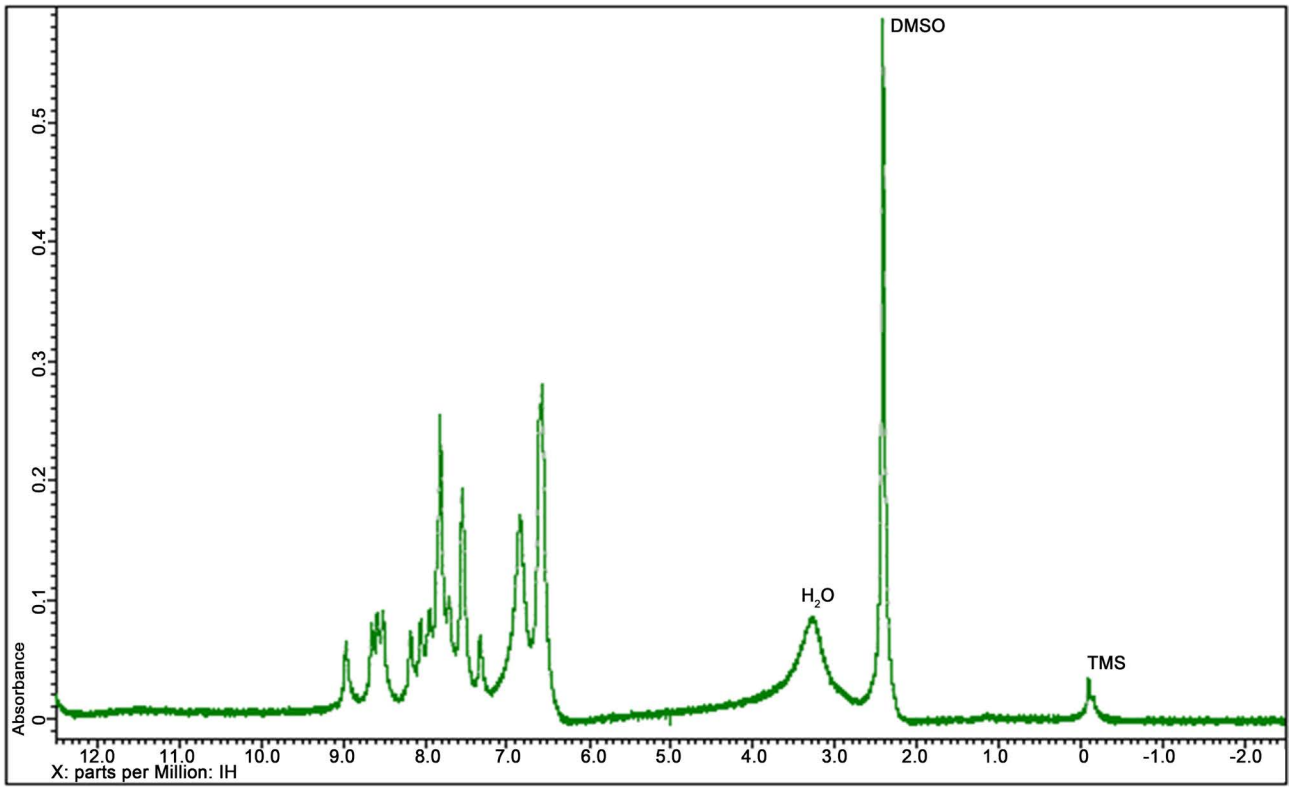

Figure 3. [1]HNMR spectrum of 2-(*α*-pyridyl)-thioquinaldinamide (PTQA).

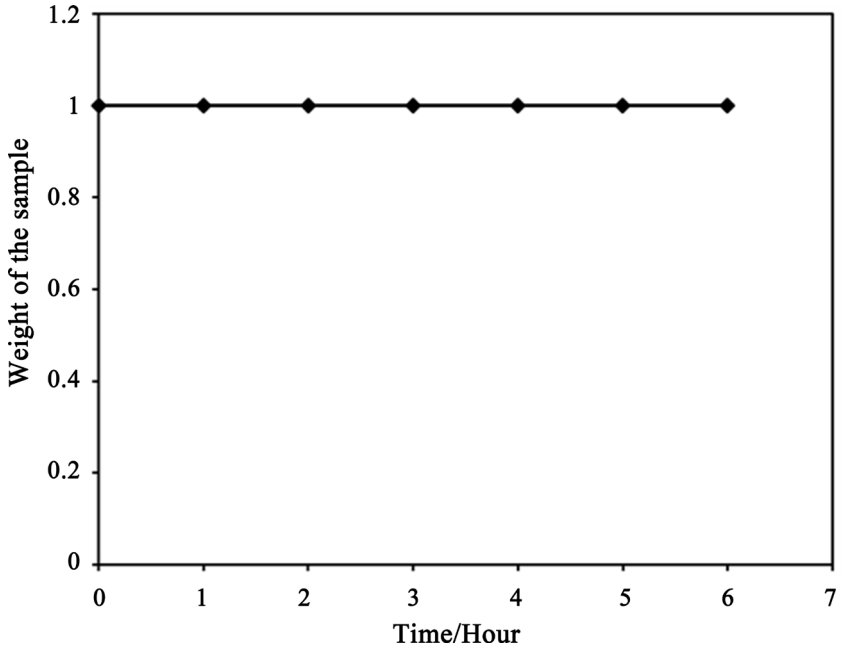

Figure 4. Thermogravimetric curve of 2-(*α*-pyridyl)-thioquinaldinamide at 80˚C - 90˚C.

with FTIR spectrophotometer Shimadzu (Kyoto, Japan) (Model-IR Prestige 21, Detector DTGS KBr) in the range 7500 - 350 cm^{-1} from our laboratory and [1]HNMR spectrum was recorded [1]HNMR spectrophotometer, Model: JEOL 500SS from University of Kanazawa, Japan.

3. Reagents and Solutions

All the chemicals used were of analytical reagent grade of the highest purity available. High-purity absolute ethanol and high-purity de-ionized water was used throughout. High-purity water was obtained by passing tap water through cellulose absorbent and to mixed-bed ion exchange columns, followed by distillation in a corning AG-11 unit. Glass vessel were cleaned by soaking in acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$ followed by washing with concentrated HNO_3 and rinsed several times with high purity de-ionized water. Stock solutions and environmental water sample (1000-mL each) were kept in polypropylene bottles containing 1-mL concentrated HNO_3. More rigorous contamination control was used when the vanadium levels in the specimens were low.

3.1. PTQA Solution (3.77×10^{-3} M)

The reagent solution was prepared by dissolving the requisite amount (0.0026 g) of PTQA, in a known volume (10-mL) of absolute ethanol. A freshly prepared reagent solution (3.77×10^{-4} M) was used whenever required.

3.2. Vanadium (V) Standard Solution (1.96×10^{-3} M)

A 100-mL amount of stock solution (1-mg·mL^{-1}) of penta valent vanadium was prepared by dissolving 229.6 mg of ammonium metavanadate, (NH_4VO_3), (Sigma-Aldrich, Merck KGaA, Germany, pro-analysis grade, 99.5%) in doubly distilled de-ionized water containing 1-2-mL of concentrated nitric acid (1:1). Aliquots of this solution were standardized with EDTA [66]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required. A freshly standardized solution was always used.

3.3. Vanadium (IV) Standard Solution (1.96×10^{-3} M)

A 100-mL amount of stock solution (1-mg·mL^{-1}) of trivalent vanadium was prepared by dissolving 390.7-mg of vanadyl sulfate (Fisher Scientific, pro-analysis grade 99.6%) in doubly distilled de-ionized water containing 1-2-mL of concentrated nitric acid (1:1). Aliquots of this solution were standardized with EDTA [66]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required. A freshly standardized solution was always used.

3.4. Potassium Dichromate Solution

A 100-mL amount of stock solution (0.1 N) was prepared by dissolving 500 mg of finely powdered $K_2Cr_2O_7$ (Merck) in 100-mL deionized water.

3.5. Ammonium Persulfate Solution

Ammonium persulfate solution (2% w/v) (A.C.S-grade 99% pure) was freshly prepared by dissolving 2 g in 100-mL of deionized water.

3.6. Tartrate Solution

A 100-mL stock solution of tartrate (0.01% w/v) was prepared by dissolving 10-mg of A.C.S.-grade (99% pure) potassium sodium tartrate tetrahydrate in (100-mL) de-ionized water.

3.7. Aqueous Ammonia Solution

A 100-mL solution of an aqueous ammonia solution was prepared by diluting 10 mL concentrated NH_4OH (28% - 30%, A.C.S.-grade) to 100-mL with de-ionized water. The solution was stored in a polypropylene bottle.

3.8. EDTA Solution

A 100-mL stock solution of EDTA (0.01% w/v) was prepared by dissolving 10-mg A.C.S.-grade (≥99% pure) ethylene diamine tetra acetic acid as disodium salt dehydrate in (100-mL) de ionized water.

3.9. Other Solutions

Solutions of a large number of inorganic ions and complexing agents were prepared from their AnalaR grade or equivalent grade water-soluble salts (or the oxides and carbonates in hydrochloric acid); those of Niobium, Tantalum, Titanium, Zirconium and Hafnium were specially prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the recommended procedures of Mukharjee [67]. In the case of insoluble substances, special dissolution methods were adopted [68].

4. Procedure

To 0.1 - 1.0-mL of a neutral aqueous solution containing 0.01 - 6000-ng of vanadium (V) in a 10-mL calibrated flask was mixed with a 1:130 - 1:300-fold molar excess (preferably 1.5-mL) of 3.77×10^{-3} M of the 2-(*α*-pyridyl)-thioquinaldinamide (PTQA) reagent solution followed by the addition of 0.5 - 2-mL (preferably 1-mL) of 0.05 M of sulfuric acid. The solution was mixed well and allowed to stand for 5 min after which 2-mL of absolute ethanol was added and the mixture was diluted to the mark with de-ionized water. The fluorescence intensity of the system was measured at 371-nm against a corresponding reagent blank, prepared concurrently, keeping the excitation wavelength maximum at 319-nm and the instrument setting the same. The vanadium content in an unknown sample was determined using a concurrently prepared calibration graph.

5. Sample Collection and Preservation [69]

5.1. Environmental Samples

Water and soil samples were collected in polythene bottles from different places of Bangladesh. After collection, HNO_3 ($1\text{-mL}\cdot\text{L}^{-1}$) was added as preservative.

5.2. Blood, Urine and Milk

Blood and urine samples were collected in polythene bottles from effected persons of Chittagong Medical College Hospital, Bangladesh. Milk sample was collected from a Bangladeshi lactating mother. Immediately after collection they were stored in a salt-ice mixture and latter, at the laboratory, were at −20˚C.

5.3. Soil Samples

Soil samples were collected from different locations of Bangladesh. Samples were dried in air and homogenized with a mortar.

5.4. Food Samples

Food samples (rice, wheat, fruits and vegetables) were collected from local market of Chittagong. After collection the samples (fruits and vegetables) were stored in refrigerator for preservation. Samples (rice, wheat) were used as dry condition and homogenized with a mortar.

6. Results and Discussion

6.1. Factors Affecting the Fluorescence Intensity

Excitation and emission spectra

Vanadium (V) fluoresces strongly in PTQA solution when irradiated with ultraviolet light. The excitation and emission spectra of the fluorescent V(V)-PTQA in 0.005 M sulfuric acid medium was recorded using the spectrofluorophotometer. The excitation and emission maxima were at 319 nm and 371 nm, respectively. The reagent blank exhibited negligible fluorescence, despite having wavelength maximum in the same region. In all instances, measurements were made against the reagent blank. The spectra are shown in **Figure 5**.

6.2. Optimization of Some Parameters on the Fluorescence Intensity

6.2.1. Effect of Solvent

Because PTQA is insoluble in water, an organic solvent was used for the system. Of the various solvents [chloroform, benzene, carbon tetrachloride, n-butanol, isobutanol, ethanol, 1, 4-dioxane and N,N-dimethylformamide (DMF)] were tested for the system, ethanol was found to be the best solvent for the system. The effect of ethanol on the fluorescence intensity was studied and no adverse effect was observed over a wide range (20% - 70%) of ethanol concentrations. It was observed that V^{V}-PTQA system with 10-μg·L^{-1} of V^{V} in absolute ethanol solution produced constant fluorescence intensity as shown in **Figure 6**. A concentration of 20% v/v ethanol in the final volume was sufficient to prevent any precipitation or turbidity and to allow accurate measurements. Therefore, a 20% v/v ethanolic solution was used in the recommended procedure.

6.2.2. Effect of Acidity

The oxidation reaction was conducted in acid medium to avoid the formation of

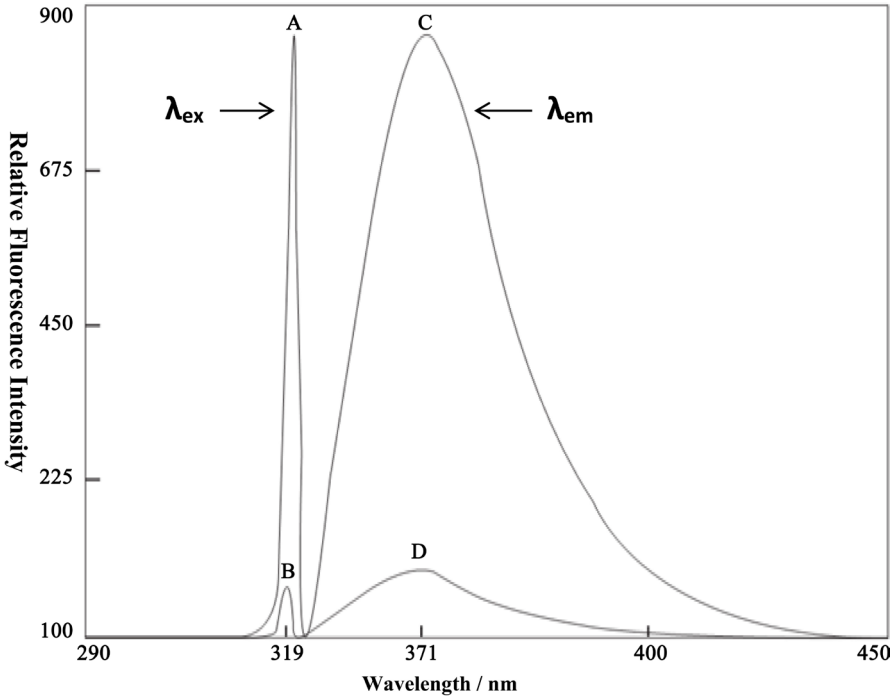

Figure 5. Spectra A & B are the excitation spectra of V^{V}-PTQA system and reagent blank (λ_{ex} = 319 nm), respectively; C and D are the corresponding emission spectra (λ_{em} = 371 nm) in aqueous solutions.

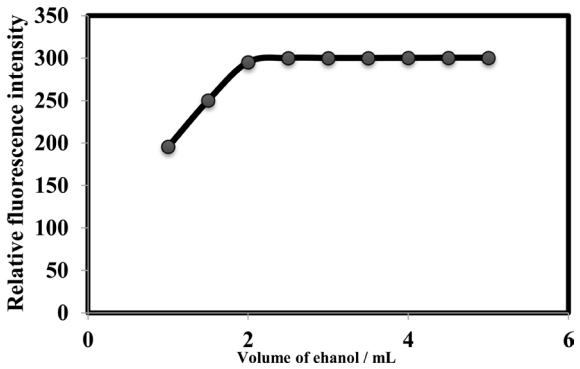

Figure 6. Effect of solvent (Ethanol) on the fluorescence of V^{V}-PTQA system.

precipitation of vanadium. In order to determine the most suitable acid for the reaction, different acids (nitric, sulfuric, hydrochloric and phosphoric) were studied. But, sulfuric acid was found to be the best acid than any other mineral acids for the system. The fluorescence intensity was at maximum and constant when the 10-mL of solution (10-μg·L^{-1} of V^{V}) contained 0.5 - 2-mL of 0.05 M sulfuric acid at room temperature (25 ± 5)˚C. Outside this range of acidity, the fluorescence intensity decreased (**Figure 7**). The optimum acidity range in the final solution is therefore 0.0035 - 0.0085 M (preferably 0.005 M) H_2SO_4. Therefore, 1-mL of 0.05 M sulfuric acid solution was used for all subsequent measurements.

6.2.3. Effect of Temperature

The influence of temperature was studied between 10˚C - 80˚C. It could be

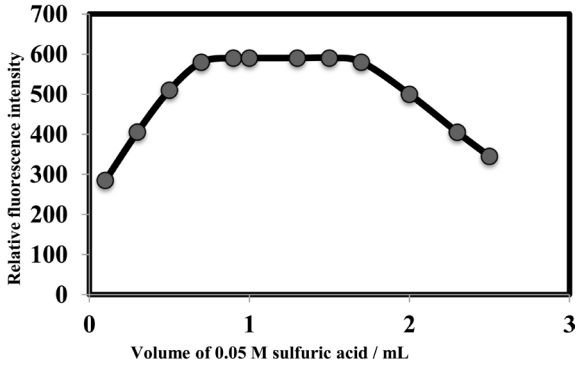

Figure 7. Effect of acidity on the fluorescence of V^{V}-PTQA system.

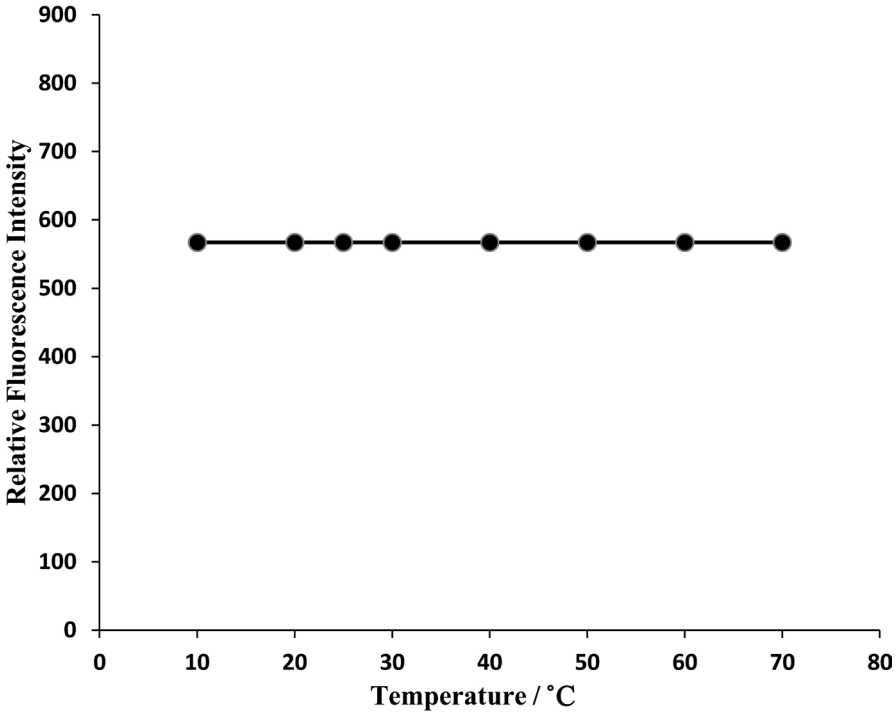

Figure 8. Effect of temperature on the fluorescence of V^{V}-PTQA system.

observed from Figure 8 that temperature effects not pronounced between 10˚C - 80˚C and so room temperature (25 ± 5)˚C is recommended for all subsequent measurements.

6.2.4. Effect of Time

The reaction is instantaneous. The V^{V}-PTQA system attained maximum and constant fluorescence intensity immediately (within 5 min) after dilution of the solution to the final volume, which then remained strictly unaltered for 24 h at room temperature (25˚C ± 5˚C) shown in Figure 9.

6.2.5. Effect of Reagent Concentration

The intensities of the fluorescence of a series of solutions containing a constant amount of V (V) with varying amounts of PTQA were measured in order to establish the optimum concentration of PTQA. The change of fluorescence inten-

sity with PTQA concentration was shown in Figure 10, while the concentration of V^V was kept constant. It was found that the fluorescence intensity increase at first as the PTQA concentration rises and reaches a maximum, but further addition of PTQA hardly effect the intensity even PTQA is more times concentrated than V^V. It was observed that at 10-μg·L^{-1} V^V metal and the reagent molar ratios of 1:130 - 1:300 produced a constant fluorescence intensity of the oxidized product. Outside this range of reagent, the fluorescence intensity get decreased (Figure 9). At different V^V concentrations (0.5 and 1-μg·L^{-1}), the effect of varying the reagent concentration was similar. For all subsequent measurements 1.5-mL of 3.77×10^{-3} MPTQA reagent was added.

6.2.6. Calibration Curves (Beer's Law)

The calibration graphs for the determination of V (V) were constructed under optimum conditions. The well-known equation for spectrofluorimetric analysis in very dilute solutions derived from Beer's law. The effect of metal concentration was studied over 0.001 - 1000-μg·L^{-1} distributed in six different sets (0.001 - 0.01, 0.01 - 0.1, 0.1 - 1, 1 - 10, 10 - 100 and 100 - 1000-μg·L^{-1}) for convenience of

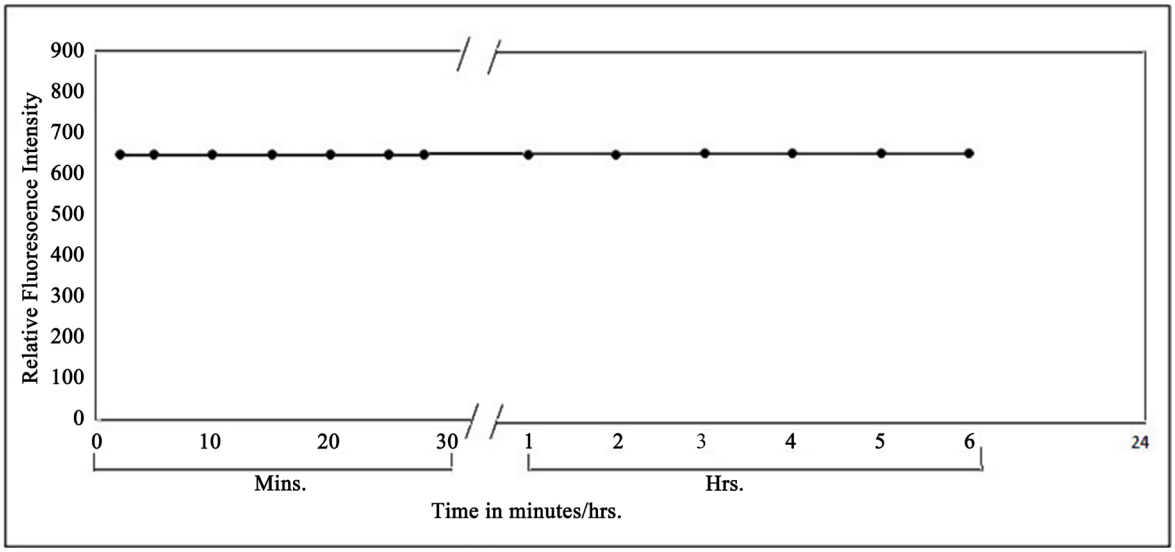

Figure 9. Effect of the time on the fluorescence of V^V-PTQA system.

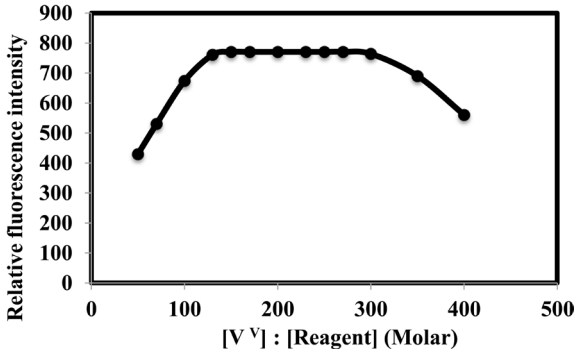

Figure 10. Effect of reagent (PTQA: V^V molar concentration) on the fluorescence of V^V-PTQA system.

measurement. The fluorescence intensity was linear over a wide range 1 pg·mL^{-1} to 600-μg·mL^{-1} for 0.001 - 600-μg·L^{-1} of vanadium(V) at excitation wavelength at 319 nm and emission wavelength at 371 nm, representing six linear graphs (0.001 - 0.01, 01 - 0.1, 0.1 - 1.0, 1.0 - 10, 10 - 100, 100 - 1000-μg·L^{-1}) as shown in Figures 11-16, respectively. Of six calibration graphs, the one showing the limit of the linearity range (Figure 16); the remaining five (Figures 11-15) were straight-line graphs passing through the origin (R^2 = 0.9998). The limit of detection

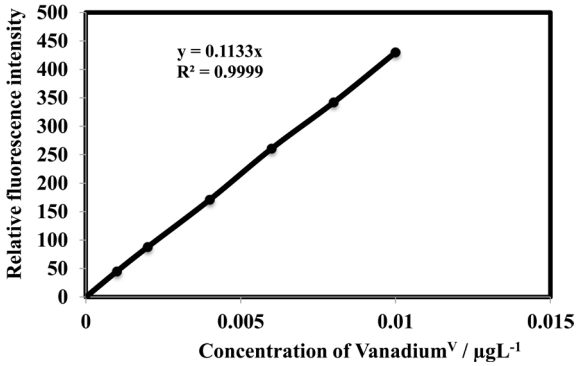

Figure 11. Calibration graph A: 0.001 - 0.01-μg·L^{-1} of vanadium (V). Bandwidth: Ex.slit-3, Em.slit-3. Sensitivity: High.

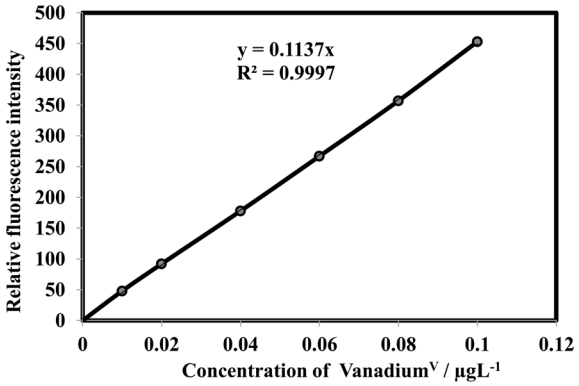

Figure 12. Calibration graph B: 0.01 - 0.1 μg·L^{-1} of vanadium (V). Bandwidth: Ex.slit-1.5, Em.slit-3. Sensitivity: High.

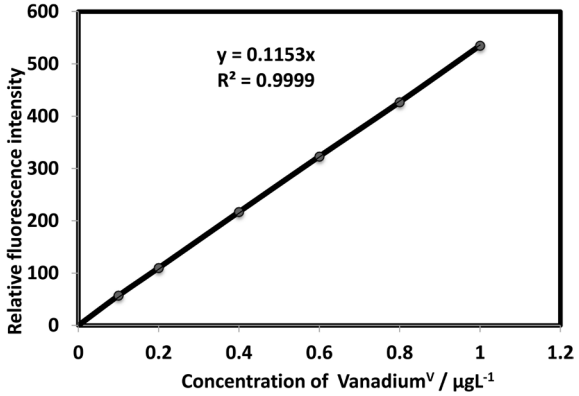

Figure 13. Calibration graph C: 0.1 - 1-μg·L^{-1} of vanadium (V). Bandwidth: Ex.slit-5, Em.slit-1.5. Sensitivity: High.

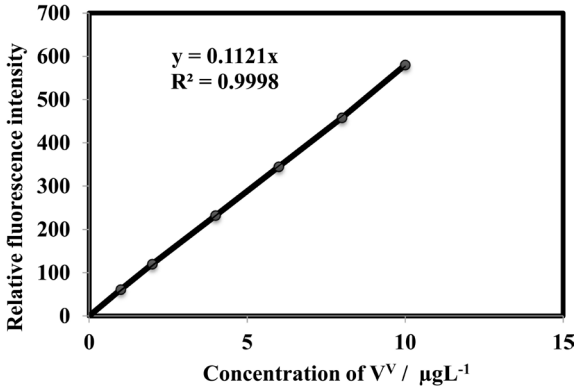

Figure 14. Calibration graph D: 1 - 10-µg·L^{-1} of vanadium (V). Bandwidth: Ex.slit-3, Em.slit-1.5. Sensitivity: High.

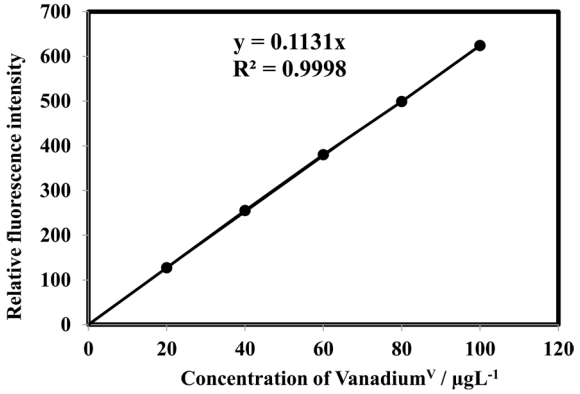

Figure 15. Calibration graph E: 10 - 100-µg·L^{-1} of vanadium (V). Bandwidth: Ex.slit-5, Em.slit-1.5. Sensitivity: High.

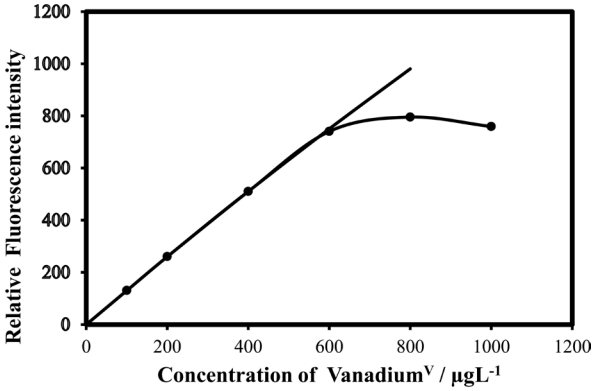

Figure 16. Calibration graph F: 100 - 600-µg·L^{-1} of vanadium (V). Bandwidth: Ex.slit-1.5, Em.slit-1.5. Sensitivity: High.

and limit of quantization were found to be 0.3-ng·L^{-1} and 3-ng·L^{-1}, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 2.

6.3 Effect of Foreign Ions

In order to apply the proposed method to the determination of the concentra-

tion of V (V) in the real sample, the effect of some co-existing species was investigated using 10-μg·L^{-1} of vanadium (V). More than 60 anions, cations and complexing agents were studied individually to investigate their effect on the determination of 10-μg·L^{-1} of vanadium (V). The criterion for interference [70] was a fluorescence intensity value varying by more than ±5% from the expected value for vanadium alone. The results are summarized in **Table 2**. As can be seen a large number of ions have no significant effect on the determination of vanadium. The most serious interference was from Se (IV), Cr (VI) and Mn (VII).

Most of the ions were tolerated over 1000 exceeds. Ascorbic acid, oxalate, citrate, tartrate, EDTA and fluoride ions etc. were tolerated over 5000 folds. In order to eliminate the interference of Se (IV), Cr (VI) and Mn (VII) ions, EDTA and tartrate can be used as masking agents, respectively [71]. A 100-fold excess of Se (IV), Cr (VI) and Mn (VII) ions could be masked with EDTA and tartrate respectively. During the interference studies, if a precipitated was formed, it was removed by centrifugation. Strong reducing agents such as, tin (II), chloride, iron (II), sulfate, hydroxylamine, hydrochloride and sodium azide, which would otherwise reduce vanadium (V), undergo oxidation during the treatment of the vanadium (IV) solution with persulphate and hence are not a problem [72]. The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limits have been studied, their tolerance ratios are mentioned in **Table 3**.

Table 2. Selected analytical parameters obtained with the optimization experiments.

Parameters	Studied range	Selected value
Excitation wavelength maximum/λ_{ex} (nm)	200 - 700	319
Emission wavelength maximum/λ_{em} (nm)	200 - 700	371
Solvent/amount of ethanol/mL	0 - 5	2 - 5 (Preferably 2)
Acidity/M H_2SO_4/mL	0.0005 - 0.02	0.0035 - 0.0085 (Preferably 0.005)
pH	3.30 - 1.70	2.35 - 2.07 (Preferably 2.30)
Time/h	0 - 72	1 min - 24 h (Preferably 5)
Temperature/˚C	10 - 70	15 - 50 (Preferably 25 ± 5)
Reagent (fold molar excess, M:R)	1:50 - 1:400	1:130 - 1:300 (Preferably 1:150)
Linear range/μg·L^{-1}	0.0001 - 1000	0.001 - 600
Limit of quantization/ng·L^{-1}	0.1 - 100	3.0
Detection limit/ng·L^{-1}	0.01 - 10.0	0.3
Reproducibility (% RSD)	0 - 10	0 - 2%
Regression Coefficient (R^2)	0.9995 - 0.9999	0.9998

Table 3. Table of tolerance limits of foreign ions[a], tolerance ratio [species (x)/V^V (w/w)].

Species x	Tolerance Ratio x/V (w/w)	Species x	Tolerance Ratio x/V (w/w)
Ammonium	1000	Iron (III)	500[b]
Aluminium	1000	Lead (II)	1000
Azide	1000	Magnesium	1000
Arsenic (III)	1000	Manganese (II)	1000
Arsenic (V)	100[b]	Manganese (VII)	300[c]
Ascorbic acid	1000	Mercury (II)	1000
Antimony	1000	Molybdenum (VI)	500[b]
Barium	1000	Nitrate	1000
Bromide	1000	Nickel	1000
Bismuth (III)	1000	Oxalate	1000
Beryllium (II)	1000	Potassium	1000
Calcium	1000	Selenium (IV)	100[c]
Chloride	1000	Selenium (VI)	1000
Cobalt (II)	1000	Silver	1000
Cobalt (III)	1000	Sodium	1000
Copper (II)	1000	Strontium	1000
Chromium (III)	1000	Sulfate	1000
Chromium (IV)	100[c]	Tellurium (IV)	1000
Cadmium	1000	Tellurium (VI)	1000
Carbonate	1000	Titanium (IV)	1000
Cesium	1000	Tartrate	1000
Citrate	1000	Thiocyanate	1000
Cerium (III)	1000	Thiourea	1000
Cerium (IV)	100[b]	Tungsten (VI)	1000
Cyanide	1000	Tin (II)	1000
EDTA	1000	Tin (IV)	1000
Fluoride	1000	Uranium (VI)	1000
Iodide	1000	Thallium (III)	100[c]
Iron (II)	1000	Zinc	1000

[a]Tolerance limit was defined as ratio that causes less than ±5 percent interference. [b]With 10 mg·L^{-1} tartrate. [c]With 10 mg·L^{-1} EDTA.

6.4. Precision and Accuracy

The precisions of the present method were proved by measuring 10 solutions of same sample (each analyzed at least five times). The relative standard deviation (n = 5) was 0% - 2% for 0.01 - 6000-ng of vanadium (V) in 10-mL, indicating that this method is highly precise and reproducible (Table 2). The detection limit (3 s

of the blank) and limit of quantization (10 times of detection limit) for vanadium (V) were found to be 0.3-ng·L^{-1} and 3-ng·L^{-1}, respectively. The method was also tested by analyzing several synthetic mixtures containing vanadium (V) and diverse ions (Table 4). The results for total vanadium were in excellent agreement with certified values (Table 5). The reliability of the procedure was tested by recovery studies. The average percentage recovery obtained for addition of vanadium (V) spike to some environmental water samples was quantitative, as shown in Table 6. The results of biological analyses by the spectrofluorimetric method were in excellent agreement with those obtained by ICP-OES (Table 7). The results of soil analyses by the spectrofluorimetric method were excellent agreement with those obtained by AAS (Table 8). The results of food and vegetables analyses by spectrofluorimetric method were also found to be in excellent agreement with those obtained by ICP-OES (Table 9). The results of speciation of vanadium (IV) and vanadium (V) in mixtures were highly reproducible (Table 10). Hence, the precision and accuracy of the method were found to be excellent.

6.5. Nature of the Fluorescent Species

The non fluorescent reagent, PTQA, produced the same spectral characteristics with excitation and emission wavelengths almost invariably around 319 nm and 371 nm, with vanadium (V), manganese (VII), chromium (VI), selenium (IV) and with persulphate, hydrogen peroxide, and triiodide in acidic media. This indicates that the fluorescence species is an oxidized product of the reagent itself and not a chelate. Similar oxidative fluorescent reactions have been utilized previously [65] [72]. The PTQA reagent produced here has so many potential reaction sites that the structure of the oxidized fluorescent species is difficult to

Table 4. Determination of vanadium in some synthetic mixtures.

Sample	Composition of Mixtures (μg·L^{-1})	Vanadium/μg·L^{-1}		
		Added	Found[a] (n = 5)	Recovery ± SD[b] (%)
A	V (V)	1.0 50	0.99 50.0	99 ± 0.5 100 ± 0.0
B	As in A + Cr^{VI} (50) + As^{V} (50) + Ti^{IV} (50) + Fe^{3+} (50) + EDTA (50)	1.0 50	1.0 49.5	100 ± 0.0 99 ± 0.6
C	As in B + Pb^{2+} (50)+ Bi^{3+} (50) + Hg^{2+} (50) + Se^{IV} (50)+ Cu (50)	1.0 50	1.01 51.0	101 ± 1.0 102 ± 1.5
D	As in C + Sb^{3+} (50) +Ni^{2+} (50) + Ca (50) + Cd (50) + Te^{IV} (50)	1.0 50	1.02 50.5	102 ± 1.6 101 ± 1.0
E	As in D + Mg (50) + Mn^{VII} (50) + W^{VI} (50) + Ba (50) + Ag (50)	1.0 50	1.03 51.5	103 ± 1.8 103 ± 1.5
F	As in E +Ce^{III} (50)+ Na (50) + K (50) + Zn (50) + Ce^{IV} (50)	1.0 50	1.05 52.5	105 ± 2.1 105 ± 2.0

[a]Average of five analyses of each sample. [b]The measure of precision is the standard deviation (SD).

predict. Given that ring closure can lead to intense fluorescent in some circumstances, it seems likely that photo-oxidative cyclization takes place, leading to the formation of structure (A) in resonance with structure (B) (Figure 17).

7. Applications

The procedure was applied for determination of trace amounts of vanadium in some synthetic mixtures of various compositions (Table 4) and also in a number of real samples e.g. several Certified Reference Materials (CRMs) (Table 5). The method was also extended to the determination of vanadium in a number of environmental, biological, soil, food, vegetables and fruit samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample were analyzed for vanadium content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in excellent agreement (Table 6). The results of biological analyses by spectrofluorimetric method were found to be in excellent agreement with those obtained by ICP-OES (Table 7). The results of soil analyses by the spectrofluorimetric method were found to be in excellent agreement with those obtained by AAS (Table 8). The results of food analyses by spectrofluorimetric method were also found to be in excellent agreement with those obtained by ICP-OES (Table 9). The results of speciation of vanadium (IV) and vanadium (V) speciation in mixtures were highly reproducible (Table 10).

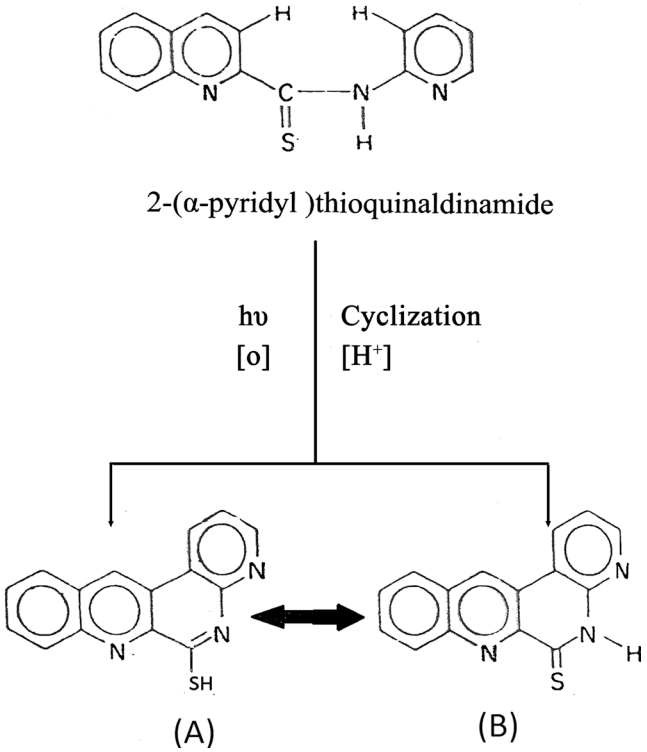

Figure 17. Mechanism of oxidative cyclization reaction of 2-(*α*-pyridyl)-thioquinaldinamide (PTQA).

7.1. Determination of Vanadium in Synthetic Mixtures

The procedure was applied to determine trace amounts of vanadium (V) in some synthetic mixtures with good recovery being achieved. The result indicates the proposed method is suitable and can be successfully applied for determination of V (V). Several synthetic mixtures of varying compositions containing vanadium (V) and diverse ions of known concentrations were determined by the present method using EDTA as masking agent. The results were found to be highly reproducible as shown in Table 3. Accurate recoveries were achieved in all solutions in the range 99.6 ± 1.5 to 99.9 ± 0.6. The reliability of our vanadium-PTQA oxidation procedure was approved by quantitative recovery of vanadium (V) spiked in several synthetic mixtures containing vanadium (V) and diverse ions. This method has high precision and accuracy (s = ±0.01 for 0.5-μg·L^{-1}).

7.2. Determination of Vanadium in Some Certified Reference Materials

A 0.1-g amount of an alloy or steel sample containing 0.83% - 1.99% of vanadium was weighed accurately and placed in a 50-mL Erlenmeyer flask in presence of excess oxidizing agent to oxidize vanadium (IV) to vanadium (V) following a method recommended by Mitra [69]. To it, 10-mL of 20% (w/v) sulfuric acid was added and while carefully covering with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 10-mL of concentrated HNO_3 until all residual carbides were decomposed. Then a further 2-mL of 1 + 1 H_2SO_4 and 2-mL 2% (w/v) freshly prepared ammonium persulphate were added and the solution was evaporated carefully to dense white fumes of sulphur trioxide, then cooled to room temperature (25˚C ± 5˚C). After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed so as to dissolve the soluble salts. The solution was then cooled and neutralized with dilute NH_4OH solution in presence of 1 - 2-mL of 0.01% (w/v) EDTA solution. The resulting solution was filtered if necessary, through a Whatman No. 40 filter paper into a 100-mL calibrated flask. The residue (silica and tungstenic acid) was washed with a small volume of hot 1 + 99 H_2SO_4, followed by water; the volume was made up to mark with de-ionized water.

An suitable liquot (1 - 2-mL) of the above-mentioned solution was taken into a 10-mL calibrated flask and the vanadium (V) content was determined; as described under procedure using tartrate or EDTA as masking agent. The proposed procedure for the spectrofluorimetric determination of vanadium was applied to the analysis of single element CRMs of V, estuarine sediment (CRM-MESS-3), Soil (CRM 029), human serum (CRM-ASTMRCVD-74231), Bovine liver (NIST@SRM-1577c) and drinking water (NIST-CRM-TMDW) the CRMs obtained from the National Research Council, Govt. of Canada, using tartrate or EDTA as masking agents, following a method recommended by Sun *et al.* [73]. Based on five replicate analyses, average vanadium concentration determined by the spectrofluorimetric method was in an excellent agreement with the certified

values. The results are given in Table 5.

7.3. Determination of Vanadium in Environmental Water Samples

Each filtered (with Whatman No. 40) environmental sample (25-mL) contained in a 50-mL Pyrex beaker were added 1-mL of concentrated H_2SO_4 and 2-mL of concentrated HNO_3 in the presence of freshly prepared excess ammonium persulphate solution in a fume cupboard to oxidize vanadium (IV) to vanadium (V) and the mixture was heated on a hot plate until white fumes of sulfur trioxide, following a method recommended by Greenberg *et al.* [74]. The solution was cooled and neutralized with dilute NH_4OH solution in presence of 1 - 2-mL of 0.01% (w/v) EDTA solution. Resulting solution was then filteredthrough a Whatman No. 40 filter paper and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1 - 2-mL) of this water sample was pipetted into a 10-mL calibrated flask and the vanadium content was determined as described under the general procedure using tartrate or EDTA as masking agent. To test the validity of our method, we have analyzed different types of portable and polluted waters in spike and un-spike conditions. The reliability of our spectrofluorimetric method was tested by recovery studies. The average percentage recovery obtained for the addition of a vanadium (V) spike to some environmental water samples

Table 5. Determination of vanadium in some certified reference materials.

Sample no	Certified Reference Materials (Composition, %)	Vanadium (%)		
		In C.R.M. Sample	Found (n = 5)	RSD[b]
1	BAS-CRM-646: High-speed steel (Cr, Mo, V and Tc)	1.99	1.985	1.5
2	BCS-CRM-220/L: High-speed steel (C, Si, S, P, Mn, Mo, V, Cr, Ni, Co, W and Cu)	2.09	2.095	1.8
3	BSC-CRM-241/L: High-speed steel (Cr, V, W, Co, Mo, Mn, C, Si, P and S)	1.57	1.578	1.5
4	GSBH-40101-96: Cr_{12}MoV: Dies steel (Cr, Mo, V, Ni, Cu, Co)	0.411	0.410	2.0
5	YSBC-1013-1-95: 9Cr_{17}MoVCo: High-tensil steel (C, Cr, Mo, V, Si, Mn and Co)	0.24	0.25	2.5
6	CRM-ASTMRCVD-74231: Human Serum[a] (Quest-Diagonisstics, ISO-17025)	0.83[e]	0.81	1.0
7	CRM 029: Sigma-Aldrich: Soil (ISO/17025)	71.0[d]	70.5	1.8
8	CRM-MESS-3: Sediments	234 ± 10[c]	233 ± 3.0	2.0
9	NIST@SRM-1577c-Bovine liver	8.17 ± 0.66[d]	8.15 ± 0.5	2.0
10	NIST-CRM-TMDW: Drinking water	30.0[f]	29.8	1.5

[a]The CRMs were obtained from the National Research Council, Govt. of Canada. [b]The measure of precision is the relative standard deviation(RSD). [c]Values in $\mu g \cdot g^{-1}$, [d]Values in $mg \cdot kg^{-1}$. [e]Values in $\mu g \cdot kg^{-1}$, [f]Values in $\mu g \cdot L^{-1}$.

was quantitative. The results of analyses of environmental water samples from various sources for vanadium are shown in Table 6.

Table 6. Determination of vanadium in some environmental water samples.

Sample		Vanadium/μg·L^{-1}		Recovery ± s (%)	S_r^b (%)
		Added	Found		
River Water	Tap water	0	2.5		
		10	12.5	100 ± 0.00	0.00
		50	53.0	106 ± 0.8	0.35
	Rain water	0	1.5		
		10	10.5	100 ± 0.00	0.00
		50	52.5	105 ± 0.5	0.19
	Well water	0	6.5		
		10	16.0	97 ±0.8	0.33
		50	58.0	102.6 ± 1.0	0.39
	Karnaphully (upper)	0	35.0		
		10	45.0	100 ± 0.00	0.00
		50	88.0	103.5 ± 0.8	0.29
	Karnaphully (lower)	0	38.5		
		10	48.0	99 ± 0.5	0.25
		50	88.5	100 ± 0.00	0.00
Sea Water	Bay of Bengal (upper)	0	5.0		
		10	15.0	100 ± 0.00	0.00
		50	58.0	105 ± 0.8	0.24
	Bay of Bengal (lower)	0	7.5		
		10	18.0	102.8 ± 0.6	0.27
		50	57.5	100 ± 0.00	0.00
Drain water	Eastern refinery[c]	0	95.0		
		10	105.0	100.0 ± 0.0	0.00
		50	145.8	100.5 ±0.8	0.25
	PHP glass[d]	0	75.6		
		10	87.0	98.4 ± 0.5	0.65
		50	126.0	99.7 ± 0.3	0.45
	BSRM steel[e]	0	85.0		
		10	95.0	100.0 ± 0.0	0.00
		50	140.0	96.4 ± 1.0	0.29
	KPM[f]	0	55.0		
		10	65.0	100.0 ± 0.0	0.00
		50	108.0	97.2 ± 0.8	0.48
	Eastern cables Ltd.[g]	0	78.0		
		10	88.0	100.0 ± 0.0	0.00
		50	129.0	99.2 ± 1.0	0.26
	Berzerpaint[h]	0	85.30		
		10	95.0	100.0 ± 0.0	0.00
		50	138.0	97.8 ± 0.8	0.27

[a]Average of five replicate determinations of each sample. [b]The measure precision is the relative standard deviation. [c]Eastern Refinary Patenga, Chittagong. [d]PHP Glass factory, Chittagong. [e]Bangladesh Steel Re-rolling Mills Ltd. (BSRM), Baizid Bosthami, Chittagong. [f]Karnaphully Paper Mills, Chandraghona, Chittagong. [g]Eastern Cabbles Ltd., Patenga, Chittagong. [h]Berger Paints Bangladesh Limited, Kalurghat, Chittagong.

Most spectrofluorimetric methods for determination of vanadium in natural and sea-water require preconcentration or standard addition of vanadium [75]. The concentration of vanadium in natural and sea water is a few $\mu g \cdot L^{-1}$ in Japan [76]. The mean concentration of vanadium found in US drinking waters is 6-$\mu g \cdot L^{-1}$ [75].

7.4. Determination of Vanadium in Biological Samples

Human blood or milk (2 - 3-mL) or urine (10 - 20-mL) or hair (3 - 5-g) sample was taken into a 100-mL micro-Kjeldahl flask. A glass bead and 10-mL of concentrated nitric acid were added, and the flask was placed on the digester under gentle heating. The sample was digested in the presence of an excess freshly prepared ammonium persulphate solution (2-mL of 2% w/v) to oxidize vanadium (IV) to vanadium (V) according to the method recommended by Stahr [77]. As the heating process continued 1-mL of H_2SO_4 is added and heated for about 0.5 hour to dense white fumes of sulphur trioxide. When the initial brisk reaction was completed, the solution was removed and cooled at room temperature and neutralized with dilute NH_4OH solution in presence of 1 - 2-mL of 0.01% (w/v) EDTA solution. Resulting solution was then filteredthrough a What-man No. 40 filter paper and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1 - 2-mL) of the final solution was pipetted out into a 10-mL calibrated flask and the vanadium content was determined as described under the general procedure using EDTA or tartrate as masking agent. The results of biological analyses by the spectrofluorimetric method were found to be in excellent agreement with those obtained by ICP-OES. The results are shown in **Table 7**.

The abnormally high values for the human lung cancer patient are probably due to the involvement of high vanadium concentration with As and Zn. The occurrence of such high vanadium contents are also reported in lung cancer patients from some developed countries [78]. The low value for the heart-disease patient is probably due to a low vanadium concentration in the environment. There is an inverse correlation between human heart-disease and vanadium concentration in the environment [79].

7.5. Determination of Vanadium in Some Surface Soil Samples

An air-dried homogenized soil sample (10-g) was accurately weighed and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in the presence of an excess oxidizing agent (2-mL of 2% freshly prepared ammonium persulfate solution) to oxidize vanadium (IV) to vanadium (V) following method recommended by Jackson [80]. As the heating process continued 1-mL of H_2SO_4 is added and heated for about 5 minutes to dense white fumes of sulphur trioxide. The solution was then cooled at room temperature and neutralized with dilute NH_4OH solution in presence of 1 - 2-mL of 0.01% (w/v) EDTA solution. The content of

Table 7. Determination of vanadium in some human fluids and hair sample.

Serial No.	Sample	Vanadium/μg·L^{-1}				Sample Source[a]
		ICP-OES (n = 5)		Proposed Method (n = 5)		
		Found	RSD (%)	Found	RSD (%)	
1	Blood	15.0	1.5	15.5	1.5	Heart disease patient (Female)
	Urine	5.8	1.2	5.9	1.3	
2	Blood	225.5	2.5	228.0	2.0	Liver cirrhosis patient (Male)
	Urine	57.8	1.8	58.5	1.5	
3	Blood	361.0	2.8	368.0	2.5	Lung cancer patient (Male)
	Urine	95.0	1.8	96.5	1.7	
4	Blood	209.0	2.6	211.5	2.2	Kidney damage patient (Female)
	Urine	53.0	1.7	55.5	1.8	
5	Blood	132.5	2.0	135.0	2.0	Skin disease patient (Female)
	Urine	34.0	1.5	35.5	1.8	
6	Blood	18.0	2.2	18.5	2.0	Manic disease patient (Male)
	Urine	5.0	1.5	4.8	1.4	
7	Blood	8.0	2.0	10.5	1.8	Diabetic patient (Male)
	Urine	2.0	1.3	2.5	1.0	
8	Blood	10.0	1.8	12.0	1.5	Normal Adult (Female)
	Urine	3.0	1.5	3.5	1.3	
9	Hair sample	100.0[b]	1.5	103.0	1.6	Human hair (Female)
10	Milk	5.0[b]	0.8	6.0	1.0	Lactating Mother

[a]Samples were collected from Chittagong Medical College Hospital; [b]Values in μg·g^{-1}.

the flask was then filtered through a What-man No. 40 filter paper and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1 - 2-mL) of the final solution was pipetted out into a 10-mL calibrated flask and the vanadium content was determined as described under the general procedure using tartrate or EDTA as masking agent. The vanadium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results of soil analyses by spectrofluorimetric method were also found to be in excellent agreement with those obtained by AAS. The average value of vanadium in the Chittagong region surface soil was found to be 53.27-μg·kg^{-1}. The results are shown in Table 8.

7.6. Determination of Vanadium in Some Vegetable, Food and Fruit Samples

The vegetable and fruit samples collected prior to the determination were

Table 8. Determination of vanadium in some surface soil samples.

Serial No.	Vanadium/μg·kg^{-1}			Sample Sources[c]
	AAS (n = 5)	Proposed method (n = 5)	RSD[b] (%)	
S_1	52.0	51.8	1.5	Esturine soil (Sediment) (River Karnaphully Chittagong)
S_2	20.0	19.8	1.0	Marine soil (Bay of Bengal)
S_3	36.0	35.8	1.2	Clevendon Tea Estate (Moulabi Bazar, Sylhet)
S_4	13.0	12.5	1.0	Agricultural soil (Chittagong university campuse)
S_5	46.5	45.6	1.5	Industrial soil (Berger paint)
S_6	160.0	157.5	1.8	Industrial soil (Eastern Refineries)
S_7	58.0	56.8	1.6	Industrial sol (Eastern Cable)
S_8	67.0	65.5	1.8	Industrial soil (PHP glass)
S_9	32.5	31.8	1.2	Industrial soil (Ship-breaking area)
S_{10}	57.0	1.5	1.5	Industrial soil (BSRM Steel Mill)

[a]Average of five analyses of each sample. [b]The measure of precision is the relative standard deviation (RSD). [c]Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Ce, Cu, Mo, Fe, Pb, V, Zn, Mn, Co, NO_3, SO_4 *et al.*

pretreated in the following way: Edible portion of samples was first washed clean with tap water followed by rewashing with de-ionized water. After removing de-ionized water from the surface of vegetables and fruits, the samples were cut into small pieces and dried at 65˚C in oven. An air dried vegetables and fruits samples (10-g) were ground in a mortar and taken in a 100-mL micro-Kjeldahl flask in presence of excess oxidizing agent and digested following a method recommended by Stahr [77] and 10-mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 1-mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2-mL of concentrated HF, and heating was continued for at least ½ hr and then cooled. In the resulting solution 2-mL of 2% (w/v) of freshly prepared ammonium persulfate is added. The mixture of each foodstuff was heated below the boiling point for 5 - 10 min to oxidize vanadium (IV) to vanadium (V). The solutions were then cooled and neutralized with dilute NH_4OH in presence of 1 - 2-mL of 0.01% (w/v) EDTA solution. The resulting solution was filtered through a Whatman No. 40 filter paper and quantitatively transferred into a 25-mL calibrated flask and mixed well and made up to the mark with de-ionized

water.

The food samples used were rice, wheat and corn and these were used under dry conditions. Each sample was first ground in a mortar. Corn and fruit samples (2-g) or rice and wheat samples (1-g) were weighed accurately and placed in a porcelain crucible and charred in an electric furnace; the sample was ashen at 555˚C in a muffle furnace in presence of excess oxidizing agent following a method recommended by Mitra [69]. To it, 2.0-mL of HCl and 10-mL of water were added to the ash. The mixture of each foodstuff was heated with 2-mL of 2% (w/v) freshly prepared ammonium persulphate was added below the boiling point for 5 - 10 min to complete oxidation from V(IV) to V(V). The solutions were cooled and neutralized with dilute NH_4OH in presence of 1 - 2-mL of 0.01% (w/v) EDTA solution and filtered. The resulting solution was quantitatively transferred into a 25-mL calibrated flask and mixed well and made up to the mark with de-ionized water.

A suitable aliquot (1 - 2-mL) of the final digested solution was pipetted into a 10-mL calibrated flask and the vanadium content was determined as described under the general procedure using tartrate as masking agent. High value of vanadium for Daucuscarota (carrot) is probably due to the involvement of high vanadium concentration in the soil. Such a high concentration of vanadium in carrot and radish is also reported in some developed countries [81]. The results of food and vegetables analyses by spectrofluorimetric method were also found to be in excellent agreement with those obtained by ICP-OES. The results are shown in **Table 9**.

7.7. Determination of Vanadium (IV) and Vanadium (V) Speciation in Mixtures

Suitable aliquots (1 - 2-mL) of vanadium (V + IV) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 250-mL Pyrex conical flask. A few drops (3 - 5 drops) of 4 M H_2SO_4, and 5 - 10-mL of 2% (w/v) freshly prepared ammonium persulphate were added to oxidize tetravalent vanadium to pentavalent vanadium and the mixture was heated gently with further addition of 10-mL water, if necessary, for 5 minutes to drive off the excess persulphate, then the mixture was cooled to room temperature (25 ± 5)˚C. The reaction mixture was then cooled and neutralized with dilute NH_4OH in presence of 3 - 5-mL of 0.01% (w/v) EDTA solution. The solution was transferred quantitatively into a 25-mL volumetric flask and 3.75-mL of 3.77×10^{-3} M PTQA reagent solution was added followed by the addition of 2.5-mL of 0.05 M H_2SO_4. It was made up to the mark with de-ionized water. The fluorescence intensity was measured then being cooled at room temperature, (25 ± 5)˚C, at 371 nm when excited at 319 nm, against a reagent blank. The total vanadium content was calculated with the help of a calibration graph prepared concurrently.

An equal aliquot (1 - 2-mL) of the above vanadium (V + IV) mixture was taken into a 250-mL Pyrex conical flask. The solution was neutralized with dilute

Table 9. Determination of vanadium in some food, fruit and vegetable samples.

Serial No.	Sample	Vanadium/μg·kg^{-1} Found[a] ± s (n = 5)				Sample Source
		ICP-OES (n = 5)		Proposed Method (n = 5)		
		Found	RSD[b] %	Found	RSD[b] %	
1	Ginger (*Zingiber officinale*)	175.0	1.6	179.0	1.8	Local Market, Chittagong
2	Carrot (*Daucus carota*)	180.0	1.8	183.0	2.0	Local Market, Chittagong
3	Garlic (*Allium sativum*)	145.0	1.9	148.5	2.0	Local Market, Chittagong
4	Onion (*Allium cepa*)	80.0	1.0	82.8	1.3	Local Market, Chittagong
5	Tomato (*Lycopersicon esculentum*)	100.0	1.2	105.0	1.5	Local Market, Chittagong
6	White cabbage (*Brassica oleracea* capitata)	535.0	2.0	538.0	2.0	Local Market, Chittagong
7	Radish (*Raphanus sativus*)	165.8	1.5	170.0	1.7	Local Market, Chittagong
8	Rice (*Oryza sativa*)	25.0	1.3	24.8	1.5	Local Market, Chittagong
9	Tea (*Camellia sinensis*)	16.8	2.3	18.5	2.5	Local Market, Chittagong
10	Wheat (*Triticum aestivum*)	35.0	1.5	38.0	1.6	Local Market, Chittagong
11	Shellfish	100.0	1.5	103.0	1.5	Local Market, Chittagong
12	Milk (Cow milk)	30.0[c]	1.5	31.5[c]	1.6	Local Market, Chittagong
13	Mushrooms (*Agaricus bisporus*)	431.0	1.8	435.0	2.0	Local Market, Chittagong

[a]Average of five replicate analyses of each sample. [b]The measure of precision is the relative standard deviation (RSD). [c]Values in μg·L^{-1}.

NH_4OH in presence of 3 - 5-mL of 0.01% (w/v) EDTA solution. After, the content of the beaker was transferred quantitatively into a 25-mL volumetric flask, 3.75-mL of 3.77×10^{-3} M PTQA reagent solution was added, followed by the addition of 2.5-mL of 0.05 M H_2SO_4. It was made up to the mark with de-ionized water. After 5 min the fluorescence intensity was measured following the general procedure at 371 nm when excited at 319 nm against a reagent blank, as before. The vanadium concentration was calculated in μg·L^{-1} or ng·L^{-1} with the aid of a calibration graph. This gives a measure of vanadium (V) originally present in the mixture. This value was subtracted from that of the total vanadium to determine the vanadium (IV) present in the mixture. The results of the assessment of speciation of V (V) and V (IV) were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of vanadium [82]. The results of a set of determination are given in Table 10.

8. Conclusions

A new simple rapid, ultra-sensitive, highly selective and inexpensive spectrofluorimetric method with the vanadium-PTQA system was developed for the determination of vanadium in some real, environmental, biological, food, vegetables and soil samples. Compared with other existing methods [35]-[60], the proposed method has several remarkable analytical characteristics:

Table 10. Determination of vanadium (V) and vanadium (IV) in mixtures.

Serial No.	V (V):V (IV)	V, taken ($\mu g \cdot L^{-1}$)		V, found ($\mu g \cdot L^{-1}$)		Error ($\mu g \cdot L^{-1}$)	
		V(V)	V(IV)	V(V)	V(IV)	V(V)	V(IV)
1	1:1	10	10	9.99	9.98	0.01	0.02
1	1:1	10	10	10.0	9.98	0.00	0.02
1	1:1	10	10	9.98	10.0	0.02	0.00
	Mean error: V (V) = ±0.0067 Standard deviation: V (V) = ±0.0058			V (IV) = ±0.013 V (IV) = ±0.011			
1	1:5	10	50	9.99	49.98	0.01	0.02
1	1:5	10	50	9.98	49.98	0.02	0.02
1	1:5	10	50	9.99	49.99	0.01	0.01
	Mean error: V (V) = ±0.013 Standard deviation: V (V) = ±0.0058			V (IV) = ±0.016 V (IV) = ±0.006			
1	1:10	10	100	10.00	99.99	0.00	0.01
1	1:10	10	100	9.98	99.98	0.02	0.02
1	1:10	10	100	9.98	99.98	0.02	0.02
	Mean error: V (V) = ±0.015 Standard deviation: V (V) = ±0.005			V (IV) = ±0.016 V (IV) = ±0.006			

1) The proposed method is highly sensitive that the amount, in $ng \cdot L^{-1}$, of vanadium can be determined without any preconcentration or standard addition in diluted biological and environmental solutions.

2) The low detection limit, 0.3-$ng \cdot L^{-1}$ *i.e.* $pg \cdot g^{-1}$ (10^{-12} g) or $pg \cdot mL^{-1}$ levels can be measured without preconcentration or standard addition technique.

3) The method has added advantages of determining individual amounts of vanadium (IV) and vanadium (V).

With suitable masking agents, the reaction can be made highly selective and better reproducibility has been achieved (RSD = 0% - 2%).

Although many sophisticated techniques such as pulse polarography, High Performance Liquid Chromatography (HPLC), Atomic absorption spectroscopy (AAS), Inductively coupled plasma optical emission spectrometry (ICP-OES) and Inductively coupled plasma mass spectrometry (ICP-MS), are available for the determination of vanadium at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrofluorimetry to remain a popular technique, particularly in laboratories of developing countries with limited budgets.

The sensitivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of vanadium in real samples down to $pg \cdot g^{-1}$ (10^{-12} $g \cdot g^{-1}$) levels in aqueous medium at room temperature (25 ± 5)˚C. It is a new method needs neither heating nor extraction to organic phase, works satisfactorily and could be an alternative method for the rapid de-

termination of vanadium in a wide variety of sample matrices and found superior to existing spectrofluorimetric methods reported in different literature [35]-[60].

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Conflicts of Interest

All authors report no conflicts of interest relevant to this article.

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