Two-Steps Cloud Point Extraction-Spectrophotomtric Method for Separation, Pre-concentration and Determination of V (IV) and V (V) Ions in Real Samples Using Laboratory-Made Organic Reagents

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Authors' contributions

The study was carried out in complete cooperation between all authors. Authors ZAAK and KJA designed and supervised the work study. Author ZAAK wrote the protocol, helped in analyzing the data statistically and wrote the final draft of the manuscript. Author KJA helped in the preparation of the new organic regents. Author ZTI carried out the most experimental works according to the cited plan and managed the literature searches. All authors have been read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: To establish a new analytical method for the extraction and pre-concentration of V (IV) and V (V) species in real samples by cloud point extraction (CPE) coupled with spectrophotometry using two newly laboratory-made chelating reagents.
Study Design: All factors affecting the extraction and determination of V (IV) and V (V) ions using

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micelle-mediation extraction were executed via a classical optimization. In addition the interferences study is also considered.

Place and Duration of Study: Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq in cooperation with Department of Chemistry, College of Science, University of *Al-Qadisiya*, Diwanya, *Al-Qadisiya,* Iraq between April 2014 and November 2014.

Methodology: The approach is based on sequential separation of two vanadium species in the same solution. First, the complexation of vanadium (IV) with 2-[(Benzo imidazolyl) azo]-4-benzyl phenol (BIABP) at pH 3.0 and then extracted into micelle phase. Second, the vanadium (V) remaining in aqueous phase after the separation of (IV) is complexed with 2-[2-(5-Nitro thiazolyl) azo]-8 hydroxyquinoline (5-NTA8HQ) and H_2O_2 in acidic medium to form a ternary complex ($V^{(V)}$-H_2O_2-NTA8HQ) which being re-extracted into micelle phase of Triton X-114. The extracted complexes in cloud point layer are dissolved in a minimum amount of ethanolic 0.1 M HNO_3, then V (IV) and V (V) are determined spectrophotometrically at their respective absorption maxima. The proposed method was applied to the estimation of the two vanadium species in various real samples with satisfactory results where the method detection limit in these matrices was of 0.120 and 0.037 µg g^{-1} for V (IV) and V (V) respectively.

Results: At established optimized conditions, a 159 and 99 fold enrichment factors and linear range of 10-100 and 1-70 ng mL^{-1}, leading the limits of detection of 1.78 and 0.75 ng mL^{-1} for V (IV) and V (V) ions respectively to be achieved in aqueous solution. The average percent recovery of 98.3±0.7 and 97.6±0.4 and a precision (RSD%, n=8) of 0.67% and 0.46%, at 40 and 30 ng mL^{-1} for V (IV) and V (V) are obtained.

Conclusion: The described method is sensitive, easy to apply and interferences-free and in that way the determination of vanadium species in different samples was easily achieved. The results of the established method were compared statistically with ETA-AAS using t-paired test showing no significant difference at 95% confidence interval and the proposed method gave comparable analytical figures of merit compared with other sophisticated techniques.

1. INTRODUCTION

In our recently published paper concerning the speciation analysis of iron by using CPE-Spectrophotometry [1], we have made clear in detail the importance of challenges facing the analysts in the analysis of various oxidation states of metals and we focused on the difficulty of choosing the appropriate analytical method, especially when metal species present at low concentration level and in the complex matrices. However, we have been able to overcome some of the difficulties in the analysis of iron species, since the results were satisfactory and worthwhile, which encouraged the authors to engage in more complicated topic, namely the separation and determination of vanadium species by using the same above methodology. The main reasons for the choice of vanadium in this work are its analysis difficulty which lies in the possibility of redistribution of vanadium species, particularly when the environment of the sample is changing [2], its biological and environmental importance and the few papers published in chemical literatures related to the use of cloud point extraction as compared to more common elements such as Fe, Hg, As and Se. Vanadium exists in various oxidation states, but the most two common species occurring in environmental and biological systems are vanadium (IV) and vanadium (V) [3-4]. Recently, vanadium is deemed to be an essential element at trace level and plays a potential role in human health especially with diabetics of type I and II [5]. However, at a high concentration level, vanadium compounds could be highly toxic to humans and animals which will affect kidney and liver functions [6]. However, its toxicity depends on its oxidation state, for which V (V) being more toxic than V (IV) and V (IV) as vanadyl sulfate is 6-10 times less toxic than V (V) as vanadate [7-8]. In fact, there is no recommended intake levels of vanadium established by international bodies so far. However, based on negative impacts observed in animal studies, has been placed accepted high intake of a standard of vanadium is 1.8 mg per day [9]. Exposure of vanadium to humans occurs via different sources, including water, which is a good indicator of urban pollution levels. Most food such as milk, grains,

cereals and vegetable oils is rich in vanadium, while fruits, meats, fish and batter are relatively poor sources of vanadium. In view of these facts, we find that the speciation analysis of vanadium is of extreme importance and represents one of the keys to the understanding of their possible harmful effects to biota and humans [10].

Vanadium speciation analysis has served modest attention by researchers by using some extraction and separation methodologies combined with instrumental techniques that manifestly appeared in the chemical literature, including, solid phase extraction-graphite furnace atomic absorption [11], reverse phase (C18) column separation-flame atomic absorption spectrometry [12] solid phase extraction-inductively coupled plasma-atomic emission spectrometry [13] micro-column packed-electrothermal vaporization inductively coupled plasma-atomic emission spectrometry [14], ion chromatography-inductively coupled plasma-mass Spectrometry [15], chromatography-inductively coupled plasma-atomic emission spectrometry [16], solid-liquid extraction–high performance liquid chromatography [17], capillary electrophoresis with direct UV detection [18], flow injection analysis-kinetic spectrophotometry [19], solid phase spectrophotometry [19] and extractive-spectrophotometry [21]. Over the past two decades, cloud point extraction (CPE) methodology coupled with spectrometric techniques has been presented as an alternative and promising method in estimating vanadium in various matrices, resulting in the emergence of few of papers including, CPE-Flow injection analysis [22], CPE-Flow injection-inductively coupled plasma optical emission spectrometry [23], CPE-graphite furnace atomic absorption spectroscopy [24-28] and CPE-Spectrophotometry [29]. In all the above methods, it has been focusing on the estimation of V (V) or V (IV) ions and mostly on total vanadium by using commercial complexing or chromgenic agents such as, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol(5-Br-PADAP), 8-quinolinol(HO$_6$Q), 2-methyl-5-octyloxymethyl-8-quinolinol, 2-(2´-thiazolylazo)-p-cresol and ascorbic acid, pyronine B and Bromopyrogallol red.

To the best of our knowledge, there is no paper dealing with the assessment vanadium in both forms using the cloud point extraction (CPE) methodology combined with spectrophotometry so far. In this work, an attempt was made to adopt two newly synthesised organic reagents prepared in our laboratory to embark on the determination of V(IV) and V(V) ions in some food and environmental samples by combined CPE-spectrophotometry, perhaps can we contribute to expand the horizons of applications of this methodology in analytical chemistry.

2. MATERIALS AND METHODS

2.1 Apparatus

Two spectrophotometer systems were used in this study namely, a PG Instrument T80+ UV/Vis spectrometer (England) and UV-7804C (China) equipped with a 10-mm quartz cell, for recording of absorption spectra of the complexes formed and absorbance measurements respectively. A double-beam Atomic Absorption Spectrophotometer novAA-400 (Analytic Jena, Germany) equipped with graphite furnace and provided with Ultra fast background correction using a Deuterium lamp and vanadium hallow cathode lamp (operated at 5 mA) as the radiation source at the wavelength of 318.4 nm with 0.5 nm spectral band pass was used for determination of V species. The mass spectrometric measurement of the prepared ligand was carried out by using an Agilent 5975C inner MSD mass spectrometer (J and W Scientific Agilent Technologies, USA) at University of Tarbiat Modares, Tahran, Iran. The mass spectrum was obtained in electron-impact mode (EI) at 70 eV and a direct insertion probe (Acq method 10 W energy) at temperature 90-110ºC. The cloud point temperature of the surfactant was monitored via using a microprocessor-controlled water bath WB 710 model with temperature accuracy of ±0.3ºC at 37ºC (OPTIMA, Japan). The pH meter Philip PW model 9421 (Holland) equipped with combined electrode was employed for solution pH during the optimization and measurement steps.

2.2 Materials and Reagents

The chemicals used in this work including, a 2-amino-benzimidazole and p-benzyl phenol (*Riedel-deHaën, Seelze, Germany*), sodium nitrite, hydrochloric acid, sodium hydroxide, acetic acid and sodium acetate (BDH, England), ethanol (GCC, England), vanadyl sulfate and ammonium metavanadate (Merck, Germany) and Triton X-114 (ACROS ORGANICS, New Jersey, USA) were used as received without any further purification. Doubly distilled and/or deionized

water used throughout. The stock solutions (1000 μg mL^{-1}) of V (IV) and V (V) ions were prepared by dissolving 4.946 g of VOSO$_4$.5H$_2$O in 1 L of 0.01 M HCl and 2.295 g of NH$_4$VO$_3$ in 1 L of 0.05 M of H$_2$SO$_4$ solution, respectively and diluted standard solutions of each metal ion were daily prepared by serial dilution of the stock solutions. A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL of concentrated solution to 100 mL of water in a 100 mL volumetric flask. The stock solutions (1x 10^{-2}M) of synthesized ligands (NTA8HQ) or (BIABP) were prepared by dissolving appropriate amounts in ethanol. The pH buffer solutions in the range (2-7) were prepared by mixing different volumes of 0.1 mol L^{-1} of acetic acid and sodium acetate in 1L.

2.3 Synthesis and Characterization of Reagents

The synthesis and characterization of the first ligand namely 2-[2-(5-Nitro thiazolyl) azo]-8 hydroxyquinoline (5-NTA8HQ) used in this work was described in our previously published paper [1]. The second ligand of 2-[(Benzo imidazolyl) azo]-4-benzyl phenol (BIABP) was synthesized according to the procedure described elsewhere [30] with some modifications as showed in Fig. 1. A 2-amino-benzimidazole (1.33 g, 0.01 mol) was dissolved in 25 mL of distilled water and 5 mL of concentrated hydrochloric acid and diazotized below 0ºC with (0.69 g, 0.01 mol) sodium nitrite and the content was left to stand for a period 15

min to complete the reaction. The resulting diazonium salt was added drop wise with cooling at constant stirring to a solution containing 1.84 g (p-benzyl phenol) and 1.2 g sodium hydroxide dissolved in 150 mL of ethanol. Thereby the clear brown color was observed. After the completion of addition, the solution left to stand for two hours followed by additional 150 mL of distilled water. The mixture was adjusted to pH = 6 with dilute HCl, forming a brown precipitate. The solid product was left for 24 hours and then washed several times with distilled water, crystallized twice from hot ethanol and dried over CaCl$_2$ to give brown crystals, Yield 82%; mp 160-162ºC; mass spectrum analysis shows that the chemical structure of the reagent is C$_{20}$H$_{16}$N$_4$O (328.23 g mol^{-1}); IR(KBr) v$_{max}$/cm^{-1}, 3224, (w, Ar-OH), 3024 (w,Ar C-H), 2916 (w,C-H aliphatic), 1596 (m, C=N), 1512 (s, N=N), 1103 (m, C-N=N-C), 846,786, 732 (s,δ C-H) H^{1}NMR [31] (DMSO-d6, 298 K,) d/ppm (1) Single peak at δ= 3.93ppm of CH$_2$ of benzyl phenol (2) Single peak at δ = 4.75 ppm for OH group in the phenolic ring and NH in benzimidazole (3) Dblat peaks at δ = 6.77-6.78 ppm for protons of phenol ring(H$_3$,H$_5$ and H$_6$) (4) Single peak at δ = 7.073 ppm for protons of benzyl ring (H$_8$,H$_9$,H$_{10}$,H$_{11}$ and H$_{12}$) (5) Dblat peaks at δ = 7.2-7.21 ppm for protons of benzimidazole ring (H$_5$ and H$_6$)(6) triplat peaks at δ = 7.28 7.31 ppm for protons of benzimidazole ring (H$_4$ and H$_7$). The mass fragmentation [32] of the free azo dye ligand (BIABP) is shown in Fig. 2.
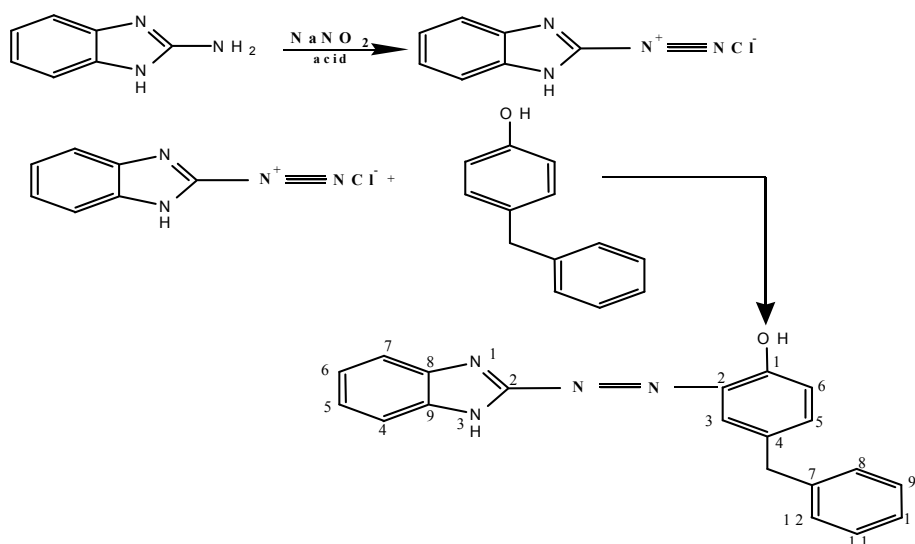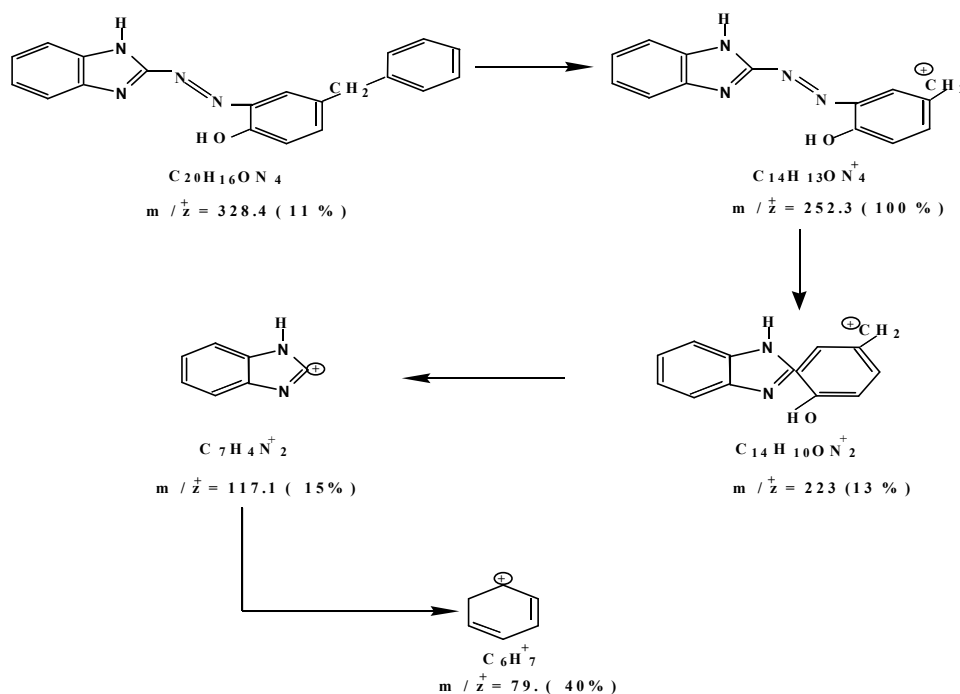

Fig. 1. Synthetic path of reagent (BIABP)

Fig. 2. Mass fragmentation of the free azo dye ligand (BIABP) by using mass spectrometry

2.4 General Procedure for CPE

A 10 mL aliquots of the standard or sample solutions containing vanadium species matched within calibration range, 0.5 mL of 1.0×10^{-2} mol L^{-1} (BIABP) reagent solution for V (IV), 0.5 mL of acetate buffer solution at pH =3.0 and 0.2 mL of (10% v/v) Triton X-114 was kept in a thermostatic water bath at 70ºC for 10 min and two phases were separated by centrifugation for 5 min at 4000 rpm min. The viscosity of the surfactant-rich phase was increased by cooling the system in an ice-bath for 20 min. The supernatant aqueous phase was carefully collected and left aside for the subsequent extraction of V (V). The surfactant-rich phase was dissolved with a 2 mL of 0.1 M nitric acid in ethanol and the concentration of V (IV) ions, was determined spectrophotometry at λ_{max} of 625 nm. The above-mentioned supernatant containing V (V) in an acidic medium (pH ≈2.5) was taken, then 0.5 mL H_2O_2 (1% v/v), 0.3 mL of 1.0×10^{-2} mol L^{-1} (NTA8HQ) reagent and 0.2 mL of (10% v/v) Triton X-114 were added and the resultant solution were held for 10 min in a thermostatic bath at 70ºC and the two phases were separated by centrifugation for 5 min at 4000 rpm. On cooling in an ice bath, the surfactant rich phase became viscous and the supernatant aqueous phase was carefully removed with a pipette. The surfactant-rich phase was dissolved as with the above-mentioned in V (IV) extraction and the concentration of V (V) ions was determined spectrophotometry at λ_{max} of 634 nm.

2.5 Preparation of Samples

2.5.1 Water [20]

The water sample was collected and preserved by the addition of 2 mL of concentrated nitric acid in a polyethylene container that had been carefully cleaned with nitric acid. The samples were stored at 4ºC prior to the measurements. 2 mL of sample was taken and the concentration of V (IV) and V (V) was determined according to the general CPE procedure.

2.5.2 Soils

The samples were dried and grounded into fine powder using a glass mortar, then an accurately amount of 1.00 g of a powdered soil sample was transferred into a 25 mL platinum crucible and digestion procedure was carried out in accordance to the protocol of Molathegi [33]. The sample was first treated with 10.0 mL of HF and 2.0 mL of $HClO_4$ and evaporated till near dryness. Subsequently a 2 mL HF and 1 mL of $HClO_4$ were added and the mixture again

evaporated to near dryness. Finally, $HClO_4$ (1 mL) was added and the sample was evaporated until white fumes appeared. The residue was then dissolved in 5 ml of 6 M HCl and diluted to 50.0 mL with de-ionized water. Extraction and determination of vanadium species were performed by the proposed method.

2.5.3 Rice

The rice sample solution was prepared according to the procedure adopted by Swetha el at. [34] by taking 1.00 g of dried rice at 110ºC and digested with 10 mL of 5M HNO_3 followed by addition of 5 mL of $HClO_4$ (70% w/w). The solution was evaporated to near dryness and the residue was dissolved in 10 mL of 0.1M HCl. Then heated to boiling, cooled and filtered. The filtrate was transferred into a 50 mL volumetric flask and diluted to the mark with deionized water. An appropriate amount of this solution (i.e. 2 mL) was taken and subjected to the CPE procedure to determine vanadium species spectrophotometrically.

2.5.4 Vegetables

Three vegetables samples (potato, spinach, carrot) were collected from local markets and subjected to the procedure described by Lokeshappa et al. [35] with little modification. Dried vegetables were first air-dried in an oven at 105ºC for three days under uncontaminated conditions. A 1.00 g of each grounded powder sample were transferred into glass beaker and digested with 10 mL of HNO_3 and 2.5 mL of HCl (4:1 v/v) on a hot plate at low temperature till complete digestion. After cooling at room temperature, the solution was made to 50 mL with deionized water in a 50 mL volumetric flask. An aliquot (2 mL) of the sample solution was taken, followed CPE procedure and the amount of the two species was determined spectrophometrically.

2.6 Statistical Analysis

All mathematical and statistical computations were made using Excel 2007 (Microsoft Office) and Minitab version 14 (Minitab Inc. State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1 Absorption Spectra

The spectroscopic study was conducted by recording the absorption spectra of [$V^{(IV)}$-BIABP] complex in the presence of surfactants versus a reagent blank. Fig. 3 shows the spectra of $V^{(IV)}$ complex giving an absorption maximum of 625 nm with molar absorpitivities of 2.06×10^6 L $mol^{-1}cm^{-1}$ and the free ligand (BIABP) at λ_{max} of 426 nm. Also, the absorption spectra of the $V^{(V)}$–H_2O_2–NTAHQ and $V^{(V)}$–NTAHQ complexes in 0.05 M H_2SO_4 medium were recorded as showed in Fig. 4. For the comparison of spectra of the binary $V^{(V)}$–NTAHQ complex and ternary $V^{(V)}$–H_2O_2–NTAHQ complex.

Accordingly, the absorption spectrum of $V^{(V)}$–H_2O_2–NTAHQ complex extracted into the surfactant-rich phase exhibited a maximum absorption band at 634 nm with a high molar absorpivitity of 1.6×10^6 L mol^{-1} cm^{-1} which is distinctly different from that of the binary complex, indicating that $V^{(V)}$ should be extracted as a ternary complex. Whilst, ligand (NTA8HQ) given the absorption maxima of 520 nm. The mole ratio and Job's methods were used to study the stoichiometry of V (IV)-BIABP complex and both method were confirmed that the composition of the green complex was of 1:2 (M:L) at pH 3.0. Thus it can be concluded as depicted in Fig. 5 that the formula and probably chemical structure of this complex is $(BIABP)_2 V^{(IV)} O$.

Concerning the stoichiometric ratio of the ternary complex of type V (V)–H_2O_2–NTAHQ, the majority of reports have indicated that the molar ratio and continuous variation methods did not represent a true stoichiomtric value for this type of complexes. So the mathematical method described by He et al. [36] which it did not mention in detail in this study, because of the large number mathematical derivations, was conducted to determine stoichiometric ratio of V (V)/H_2O_2/NTAHQ system. This method was confirmed that the mole ratio of this type of complex is 1:1:1, from which the proposed chemical structure of the ternary complex can be deduced as shown in Fig. 6.

3.2 Factors Affecting CPE Procedure

The effect of the factors such as, pH, concentration of H_2SO_4, H_2O_2 quantity, concentration of reagents, surfactant amount, temperature and incubation time were searched using the classical optimization strategy to obtain the optimum conditions which can achieve the best analytical figures of merit for the two species. All these experiments were performed for the solutions containing 60 ng mL^{-1} V (IV) and/or 30 ng mL^{-1} V (V).
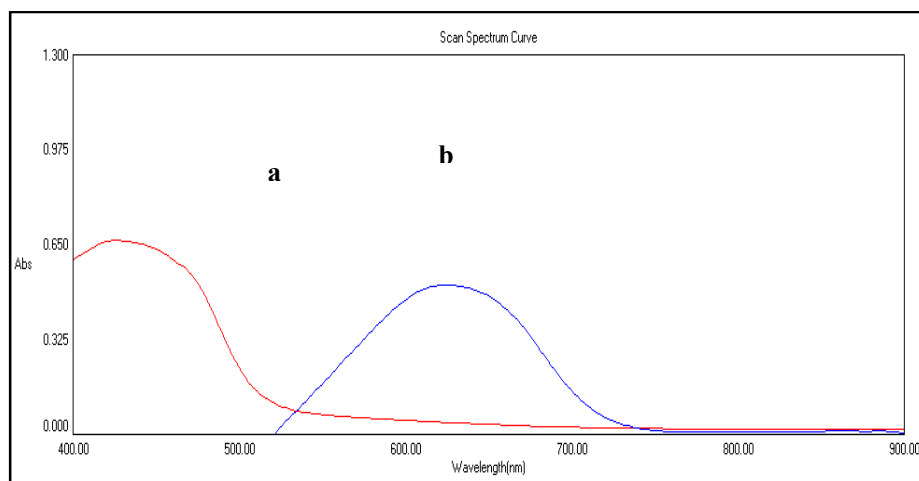
**Fig. 3. Absorption spectra (a) 5 x 10^{-4} M of (BIABP) reagent (b) V (IV-(BIABP) complex, V (IV) =70 ng mL^{-1}, 0.5 mL of (BIABP) = 1 x 10^{-2} M, Buffer pH = 3.0 (0.5 mL), 0.2 mL of 10% (v/v) Triton X-114**
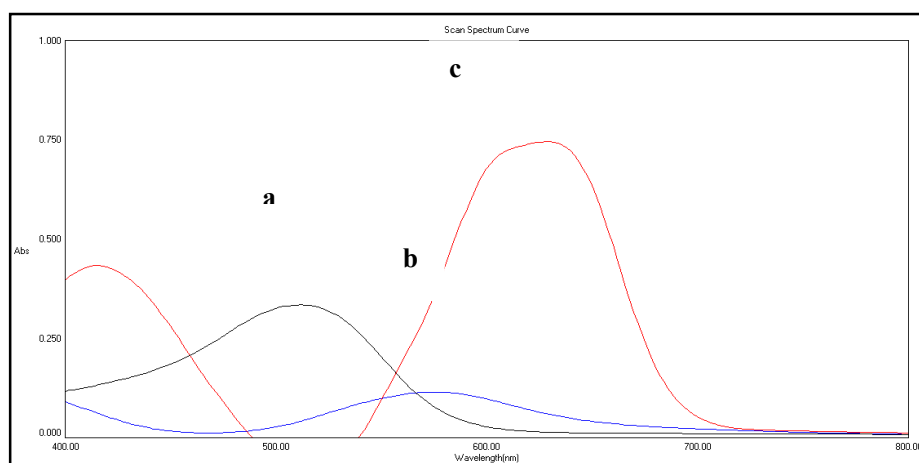
Fig. 4. Absorption spectra (a) 3 x 10^{-4} M (NTA8HQ) reagent (b) V-NTA8HQ binary complex; V (V) =50 ng mL^{-1}, 0.3 mL of 1x 10^{-2} M (NTA8HQ), 0.2 mL of 10 % (v/v) Triton X-114.(c) V-H$_2$O$_2$-(NTA8HQ) ternary complex; V(V) =50 ng mL^{-1},0.3 mL of 1x10^{-2} M (NTA8HQ), 0.5 mL of (1%) [H$_2$O$_2$], 0.2 mL of 10% (v/v) Triton X-114
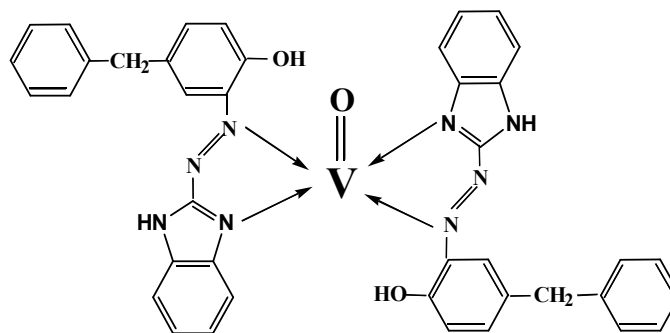
Fig. 5. The suggested chemical structures of the V (IV)-BIABP complex
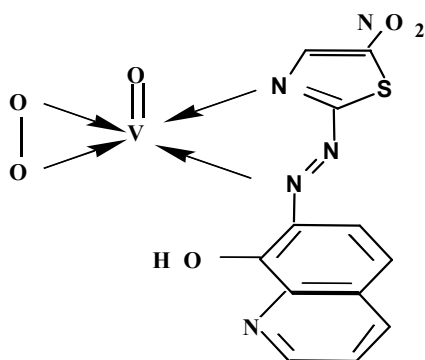
Fig. 6. The proposed chemical structure of V (V)/H$_2$O$_2$/NTAHQ system

The impact of pH on V (IV)-BIABP complex was studied in the range of 2 to 7 using different pH acetate buffer solutions. The results are depicted in Fig. 7. It is noticed that the absorbance increased suddenly with increasing pH and reached a maximum at pH 3 for V (IV) complex and then the absorbance decreased due to partial dissociation of the complexes at higher pH, which may lead to incomplete extraction of complex. Thus, pH 3.0 was chosen as the optimum pH for complete formation of for V (IV) complex. The effect of H$_2$SO$_4$ concentration on V (V)-H$_2$O$_2$-NTA8HQ ternary complex was conducted by varying concentration range of 0.01-0.1 M.

H$_2$SO$_4$. The results depicted in Fig. 8 showed that the absorbance signal reaches a maximum at 0.05 M H$_2$SO$_4$. At higher concentrations of H$_2$SO$_4$, the absorbance decreases which most probably due to the reduction of V (V) ion to (IV) thus preventing the formation of ternary complex in micelle-mediated phase. Therefore, a 0.05 M of H$_2$SO$_4$ was used for further experiments. The influence of H$_2$O$_2$ concentration on the formation of V (V)-H$_2$O$_2$-NTA8HQ ternary complex was carried out by varying volume from 0.1 to 1 mL of H$_2$O$_2$ (1% v/v) as shown in Fig. 9. It was observed that the analytical responses increase rapidly as the volume of H$_2$O$_2$ increases and reach maximum up to 0.5 mL and it remained constant for higher added concentrations. Therefore, 0.5 mL of H$_2$O$_2$ was selected as optimal. The effect of the (5-NTA8HQ) and (BIABP) concentration was conducted for the solutions containing 30 ng mL^{-1} V (V) and 60 ng mL^{-1} V (IV) and varying volume from 0.1 to 1 mL of 1 x 10^{-2} M (5-NTA8HQ) and (BIABP). In both cases, V (V) or V (IV), the analytical responses increase rapidly as the volume of (5-NTA8HQ) or (BIABP) increases and reaches maximum up to

0.3 mL and 0.5 mL of 1.0 × 10^{-2} M of (NTA8HQ) and (BIABP) respectively and decrease thereafter with further increase in the chelating agents indicating that any excessive amount of chelating reagents was unnecessary (Fig. 10). Consequently, 0.3 mL of 1 x 10^{-2} M of (5-NTA8HQ) and 0.5 mL of 1 x 10^{-2} M of (BIABP) was chosen as optimum for V (V) and V (IV) respectively. Fig. 11 shows the impact of Triton X-114 amount on extractability of the two complexes within the surfactant volume range of 0.1 – 0.4 mL of 10% (v/v) Triton X-114 at previously established optimum conditions. It can be seen that the absorbance for both ions increased by increasing the Triton X-114 concentration up to 0.2 of 10% (v/v) for V (V) and V (IV) and then suddenly decreased at higher amounts. Thus 0.2 mL of 10% (v/v) Triton X-114 was used as the optimum amount for V (V) and V (IV) for subsequent experiments.

Fig. 12 shows the influence of the equilibrium temperature ranged from 30 to 80 at 10 min on extraction of the two complexes by CPE. It was shown that a maximum absorbance signal was achieved when the temperature at 70ºC for both species. Whilst Fig. 13 displays the effect of incubation time and found to be 10 min needed for complete extraction of both species in their complexes.

3.3 Calibration Graphs and Statistical Treatments

The calibration graphs for both species were constructed at the established optimized conditions using combined CPE–Spectrophotometry by taken a series of standard V (IV) and V (V) solutions ranging from 10-100 and 1-70 ng mL^{-1} respectively. The two calibration plots were subjected to the statistical evaluation which shown that a strong correlation exists between the calibration points (r = 0.9997 and 0.9999 for V (IV) and V (V) respectively) as shown in Table 1. This was supported by ANOVA analysis (Table not shown) giving that MS$_{reg}$/MS$_{error}$ = 22870 for V (IV) and 106582 for V (V) for 1 and 8 dof and 1 and 7 dof, larger than critical value (F$_{1, 8}$ = 5.32 and F$_{1, 7}$ = 5.5 9 at 95% CI) and confirmed by the normal probability plots (Fig. 14, a and b) which revealed that an ideal linear trend indicative of normality of absorbance response being acceptable and statistically valid [37]. The calibration plots for both species have also been undergone the statistical treatments to extract new analytical figures of merit which are summarized in Table 1.
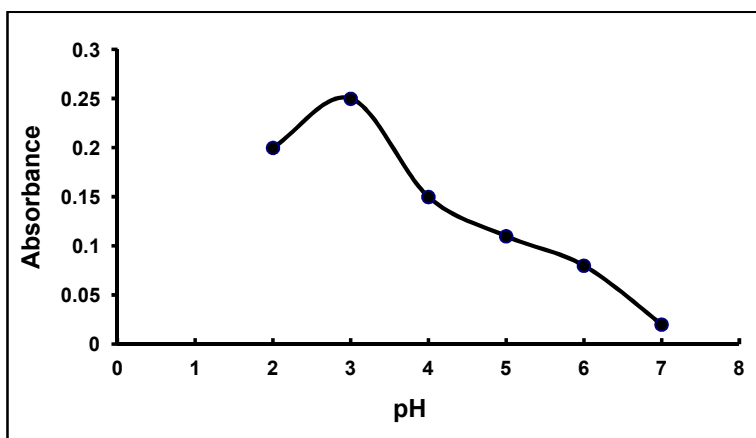
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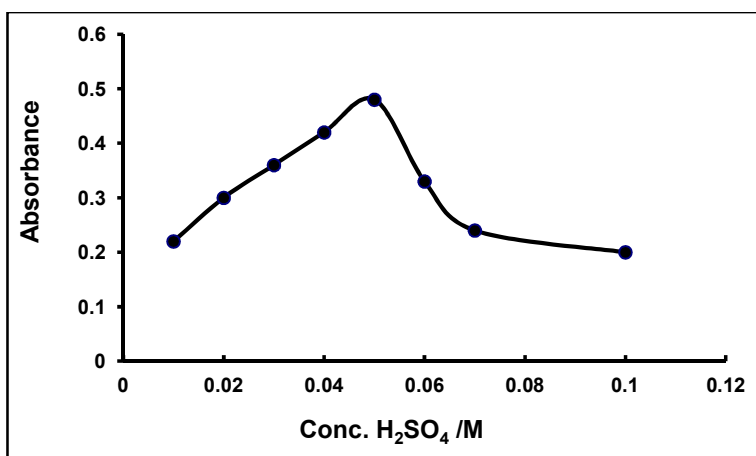**Fig. 7. Effect of pH on the formation of V (IV)-BIABP complex by CPE**

Fig. 8. Effect of H_2SO_4 concentration on V (V)-H_2O_2-NTA8HQ ternary complex by CPE
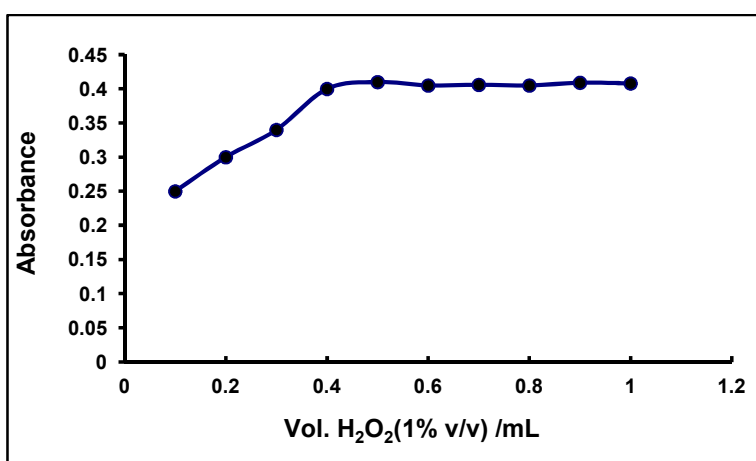

Fig. 9. Effect of H_2O_2 concentration on the formation of V (V)-H_2O_2-NTA8HQ ternary complex by CPE
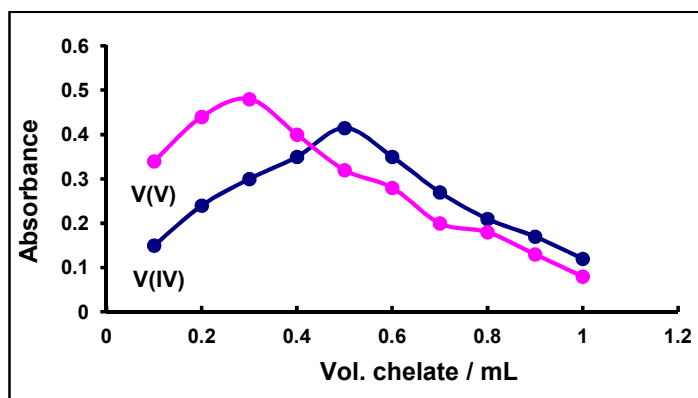
Fig. 10. **Effect of concentration of (5-NTA8HQ) and (BIABP) on the formation of V (V) -H$_2$O$_2$-NTA8HQ ternary complex and V (IV)-BIABP complex by CPE**
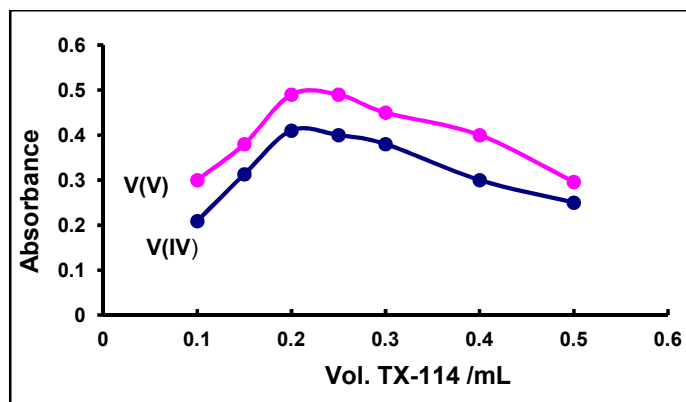
Fig. 11. Effect of Triton X-114 amount on the CPE of V (V) and V (IV) complexes
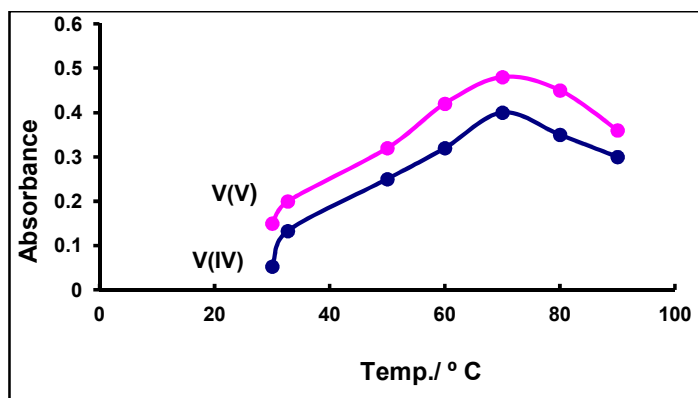
Fig. 12. Effect of temperature on the extraction of V (V)/V (IV) complexes by CPE

It can be seen from Table 1, high enrichment factors were achieved which reflected in enhancement of the sensitivity in term of molar absorptivity or Sandell sensitivity of the proposed method via using has new synthesised chelating agents. This obviously led to obtain very low detection limit in order of 2.53 and 0.72 ng mL^{-1} for V (IV) and V (V) ions in aqueous solutions respectively. These findings were much better than that obtained by other workers (Table 2) except that obtained by Fan et al. [14] and Gamage et al. [15] whom used sophisticated hyphenated techniques.
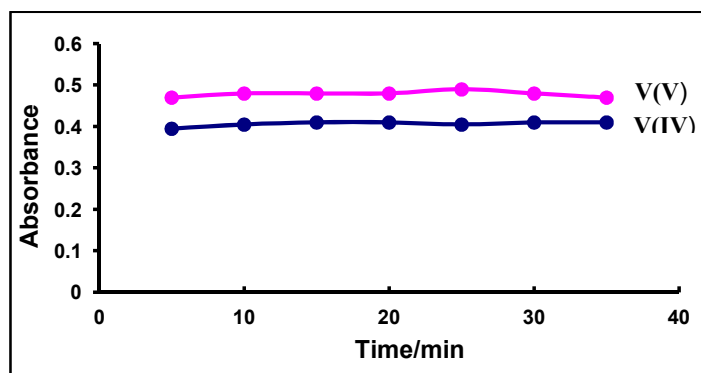
Fig. 13. Effect of time on the CPE of V (V)/V (IV) complexes
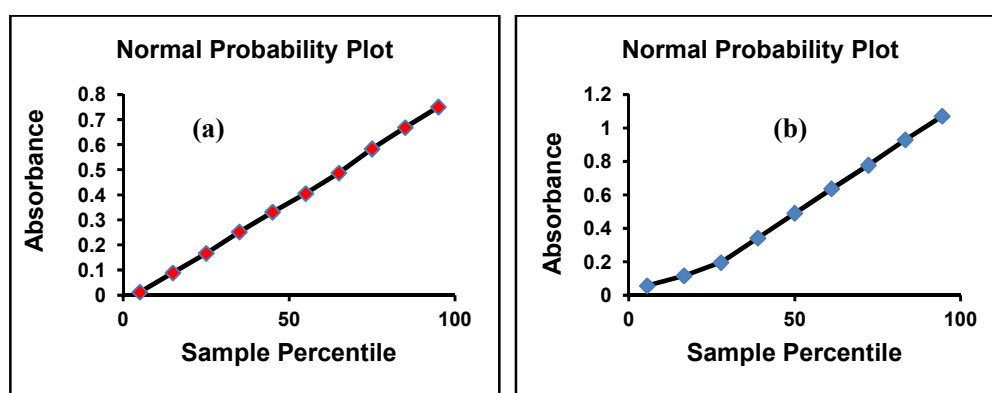
Fig. 14. Normal probability plot of absorbance data for analysis of (a) for V (IV) and (b) for V (V)

Table 1. Analytical figures of merits of V (IV)–(BIABP) and V (V)–H_2O_2-(5-NTA8HQ) by the proposed method

Parameter	V (IV)	V (V)
Regression equation	y = 0.0081x-0.077	y= 0.0146x+0.045
Correlation coefficient (r)	0.9997	0.9999
Correlation of Determination (R2%)	99.97	99.99
Std. dev. of regression line ($s_{y/x}$)	0.00682	0.00351
C.L. for the slope ($b\pm ts_b$) at 95%	0.0081±0.000124	0.0146±0.0001
C.L. for the intercept ($a\pm ts_a$) at 95%	0.077±0.0000855	0.045±0.0048
Concentration range (ng mL^{-1})	10-100	1-70
Limit of Detection (ng mL^{-1})	2.53	0.72
Limit of Quantitation (ng mL^{-1})	8.42	2.40
Sandell's sensitivity (µg cm^{-2})	12×10^{-5}	6.8×10^{-5}
Molar absorptivity (L.mol^{-1}.cm^{-1})	2.06×10^6	1.6×10^6
Composition of complex (M: L)*	1:2	1:1:1
RSD% (n=8)% at 40 ng V (IV) $ml^{-1}$and 30 ng V (V) mL^{-1}	0.670	0.461
Recovery% at 95% C.I	98.3±0.72	97.6±0.45
Preconcentration factor (PF)*	49	66
Enrichment factor (EF)**	159	99

* PF is calculated as the ratio of volumes of aqueous phase to that of surface -rich phase. **EF is calculated as the ratio of slope of calibration curves obtained with and without CPE

Table 2. The reported methods compared to the established method for the determination of V (IV) and V (V) species

Sample	Technique	Detection limit (ng mL^{-1})		Ref.
		V (IV)	V (V)	
Various	Chromatography-FASS	150	180	[12]
Natural water	micro-column separation-ETV-ICP-OES	0.068	0.068	[14]
Ground water	Chromatography-ICP-MS	0.02	0.06	[15]
Minerals processing sample	Chromatography-ICP-OES	40	30	[16]
Catalysts	LLE-Spectrophotometry	-	3.3	[21]
Foods and plants	Spectrophotometry	50	100	[38]
Industrials	IC-ICP-OES	20	50	[39]
human urine, soil and plant	Spectrophotometry	-	12.4	[40]
Synthetic	Spectrophotometry	5.5	-	[41]
Water, soil and foods	CPE-Spectrophotometry	2.53	0.72	This work

This leads us to be quite sure that the prepared ligands have had a major role in enhancing the sensitivity and lowering the detection limit of vanadium species by the proposed method, which gave an impression that there is no need to use much sophisticated instrumentation compared with CPE-Spectrophometry as described by our previous studies [42-45], concluding that this will easily motivate of using the proposed method in the detection of vanadium species in foods, drug formulations and environmental fields alike. The LOD's of the method were also calculated and found to be 0.120 and 0.037 µg g^{-1} for V (IV) and V (V) respectively, via considering a limit of detection (LOD) of 2.53 µg L^{-1} for V (IV) (II) and 0.75 µg L^{-1} for V (V) in aqueous solution and 1.0 g of solid sample in 50 mL solution. These findings have encouraged the application of the proposed method successfully in the estimation of both species in water, soil, rice and vegetable samples to test its applicability and reliability.

3.4 Accuracy Evaluation

Due to the universal lack of certified reference materials that define exactly the quantity of vanadium species, the accuracy of the established method was carried out by assessing the recovery percentage by taking a binary mixture V (V)/V (IV) solution in concentration ratio range from 0.1-2. The results are summarized in Table 3.

3.5 Interferences Study

The selectivity of the suggested method was tested against the potential of some divers' metal ions which may affect the determination of the vanadium species in the selected samples. In this regard, the effect of 1000 fold concentration of each interfering ions on the estimation of 60 ng/mL of V (IV) and 40 ng/mL V (V) solutions were studied following the general CPE procedure. The results are summarized in Table 4.

It can be concluded that there are no appreciable influences for any of interfering ions on the responses of both vanadium species (Table 4). This might be attributed to inability of the interfering ions to form complexes with the two synthesized ligands at the working pH value of the proposed method.

3.6 Applications Study

According to the considerable analytical features that have been achieved in the proposed method, such as low detection limit, high recoveries and interference-free, the method was employed for the detection of both species in water, soil, rice and vegetables samples after the digestion procedures that described in experimental work and measured in triplicate. At the same time, the sample solutions were also determined by electrothermal atomic absorption spectrometric method (ETAAS) to test the significance of the proposed method. The findings are presented in Tables 5. The statistical computations using t-paired test for all tested sample reveals that the proposed method has no significant difference compared with ETAAS method at 95% confidence level.

Table 3. The recovery percentage and relative error of vanadium in binary mixture by CPE-Spectrophotometry

V (V)/V (IV) ratio	Added (ng mL^{-1})	V (IV)			V (V)		
		Found (ng mL^{-1})	Recovery (%)	E$_{rel}$ (%)	found (ng mL^{-1})	Recovery (%)	E$_{rel}$ (%)
0.1	20	19.2	96.00	- 4.00	19.4	97.0	- 3.0
	30	29.5	98.30	- 1.70	29.2	97.3	- 2.7
	40	39.5	98.75	- 1.25	39.0	97.5	- 2.5
0.2	30	29.3	97.90	- 2.10	29.1	97.0	- 3.0
	40	39.4	98.50	- 1.50	38.9	97.2	- 2.8
1.0	40	39.5	98.75	- 1.25	39.2	98.0	- 2.0
	50	49.5	99.00	- 1.20	49.3	98.6	- 1.4
2.0	50	49.3	98.60	- 1.40	49.1	98.2	- 1.8

Table 4. Effect of interfering ions on the absorption signal of V (IV) [60 ng mL^{-1}, Abs= 0.405] and V (V) [40 ng mL^{-1}, Abs= 0.636] by proposed method

Interfering ion	V (IV)			V (V)		
	A	AΔ	% E$_{rel}$	A	AΔ	% E$_{rel}$
Na$^+$	0.410	+0.005	+1.23	0.635	-0.001	-0.15
K$^+$	0.400	- 0.005	- 1.23	0.632	-0.004	-0.62
Ca^{2+}	0.410	+0.005	+1.23	0.635	-0.001	-0.15
Mg^{2+}	0.408	+0.003	+0.74	0.631	-0.005	-0.78
Fe^{3+}	0.403	- 0.002	-0.49	0.629	-0.007	-1.10
Fe^{2+}	0.406	+0.001	+0.24	0.632	-0.004	-0.62
Al^{3+}	0.403	- 0.002	- 0.49	0.627	-0.009	-1.40
Mn^{2+}	0.404	- 0.001	- 0.24	0.625	-0.011	-1.70
Zn^{2+}	0.401	+0.005	+1.23	0.628	-0.008	-1.20
Cr^{3+}	0.400	- 0.005	- 1.23	0.637	+0.001	+0.15
Co^{2+}	0.399	- 0.006	- 1.48	0.634	-0.002	-0.31
Ni^{2+}	0.398	- 0.007	- 1.70	0.630	-0.006	-0.94
Cd^{2+}	0.396	- 0.009	+2.20	0.639	+0.003	+0.47
Cu^{2+}	0.398	- 0.007	- 1.70	0.640	+0.004	+0.62

Table 5. Results of the estimation of V (IV) and V (V) ions in different real samples with statistical paired t-test at 95% confidence level

Sample	V(IV)/ng mL^{-1}			V(V)/ng mL^{-1}		
	Prposed method*	ETAAS method*	Paired t- test**	Prposed method*	ETAAS method*	Paired t- test**
Water			$\overline{x}_d$=0.055			$\overline{x}_d$=0.26
Tap water	2.10±0.26	1.60±0.18	s$_d$=0.049	8.30±0.50	7.40±0.18	s$_d$=0.323
MBW 1	0.37±0.08	0.29±0.05	t$_{cal}$= 1.58	1.23±0.24	1.59±0.13	t$_{cal(n=6)}$=1.97
MBW 2	0.40±0.04	0.36±0.09	t$_{crit}$ at 95%	0.84±0.43	0.56±0.33	t$_{crit}$ at 95%
MBW 3	0.14±0.07	0.12±0.07	df,5=2.57	0.54±0.12	0.45±0.92	df,5=2.57
MBW 4	0.26±0.12	0.18±0.11		2.05±0.83	1.95±0.73	
MBW 5	0.31±0.17	0.26±0.31		0.82±0.13	0.77±0.26	
Soil						$\overline{x}_d$=0.445
1	<0.120	<0.120		2.35±0.23	1.92±0.16	S$_d$=0.099
2	<0.120	<0.120	-	2.82±0.50	2.26±0.13	t$_{cal(n=4)}$= 1.97
3	<0.120	<0.120		3.32±0.19	2.83±0.62	t$_{crit}$ at 95%
4	<0.120	<0.120		2.10±0.21	1.78±0.24	df,3 =3.18
Rice			$\overline{x}_d$ =0.055			*
Iraqi 1	0.35±0.28	0.25±0.072	S$_d$=0.042	3.84±0.23	2.92±0.13	
Iraqi 2	0.28±0.14	0.20±0.048	t$_{cal(n=4)}$=	3.25±0.09	2.61±0.42	

Sample	V(IV)/ng mL^{-1}			V(V)/ng mL^{-1}		
	Prposed method*	ETAAS method*	Paired t- test**	Prposed method*	ETAAS method*	Paired t- test**
Indian	0.17±0.09	0.16±0.122	2.61	2.68±0.51	2.59±0.08	
Vietnamese	0.60±0.15	0.57±0.083	t_{crit} at 95% df,3=3.18	4.42±0.05	4.37±0.12	
Potato	<0.12	<0.12	$\overline{x}_d$=0.055	10.20±0.34	9.90±0.12	$\overline{x}_d$=0.6
Spinach	0.15±0.09	0.14±0.03	S_d=0.049	5.10±0.63	4.70±0.32	S_d=0.435
Carrot	0.63±0.03	0.72±0.07	t_{cal}= 1.58	8.30±0.14	7.20±0.65	$t_{cal(n=3)}$= 2.34 t_{crit} at 95% df,2=4.3

*The mean value and its standard deviation for three replicate measurements at 95% confidence level ($\overline{x}\pm$ t. s/√n); **paired t-test: ׀ t׀= $\overline{x}_d$ √n/s$_d$;

4. CONCLUSION

In this piece of work, a new CPE coupled with traditional spectrophotometric method using two synthesized ligands was established for the determination of V (IV) and V (V) species in different samples. The separation of two vanadium species was easily conducted in two steps and single extraction by CPE using home-made organic reagents for the first time. The established method gave the distinct features which were represented by acceptable analytical figures of merit, high reliability and wide applications in different areas compared with other sophisticated techniques (Table 2).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ibrahim ZT, Khammas ZAA, Khadhim KJ. Determination of micro amounts of Fe (II) and Fe (III) in tea and rice samples by cloud-point extraction-spectrophotometry using a new chelating agent. Int J Chem Sci. 2014;12(4):1189-1207.
2. Pyrzyńska K. Some Problems in speciation Analysis of Vanadium. Adnan Menderes University, 4th AACD Congress, 29 Sept-3Oct. 2004, Kuşadası-Aydin/Turkey Proceedings Book 006.DOC; 2004.
3. Crans DC, Bunch RL, Theisen LA. Interaction of trace levels of vanadium (IV) and vanadium (V) in biological systems. J Am Chem. Soc. 1989;111:7597-7607.
4. Cornelis R, Caruso J, Crews H, Heumann K. Handbook of elemental speciation II–species in the environment, food, medicine and occupational health. John Wiley & Sons Ltd, the Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England; 2005.
5. Radmaev V, Prakash S, Majeed M. Vanadium: A review of its potential in fight against diabetes. J Altern Complement Med. 1999;5(3):273-291.
6. Liasko R, Kabanos TA, Karkabounas S, Malamas M, Tasiopoulas AJ, Stefanou D, Collery P, Evangelou A. Beneficial effects of a vanadium complex with cysteine, administered at low doses on benzo (alpha) pyrene-induced leiomyosarcomas in Wistar rats. Anticancer Res. 1998;18(5A):3609-3613.
7. Patel B, Henderson GE, Haswell SJ, Grzeskowiak R. Speciation of vanadium present in a model yeast system. Analyst. 1990;115:1063-1066.
8. Poucheret P, Verma S, Grynpas MD, McNeill JH. Vanadium and diabetes. Mol Cell Biochem. 1998;188:73-80.
9. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, D. C; 2001.
10. Ebdon L, Cornelis R, Crews H, Donald OFX, Quevauviller PH. Trace element speciation for environment, food and health. Sci Tot Environ. 2001;309(1-3):265−270.
11. Wang D, Sañudo-Wilhelmy SA. Development of an analytical protocol for the determination of V (IV) and V (V) in seawater: Application to coastal environments. Mar Chem. 2008;112:72–80.
12. Gáspár A, Posta J. Rapid and simple chromatographic separation of V (V) and V (IV) using KH-phthalate and their

determination by flame atomic absorption spectrometry. Fresenius J. Anal Chem. 1998;360:179–183.

13. Ferreira SLC, Queiroz AS, Fernandes MS, Dos Santos HC. Application of factorial designs and Doehlert matrix in optimization of experimental variables associated with the preconcentration and determination of vanadium and copper in seawater by inductively coupled plasma optical emission Spectrometry. Spectrochim Acta, Part B. 2002;57:1939–1950.

14. Zhefeng Fan Z, Hu B, Jiang Z. Speciation analysis of vanadium in natural water samples by electrothermal vaporization inductively coupled plasma optical emission spectrometry after separation/ preconcentration with thenoyltrifluoroacetone immobilized on microcrystalline naphthalene. Spectrochim Acta, Part B. 2005;60:65–71.

15. Gamage SV, Hodge VF, Cizdziel JV, Lindley K. Determination of vanadium (IV) and (V) in Southern Nevada groundwater by ion chromatography-inductively coupled plasma mass spectrometry. The Open Chemical and Biomedical Methods Journal. 2010;3:10-17.

16. Hu M, Coetzee PP. Vanadium speciation by chromatographic separation of V (IV) and V (V) in acidic solution followed by ICP-OES determination. Water SA. 2007; 33(2):291-296.

17. Takaya M. Selective determination method for vanadium (V) and vanadium (IV) controlling the pH of dediafora solid-liquid extraction column. Industrial Health. 2000; 38:91–94.

18. Chen Z, Naidu R. On-column complexation and simultaneous separation of vanadium (IV) and vanadium (V) by capillary electrophoresis with direct UV detection. Anal Bioanal Chem. 2002;374:520–525.

19. Oguma K, Yoshioka O, Norob J, Sakurai H. Simultaneous determination of vanadium (IV) and vanadium (V) by flow injection analysis using kinetic spectrophotometry with Xylenol Orange. Talanta. 2012;96:44-49.

20. Bosque-Sendra JM, Valencia MC, Boudra S. Speciation of vanadium (IV) and vanadium (V) with Eriochrome Cyanine R in natural waters by solid phase spectrophotometry. Fresenius J. Anal Chem. 1998;360(1):31–37.

21. Gavazov K, Lekova V, Patronov G, Turkyilmaz M. Extractive-spectrophotometric determination of vanadium (IV/V) in catalysts using 4-(2-Pyridylazo)-resorcinol and tetrazolium violet, Chem Anal. 2006;51:221-227.

22. Paleologos EK, Koupparis MA, Karayannis MI, Veltsistas PG. Nonaqueous catalytic fluorometric trace determination of vanadium based on the pyronine B-hydrogen peroxide reaction and flow injection after cloud point extraction. Anal Chem. 2001;73(18):4428-33.

23. Wuilloud GM, DE WUILLOUD JC, Wuilloud RG, Silva MF, Olsina RA, Martinez LD. Cloud point extraction of vanadium in parenteral solutions using a nonionic surfactant (PONPE 5.0) and determination by flow injection-inductively coupled plasma optical emission spectrometry. Talanta. 2002;58(4):619-27.

24. Ohashi A, Ito H, Kanai C, Imura H, Ohashi K. Cloud point extraction of iron (III) and vanadium (V) using 8-quinolinol derivatives and Triton X-100 and determination of 10^{-7} mol dm^{-3} level iron (III) in river water reference by a graphite furnace atomic absorption spectroscopy. Talanta. 2005; 65:525-530.

25. Zhu X, Zhu Z, Wu S. Determination of trace vanadium in soil by cloud point extraction and graphite furnace atomic absorption spectroscopy. Microchim Acta. 2008;161:143–14.

26. Khan S, Kazi TG, Baig JA, Kolachi NF, Afridi HI, Wadhwa SK, Shah AQ, Kandhro GA, Shah F. Cloud point extraction of vanadium in pharmaceutical formulations, dialysate and parenteral solutions using 8-hydroxyquinoline and nonionic surfactant. J Hazard. Mater. 2010;182:371-376.

27. Khan S, Kazi TG, Baig JA, Kolachi NF, Afridi HI, Kumar S, Shah AQ, Kandhro GA. Cloud point and solid phase extraction of vanadium in surface and bottled mineral water samples using 8-Hydroxyquinoline as a complexing reagent. J Iran Chem Soc. 2011;8(4):897-907.

28. Filik H, Aksu D. Determination of vanadium in food samples by cloud point extraction and graphite furnace atomic absorption spectroscopy. Food Anal Methods. 2012; 5:359–365.

29. Madrakian T, Afkhami A, Siri R, Mohammadnejad M. Micelle mediated extraction and simultaneous spectrophotometric determination of vanadium (V) and molybdenum (VI) in

plant foodstuff samples. Food Chem. 2011; 127(2):769-73.

30. Habeeb HA, Al-adilee KJ, Jaber SA. Synthesis, characterization, thermal and kinetic photo chemical decomposition study of new azo dye 7-[2-(BenzImidazolyl) Azo]-8-Hydroxy Quinoline and its znic (II) complex. Chem Mater Res. 2014;6(8):69–80.

31. Jarad AJ. Ph.D. Thesis. Baghdad University; 2007.

32. Elajaily MM, Abdullah FI, Akasha RA, Suliman MS. Synthesis, characterization and corrosion inhibition of cobalt (II) azo schiff base chelate. J Chem Pharm Res. 2013;5(12):1144-1151.

33. Molatlhegi RO. M.Sc. Thesis. Faculty of Natural Sciences. Tshwane University of Technology; 2005.

34. Swetha M, Raveendra RP, Krishna RV. Direct derivative spectrophotometric determination of micro amounts of Vanadium (V) by 5-bromo salicylaldehyde isonicotinoyl hydrazone (5-BrSAINH). Intern J Chem Tech Res. 2013;5(5):2322-2328.

35. Lokeshappa B, Kandarp S, Vivek T, Anil KD. Assessment of toxic metals in agricultural products. Food and Public Health. 2012;2(1):24-29.

36. He X, Tubin M, Rossi AV. The determination of the stoichiometry of the mixed complex of vanadium with hydrogen peroxide and with 4-(2-pyridilazo) resorcinol. Quimica Nova. 2000;23(3):316-319.

37. Gardiner WP. Statistical analysis methods for chemists: A software-based approach. The Royal Society Chemistry. Thomas Graham House, Cambridge; UK; 1997.

38. Jassim AH, Qassim BB, Mansoor AS. Removal interferences with spectrophotometric study for the determination of chromium, vanadium and their application. Ibn Al- Haitham J. Pure Appl Sci. 2010;23(3):205-225.

39. Hu M. Metal speciation of vanadium and thallium by IC-ICP-OES M.Sc Thesis, University of Rand Afrikaans; 2002.

40. Al-Tayar NGS, Nagaraja P, Vasantha RA, Shresta AK. A spectrophotometric assay method for vanadiumin biological and environmental samplesusing 2, 4-dinitrophenylhydrazine with imipramine hydrochloride. Environ Monit Assess. 2012;184:181–191.

41. Spinola Costa AC, Leonardo SG, Jaeger THV, Ferreira SLC. Spectrophotometric determination of vanadium (IV) in the presence of vanadium (V) using Br-PADAP. Mikrochim Acta. 1998;130:41-45.

42. Khammas ZAA, Ghali AA, Kadhim KH. Combined cloud-point extraction and spectrophotometric detection of lead and cadmium in honey samples using a new ligand. Int. J. Chem. Sci. 2012;10(3):1185-1204.

43. Khammas ZAA, Jawad SK, Ali IR. A new spectrophotometric determination of chromium (VI) as $Cr_2O_7^{2-}$ after cloud-point extraction using a laboratory-made organic reagent. Global Journal of Science Frontier Research Chemistry. 2013;13(8):9-19.

44. Khammas ZAA. Recent trends for separation and preconcentration in metal ions and organic compounds analysis after cloud-point methodology: Developments and analytical applications–A review. Eurasian J. Anal Chem. 2009;4(1):1-35.

45. Khammas ZAA, Jawad SK, Ali IR. A new approach for extraction and determination of manganese in environmental samples using cloud-point extraction coupled with spectrophotometry. Chem Sci Trans. 2014;36:255-267.

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