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REVIEW

Application of HPLC to measure vanadium in environmental, biological and clinical matrices

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Abstract Vanadate and vanadium compounds exist in many environmental, biological and clinical matrices, and despite the need only limited progress has been made on the analysis of vanadium compounds. The vanadium coordination chemistry of different oxidation states is known, and the result of the characterization and speciation analysis depends on the subsequent chemistry and the methods of analysis. Many studies have used a range of methods for the characterization and determination of metal ions in a variety of materials. One successful technique is high performance liquid chromatography (HPLC) that has been used mainly for measuring total vanadium level and metal speciation. Some cases have been reported where complexes of different oxidation states of vanadium have been separated by HPLC. Specifically reversed phase (RP) HPLC has frequently been used for the measurement of vanadium. Other HPLC methods such as normal phase, anion-exchange, cation-exchange, size exclusion and other RP-HPLC modes such as, ion-pair and micellar have been used to separate selected vanadium compounds. We will present a review that summarizes and critically analyzes the reported methods for analysis of vanadium salts and vanadium compounds in different sample matrices. We will compare various HPLC methods and modes including sample preparation, chelating reagents, mobile phase and detection methods. The comparison will allow us to identify the best analytical HPLC method and mode for measuring vanadium levels and what information such methods provide with regard to speciation and quantitation of the vanadium compounds.

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Contents

1. Introduction

The determination of vanadium in environmental, biological and clinical samples has attracted much attention in the last three decades, because of the increasing roles that various forms of vanadium are playing in the sciences (Kiss et al., 2008; Sakurai et al., 2002; Thompson et al., 2004; Lyonnet et al., 1899; Rehder, 2013; Willsky et al., 2013; Shechter and Karlish, 1980). In fact, vanadium toxicity has been discussed (Kiss et al., 2008; Cohen et al., 2010; Rehder et al., 2003; Cohen et al., 2007; Domingo, 2000; Aureliano and Crans, 2009; Zhang et al., 2006; Domingo, 1996; Crans et al., 2011) and compared to other known trace elements (Pais and Jones, 1997; Nordberg et al., 2011). Vanadium is known to have insulin mimetic properties (Kiss et al., 2008; Willsky et al., 2013; Shechter and Karlish, 1980; Rehder et al., 2003; Domingo, 2000; Aureliano and Crans, 2009; Willsky et al., 2011; McNeill et al., 1992; Thompson et al., 2009; Rehder et al., 2002; Crans et al., 2013; Crans, 2000; Rehder, 2012; Thompson et al., 1999) and found to be able to enhance the effects of insulin by lowering elevated blood glucose and lipid concentration to near normal levels and remarkably it does so without affecting glucose and lipid levels in normal subjects. Vanadium is a trace level element that may or may not have an essential role for humans (Chasteen, 1983; Nielsen, 1991; Nielsen, 2000). In addition, vanadium has anti-carcinogenic activities some of which involve some of the same proteins activated as anti-diabetic agents and as anti-oxidant agents (Evangelou, 2002; Korbecki et al., 2012; Zwolak, 2013; León et al., 2014; Wu et al., 2014; Pessoa et al., 2014; De Cremer et al., 2002; Thompson et al., 1984). In addition to these beneficial effects, vanadium is known to be anthropogenically released to the environment in large amounts, mainly from burning fossil fuel (oil), coal and other industrial activities (Sabbioni et al., 1996; Chen and Owens, 2008). Therefore the effects of high concentration of vanadium compounds on human health must be considered. Depending on pH, vanadium salts and vanadium compounds exist in different oxidation states, the most common forms found in biological systems being vanadium(IV) and vanadium(V) compounds (Crans, 2000; Thompson et al., 1999; Chasteen, 1983; Crans et al., 2004; Thompson and Orvig, 2006). In addition to the multiple number of species existing, the chemistry is even more complicated because some of these species interconvert rapidly (Crans, 2000; Chasteen, 1983; Chasteen et al., 1986; Amin et al., 2000; Crans et al., 1990); and this requires that the determination of species be carried out under the condition of the systems, that is in specific environmental, biological and clinical matrices.

The interest of vanadium speciation originates from the frequent occurrence of this metal ion, under its different forms, in various matrices (Aureliano and Crans, 2009; Crans et al., 2013; De Cremer et al., 2002; Rehder, 2003; Ortega and Metals, 2002). However, the concept of speciation varies from that used by the classical solutions chemists (Pettersson et al., 1983, 1985a,b) on one hand to that described by the IUPAC on the other hand focusing on the composition of matter (Crans et al., 2004; Rehder, 2003; Ortega and Metals, 2002). Extensive work by three IUPAC divisions have gone into the definition of terminology to encompass different groups of scientists, legislators and consumer groups (Crans et al., 2013; Templeton et al., 2000; Bernhard et al., 1986; Lobinski et al.,

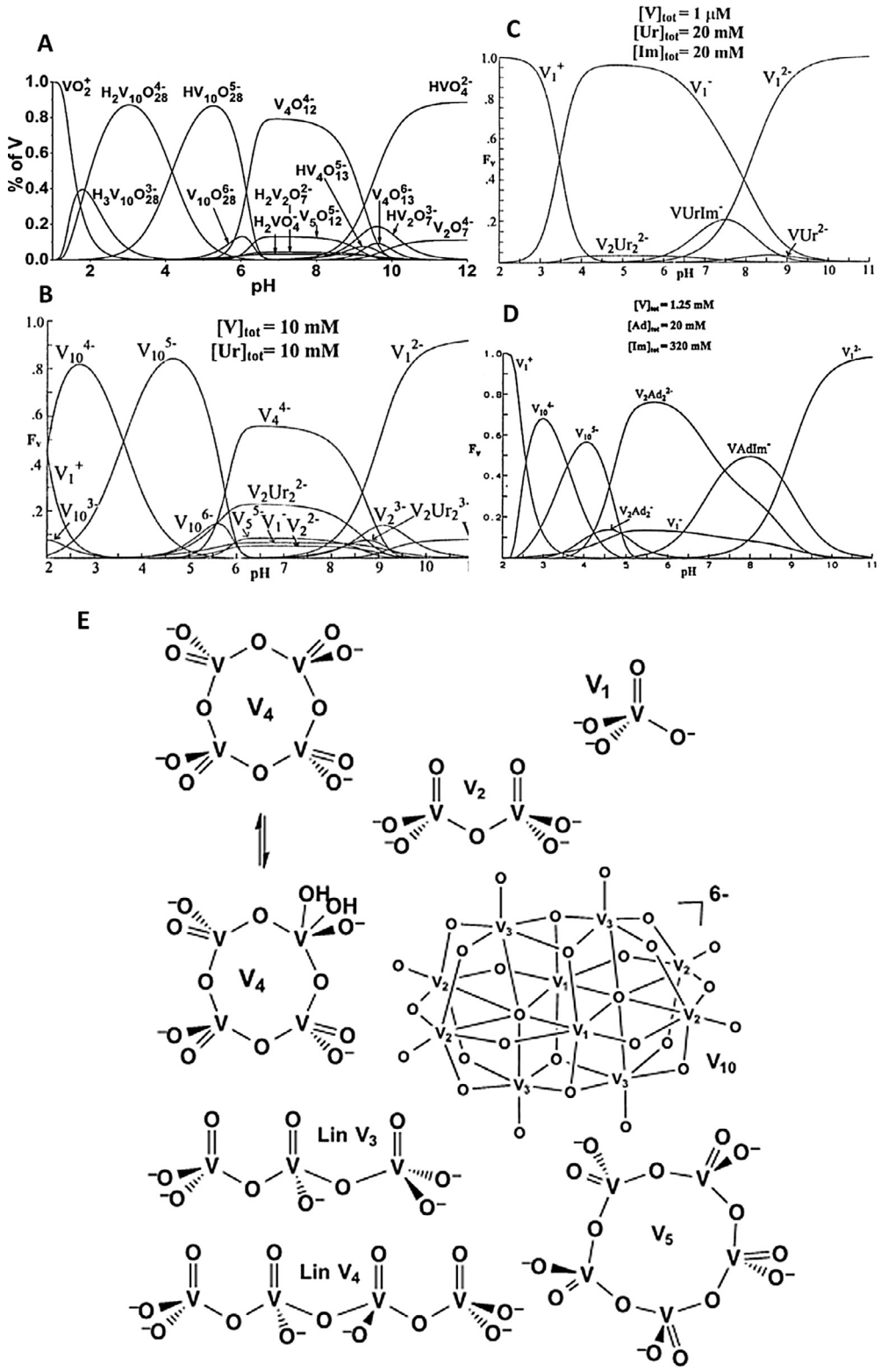

Fig. 1 (A–E) The speciation of 50 mM vanadate and 0.15 M NaCl describing the relative concentration of each vanadium species at a given pH. This speciation diagram was calculated using the HySS program and formation constants given in the literature (Crans and Levinger, 2012). For comparison 3 more speciation plots are shown containing a chelator, uridine (Elvingson et al., 1998). Panel B shows the speciation for a solution of 10 mM vanadate and 10 mM uridine. Panels C and D contains vanadate, uridine and imidazole. Panel E shows the structures for the different vanadium(V) and vanadium(IV) species that are known to form in aqueous solution (Crans et al., 2004). Diagrams were reproduced with permission from references (Crans et al., 2004; Crans and Levinger, 2012; Elvingson et al., 1998).

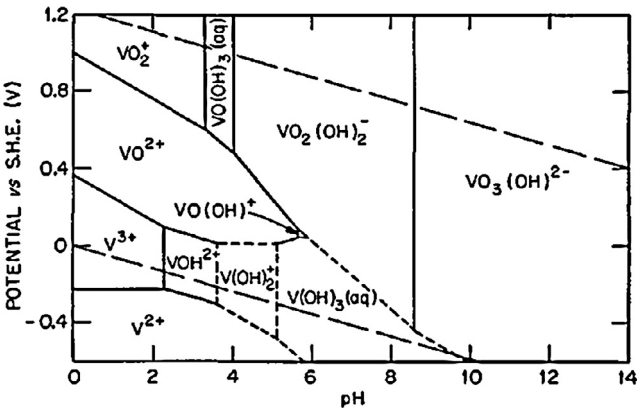

Fig. 2 The forms of vanadium are shown in a plot of pH versus redox potential. The diagram was reproduced with permission from references (Crans et al., 2004; Baes and Mesmer, 1976).

2010; Mounicou et al., 2009). Specifically IUPAC recommends that the term *chemical species* is used to describe a specific form which can be an element and its defined isotopic composition, electronic or oxidation state and/or a complex or molecular structure. It is this context that speciation analysis is defined in this manuscript. Such definition does require that the chemistry be considered, particularly when different forms (that is species) can interconvert under mild condition whereas others are stable. This is therefore a particularly challenging system because the analytical studies are conducted to measure the quantities of one or more individual chemical species in a sample. In summary, the term *speciation of a component* can be defined as the distribution of this component among defined chemical species in a system.

In order to understand in detail the biochemistry of vanadium salts and compounds, quantification of the different forms within a specific environmental, biological or clinical matrix, as well as, identification of the species within a matrix is important albeit difficult (Chen and Owens, 2008; Donard and Caruso, 1998; Rosen and Hieftje, 2004). The rich vanadium chemistry and the ability of, for example, vanadate to react even with buffers (Crans and Shin, 1994) the stability and lability (Crans et al., 2004, 1990) of each vanadium species is determined. Many studies measure the total amount of vanadium(IV) and vanadium(V) species, however, some studies have been done to demonstrate the differential interactions of these two metal ions within matrices. The aqueous speciation of vanadium(V) is very complex and several species of different nuclearity and protonation states can form as shown in the speciation diagrams (see section 2.02 below), Fig. 1A and B (Chasteen, 1983; Crans et al., 2004; Pettersson et al., 1983, 1985a,b). We highlight here the speciation in a solution of pure vanadium(V) followed by speciation schemes showing that combinations of the vanadium-uridine systems illustrate nicely the different effects that a chelating ligand and changes in vanadium concentration has on the speciation (Jakusch et al., 2011; Crans and Levinger, 2012; Elvingson et al., 1998). The addition of a chelator will concentrate the vanadium in those materials. The complex aqueous chemistry is compounded by the fact that some of these species interconvert rapidly and others slowly (Fig. 1C) (Crans, 2000; Chasteen, 1983; Chasteen et al., 1986; Amin et al., 2000). The aqueous vanadium(V) chemistry is very well understood, because these systems have been studied using ^{51}V NMR spectroscopy leading to a detailed understanding of these fundamental reactions. Corresponding reactions also take place with aqueous vanadium(IV) although the details are less understood (Kiss et al., 2008; Chasteen, 1983; Crans et al., 2004; Kiss and Odani, 2007), because dimerization of the vanadyl cation render the species EPR silent and not measurable by this technique (Chasteen et al., 1986). Vanadyl cation is also referred to by IUPAC as oxidovanadium(IV) and both names are used in this work. Quantitation of vanadium(IV) species was done, as with other vanadium species, against a calibration graph generally constructed by standards prepared in blank solutions.

In the neutral and basic pH range vanadium in lower oxidation states are not easily maintained in the presence of oxygen (Chasteen, 1983; Crans et al., 2004; Templeton et al., 2000; Bernhard et al., 1986; Kiss and Odani, 2007; Tracey et al., 2007; Meisch and Bieling, 1980). In general the coexistence of two oxidation states are common but depends on the pH, redox potential and the ionic strength. From a stability point of view, vanadium(V) and vanadium(IV) have very different speciation profiles in aqueous solution. A representation of the changes as a function of redox potential and pH is shown in Fig. 2. However, this illustration is not properly reflecting the complexity of the chemistry as the different oxidation states undergo different chemistry and do not remain as the mononuclear form. For example, it was found that a solution of vanadium(V) salts, prepared in deionized water, was stable in the pH range of 2–9. Although, depending on the pH value of the aqueous solution, different oligomeric species formed, each of which had several protonation states. Therefore, the mononuclear description of the aqueous vanadium(V) species generally used is dramatically simplifying the chemistry and only valid for systems where the concentrations are micromolar or submicromolar (Kiss et al., 2008; Chasteen, 1983; Crans et al., 2004; Pettersson et al., 1985a,b; Kiss and Odani, 2007; Pettersson, 1993). In contrast, a solution of vanadium(IV) salts were found to be stable only below pH 3. On the other hand, vanadium(IV), in the form of vanadyl (oxidovanadium(IV)) cation VO_2^+ is stable in acidic solution below pH 3, and as $VO(OH)_3^-$ above pH 12. Above pH 3 vanadium(IV) begins to oxidize unless oxygen is kept away from the solution. As pH increases the oxidation reaction rate increases and the conversion into vanadium(V) at pH 9 is much faster (Kiss et al., 2008; Crans et al., 2004). Therefore, the stability of these two oxidation states of vanadium should be carefully taken into consideration because even during characterization of a given material, some of the species may readily interconvert depending on reaction conditions. Thus, the specific parameters including time and pH should be identified during sampling, pretreatment, separation, calibration for quantification and speciation characterization (Sugiyama et al., 2001).

For analytical purposes, a variety of techniques have been used for vanadium separation, characterization, quantitation and speciation, Fig. 3. These cases are based on spectroscopic/spectrometric methods (Chen and Owens, 2008; Settle, 1997; Amorim et al., 2007), and separation methods (Chen and Owens, 2008; Settle, 1997). The spectroscopic/spectrometric techniques include atomic absorbance spectroscopy (AAS) with flame and graphite tube atomizers (Gáspár and Posta, 1998; Pyrzyńska and Wierzbicki, 2004), inductively coupled plasma optical emission spectrometry (ICP-OES) (Chen and Owens, 2008; Pyrzyńska and Wierzbicki, 2004; Pyrzyńska, 2005), inductively coupled plasma mass spectrometry (ICP-

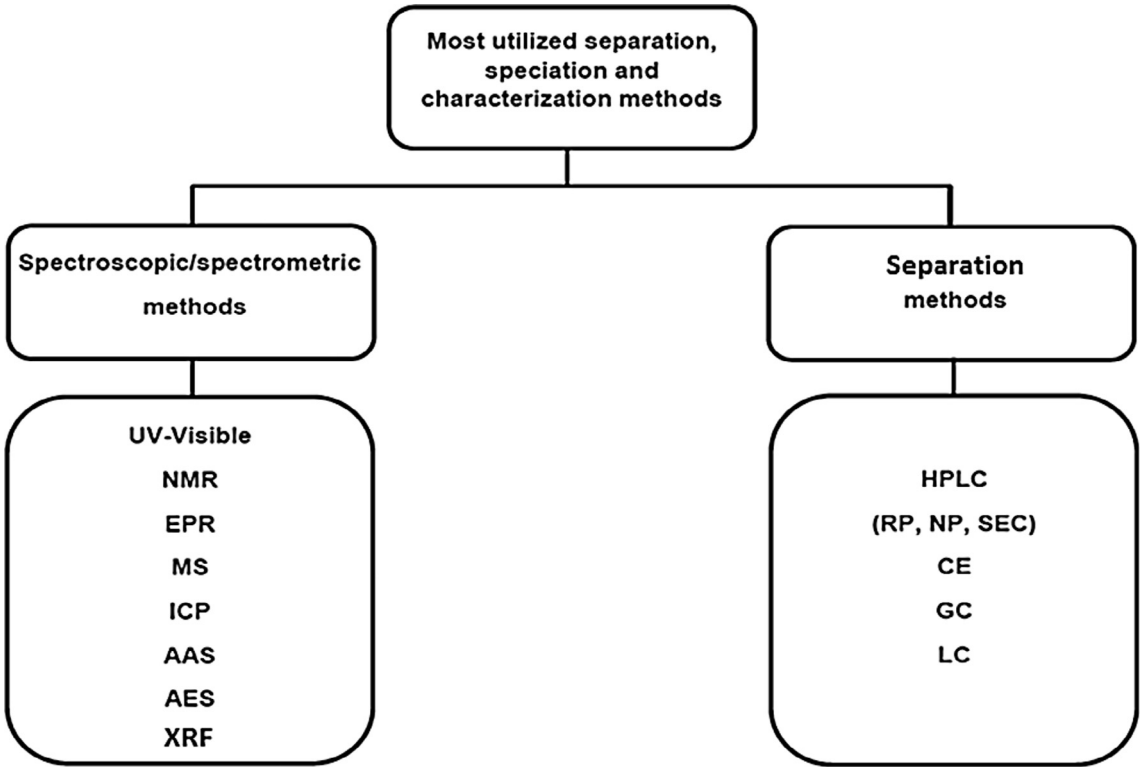

Fig. 3 Most known methods used for vanadium separation, quantitation and speciation.

MS) (Chen and Owens, 2008; Settle, 1997), and in some cases X-ray fluorescence spectrometry (XRF), Fig. 3 (Pyrzyńska and Wierzbicki, 2004; Pyrzyńska, 2006; Cornelis et al., 2005). These methods measure total vanadium because they cannot distinguish vanadium atoms in different oxidation states. With regard to the separation techniques, a variety of methods have been used including capillary electropheresis (CE), liquid chromatography and high performance liquid chromatography (LC and HPLC) (Sugiyama et al., 2001; Pyrzyńska and Wierzbicki, 2004; Pyrzyńska, 2006; Coetzee et al., 2004). In addition to spectroscopic and chromatographic methods, other methods that are based on spectrophotometric techniques (Chen and Owens, 2008; Pyrzyńska, 2005; Ayora-Cañada et al., 2000; Magda Ali et al., 2005) and potentiometry have as well been used for the characterization and speciation of vanadium compounds (Pyrzyńska, 2005; Elvingson et al., 1996, 1997; Correia et al., 2004).

HPLC facilitates good characterization and quantitation efficiency in separation of different metal ion complexes, but it also allows for flexibility and use of many detectors. Two main types of HPLC techniques have been used for separation, and metal ion speciation purposes, reversed phase (RP) HPLC and normal phase (NP) HPLC (Settle, 1997; Ali and Aboul-Enein, 2006). The reversed phase systems were the first to gain popularity due to the versatility among RP-HPLC method which allows adaptation of other methods such as: ion- pair (IP), ion-exchange and micellar. These earlier methods are applied by using the reversed phase stationary systems (Settle, 1997; Ali and Aboul-Enein, 2006; Wang and Lee, 1997). Ultraviolet spectrophotometric detection is most frequently used in combination with HPLC (Chen and Owens, 2008; Wang and Lee, 1997). Alternative detection techniques include inductively-coupled plasma atomic emission spectrometry (ICP-AES) (Cornelis et al., 2005; Hill et al., 1993), inductively-coupled plasma mass spectrometry (ICP-MS) (Cornelis et al., 2005; Hill et al., 1993) and inductively-coupled plasma optical emission spectrometry (ICP-OES). Nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) have also been used for detection in separation, characterization, quantitation and speciation studies (Chen and Owens, 2008; Cornelis et al., 2005; Wang and Lee, 1997; Hill et al., 1993).

This review describes vanadium separation, characterization and speciation by the HPLC technique and the many variations of the systems for characterization of vanadium speciation in a variety of environmental, biological and clinical matrices. First, sample preparation, pretreatment and preconcentration details are discussed. Second, various HPLC methods and modes used for the separation, and characterization of vanadium compounds are described. Third, general aspects of vanadium characterization in environmental and biological samples are described. Finally, clinical samples and the key features such as stability, coordination complexes, complex lability and their impact on the characterization of the vanadium systems are highlighted. Because the different HPLC methods facilitate separation of metal ions most studies determined the total amount of vanadium in the sample, however, a few studies have demonstrated that oxidation state could be identified and vanadium(IV) and vanadium(V) species have been reported.

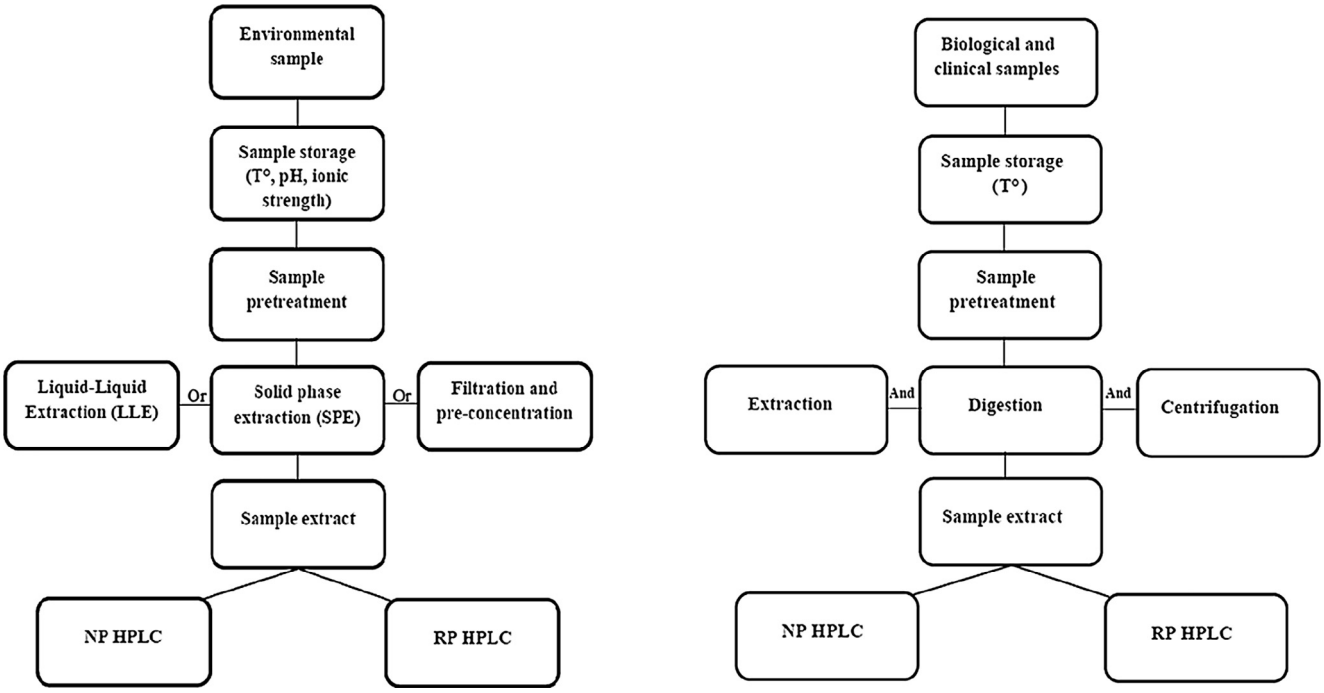

Fig. 4 Procedure of environmental (A), biological and clinical (B) sample preparation prior to HPLC analysis.

2. Sample preparation

2.1. Pretreatment and pre-concentration

Sample preparation is key step to any analysis of vanadium compounds in complex samples. All analytical methods, including separation, speciation and quantitation, require sample preparation whether they were carried out to quantify or identify a species or its various forms present in the samples under investigation. Sample preparation is a process which requires several steps including sampling, storage, sample pretreatment prior to sample analysis. The pretreatment often involves filtration, acidification and extraction depending on the nature of the sample (environmental, biological or clinical) (Kotaś and Stasicka, 2000). Prior to analysis of the various matrices, the steps in sample preparation will include elimination of contaminants, removal of interfering components, preconcentration of the sample and the selection of the solvent that may be needed later in the analysis phase (as the HPLC mobile phase for example) (Gilar et al., 2001). Importantly, these treatments will result in changes of the speciation for all but the most robust classes of vanadium compounds. However, if the experiments are designed so that the vanadium oxidation states will be preserved after chelation, the samples must be processed under inert atmosphere because vanadium (IV) will oxidize to vanadium(V) at pH values above 3. If the integrity of the samples remain then the chromatographic separation of the vanadium(IV) and (V) complexes will accurately describe the oxidation state distribution in the original samples and as such serve to provide important information regarding the nature of the sample prior to analysis.

One popular method for sample pretreatment is liquid-liquid extraction (LLE) which is based on partitioning between two solvents (Buszewski and Szultka, 2014; Pena-Pereira et al.,

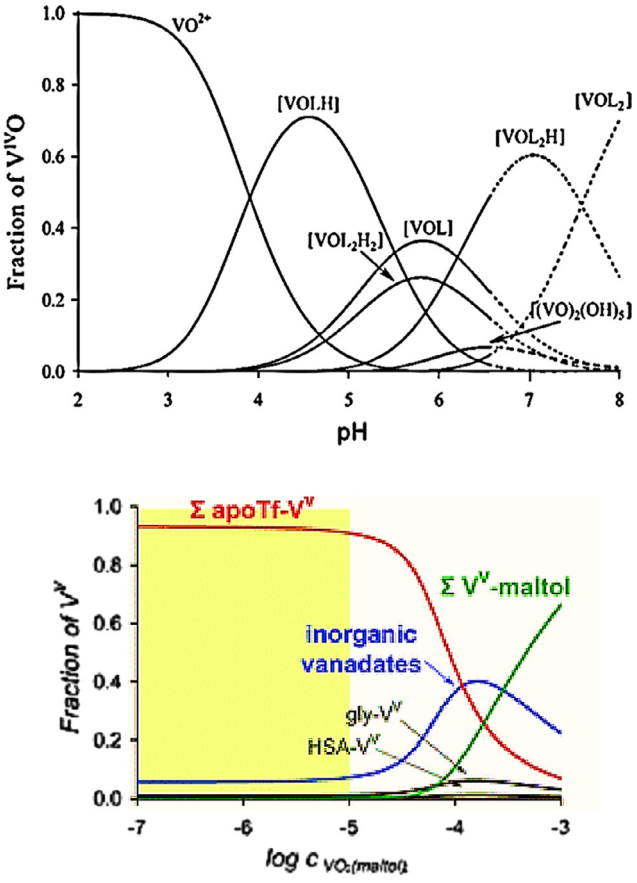

Fig. 5 A speciation diagram for vanadium(IV) showing the species presence as a function of pH. The diagram was reproduced with permission from references (Correia et al., 2004).

2009). Alternatively, solid phase extraction (SPE) involves partitioning of the species between a solid phase and a liquid phase. The method involves four steps: column preparation, sample injection, column post wash and sample desorption (Gilar et al., 2001; Buszewski and Szultka, 2014; Wang and Sañudo-Wilhelmy, 2008).

Other sample preparation procedures involve digestion and centrifugation. These are mainly used for biological and clinical matrices. The microwave digestion method results in a significant reduction in the usage of organic solvent compared to other conventional sample preparation methods (Ali and Aboul-Enein, 2006; Eskilsson and Björklund, 2000; Kuo and Jiang, 2007; Vachirapatama et al., 2002). Centrifugation is a reliable, contamination-free, fast and inexpensive technique. Different variables of this technique include, the relative force of centrifugation and the time of centrifugation (Kuo and Jiang, 2007; Elkhatib et al., 1987; Nischwitz et al., 2013; Tadayon et al., 1999; Smith and Azam, 1992). The speciation in these samples will however, as described for the samples above, change for all but the most resistant forms of vanadium species, but the complexation chemistry have been designed such that the oxidation state distribution is accurately reflected. In Fig. 4B the general procedure taken to analysis of biological samples are illustrated.

It is important to recognize direct analysis of vanadium species from various matrices by either spectroscopic/spectrometric or separation techniques is almost impossible without any sample preparation and pretreatment. The relatively low and generally submicromolar vanadium in most matrices compared to other metal ions, and to the complex nature of environmental, biological and clinical matrices makes the preconcentration of the vanadium in a pretreatment step a very critical step (Pyrzyńska and Wierzbicki, 2004). If these samples did contain any labile form of vanadium(V) such a type of vanadium would not remain after pretreatments but the compositions would alter and speciation results would not be an accurate reflection of the speciation in any matrices analyzed. At this point it is therefore important to consider the aqueous speciation of vanadium.

2.2. Aqueous vanadium speciation

Vanadium(V) is found as VO_2^+ in acid media (pH < 3) and VO_4^{3-} in alkaline solution (pH > 12). For the pH ranges in between, some different species such as HVO_4^{2-} and $H_2VO_4^-$ persist in the systems and the spectral changes can be observed and thus the protonation state can be followed, Fig. 1A and B (Kiss et al., 2008; Crans et al., 2013; Chasteen, 1983; Crans et al., 2004, 1990; Pettersson et al., 1983, 1985a,b). In addition, oligomeric forms can be observed. These are species in which the VO_3^- units are combined as illustrated by having two molecules of $H_2VO_4^-$ reacting to form the pyrophosphate analog dimeric vanadate. The structures of the oligomeric species are shown in Fig. 1C. Interestingly, the major species in solution varies with pH, however, the nuclearity of the most prominent species are one, two, four, five and ten. This diverse chemistry can readily be illustrated by speciation diagrams in which the amounts of each species are determined at each pH value. As shown in Fig. 1A and 1B these diagrams depend on concentration reflecting the fact, that these interconversions take place under mild conditions for many of the species

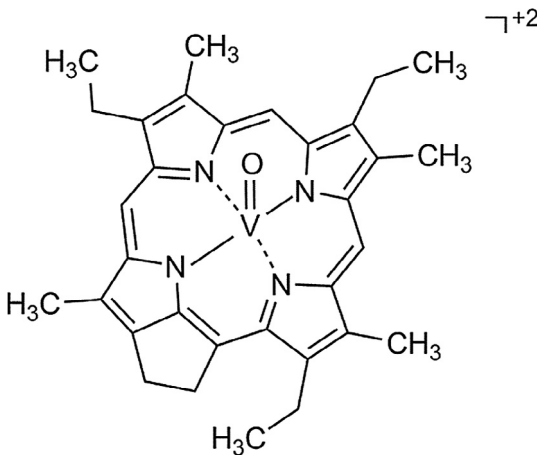

Fig. 6 The structure of vanadyl-porphyrin.

(Jakusch et al., 2011; Crans and Levinger, 2012; Elvingson et al., 1998).

The chemistry of aqueous vanadium(IV), vanadyl cation (oxidovanadium(IV)), in contrast is very different. Aqueous VO^{2+} undergo oligomerization chemistry but the resulting structures are polymeric and dimeric systems, Fig. 5 (Kiss et al., 2008; Chasteen, 1983; Chasteen et al., 1986; Kiss and Odani, 2007). In addition, it readily polymerizes and also reacts with buffers to form coordination complexes or clusters. The EPR spectrum is lost in these systems because these allow for pairing of the free signal for the species, and as such there is less information available on these systems. However, use of absorbance spectroscopy and potentiometry have led to the characterization of these systems as we know it today. For example, these study methods have been used to characterize the vanadium speciation in blood (Correia et al., 2004).

For the first order transition metal ion coordination complexes, the metal ion in most cases undergoes rapid ligand exchange (Kustin and Inorg, 2014), a process that varies with ligand, pH and the oxidation state of the metal ion (Kustin and Inorg, 2014; Crans et al., 1991). For example, vanadium (V) complexes are very labile and ligands readily exchange (Kustin and Inorg, 2014), and this process has been followed using ^{51}V NMR, UV–Vis, ^{51}V NOSEY(Nuclear Overhauser Effect Spectroscopy), ^{1}H and ^{13}C NOESY (Crans et al., 1990; Crans and Shin, 1994). There are however, some vanadium compounds that do not exchange rapidly such as decavanadate ($V_{10}O_{28}^{6-}$) (Aureliano and Crans, 2009). The vanadium(IV) complexes are generally less labile and ligand exchange is significantly slower (Crans et al., 2013). The type of complexes present are therefore subject to change, unless specific care and precautions are made to secure that the composition while conducting the speciation and separation studies remains consistent (Crans et al., 1995). In most studies speciation is done directly on the sample, whereas quantitation can be done after speciation studies. However, it is important to point out that any pretreatment may alter the original speciation in the biological system and that important information such as the distribution of oxidation states can still be answered if the processing reactions will be done under inert atmosphere.

In the following subsections examples of how sample preparations from different matrices prior to HPLC separation and characterization differ are explained.

2.3. Samples isolated from an environmental matrix

2.3.1. Crude oil

The vanadyl-porphyrin (oxidovanadium(IV)-porphyrin) complexes are very stable porphyrin complexes and thus can be a major component in oils and coal. Since these compounds are very stable they may survive the hard sample processing conditions and actually lead to identification of vanadium-porphyrin complexes after HPLC analysis. That is should the pre-treatment include addition of chelator, then the HPLC analysis may yield both vanadium-porphyrin complex as well as vanadium-chelator complex.

Several of crude oil samples were prepared for analysis and all of them were using either liquid-liquid extraction (LLE) or solid-liquid extraction (SLE). Crude oil contains a class of vanadium compounds in which porphyrins form coordination complexes to the vanadium as shown in Fig. 6. In one study the sample preparation of the vanadium-porphyrin complexes from crude oil consisted first of an extraction of the complex using pentane, then, subsequently an adsorption of the complex on alumina or silica gel. Vanadyl-porphyrins (oxidovanadium(IV) porphyrrins) were desorbed from silica gel with benzene-acetone. The procedure was particularly effective in removing nonpolar impurities from the porphyrins. The products recovered from the column were analyzed using RP-HPLC to determine the speciation of petroporphyrins (Saitoh et al., 2001). Another study reported the crude oil sample was consecutively extracted with a mixture of pyridine–water–toluene (4:1:1). The extract was then evaporated to dryness and the resulting residue was re-dissolved in toluene and then methanol was added. The mixture was sonicated for about 3 min and centrifuged approximately at 3000 rpm for 5 min (Tadayon et al., 1999).

2.3.2. Water

Another example of an environmental matrix is water samples. In these systems the vanadium species are not directly extracted, but there are converted into complexes. In a study where river water was analyzed, the sample was collected and then nitric acid was added. This was followed by a filtration. A portion of the filtered sample solution was taken and nitric acid solution was added and heated to almost dryness. Then water, sodium 1,2-dihydroxybenzene- 3,5-disulfonic acid and appropriate amounts of hydrochloric acid (HCl) were added to the dry sample solution, and the total volume was then made up with water. This solution/suspension was mixed with PBHA (*N*-phenylbenzohydroxamic acid) and 8-hydroxyquinoline solution in acetonitrile at a 1:1 volume ratio in order to complex the vanadium (Nagaosa and Kimata, 1996). For a lake water sample, similar sample preparation was followed. The lake water was collected and stored refrigerated. Then sample was filtered to remove particulate matter, then EDTA, TBA, phosphoric acid and methanol were added, respectively. The lake water sample was removed for the analysis. This process lead to the separation and identification of vanadium in the form of vanadium(IV)- and vanadium(V)-EDTA complexes (Kilibarda et al., 2013).

Another study has reported sea water sample preparation prior to HPLC analysis. The sea water sample was filtered through 0.45 μm Millipore filter and acidified using HCl. Then to 50 mL of the solution, 0.5 mL of 5.0 mM Br-PAPS solution and 2.5 mL of 2.0 M sodium acetate solutions were added. The pH was adjusted to 4.50 by 1.0 M Ammonium solution. The mixture was transferred through a separatory funnel and 0.2 mM of capriquat-xylene was added to it. After shaking and centrifugation, the aqueous phase of the mixture was removed by a pipette and 20 μl of the solution was subjected to analysis using the HPLC method for the characterization of total vanadium metal ion present in sea water (Shijo et al., 1996).

2.4. Samples isolated from a biological matrix

2.4.1. Leaves of Persea americana

For biological matrices the sample preparation is based on extraction and material digested to release the vanadium. Leaves of the plant *Persea americana* were collected (Tainan, Taiwan), dried, ground and sieved to obtain small particles. Microwave digestion was used as the extraction device. Placing the sample in centrifuge tubes and adding sequentially HF, EDTA, TBAP and methanol were added before processing to the HPLC column to separate, speciate and characterize the metal ion (Kuo and Jiang, 2007). In this case, vanadium (IV,V)-EDTA complexes were successfully isolated.

2.4.2. Tomato plants (Lycopersicon esculentum)

The plant was separated into leaves, stem and roots, which were oven dried. The resulting dried plant were ground and passed through a sieve to form a powdered plant sample. This powder was later placed and treated with few drops of water, followed by concentrated HNO_3 and HCl. The sample was then digested on a hotplate and the sample solution was evaporated to dryness and then water was added followed by concentrated HNO_3. The digestion was carried out a second time on a hotplate. The solution was then transferred to a container, made up with water before final filtration. The ligand added was PAR in the presence of H_2O_2. And the HPLC analysis has led to the isolation the vanadium(V)-PAR-H_2O_2 complex (Vachirapatama et al., 2005).

2.4.3. Mussel homogenate

Biological sample preparations use mostly digestion, filtration and complexation prior to HPLC analysis but the samples described above were relatively different. Many articles have published using tissues of mussel sample preparation (Colina et al., 2005; Rivaro, 1997; Nóbrega et al., 2002; Wang et al., 2007; Baumard et al., 1997). Here, we are presenting an example of these studies that has reported preparation for mussel homogenate samples. The procedure has started with dissolving the mussel homogenate in nitric and perchloric acid (Gorsuch, 1970; Miller and Kalra, 1998). Pure water was added after decomposition and the solution was filtered. The pH was adjusted to 2.5, and the resulting solution was mixed with PBHA and HQ solution prior to HPLC analysis of vanadium(V) in this mussel homogenate sample (Nagaosa and Kimata, 1996).

2.5. Samples isolated from a clinical matrix

Unlike environmental and biological matrices, samples from a clinical matrix require little preparation (Ash and Komaromy-Hiller, 1996). For clinical matrices the sample storage and preparation should be as short as possible and preferably at

a low temperature (4 °C) (Michalke and Caroli, 2013). Body fluids are normally diluted, whereas tissue samples are prepared through dissolution, digestion and centrifugation. Clinical samples can include urine, blood, plasma and serum, hair, bones and teeth and other human tissues such as liver, kidney, placenta and human breast milk (Nischwitz et al., 2013; Ash and Komaromy-Hiller, 1996; Michalke and Caroli, 2013; Nagaoka et al., 2002; Cornelis et al., 2005). We are presenting here few examples of sample preparation from a clinical matrix.

2.5.1. Human serum transferrin (hTf)

Many studies have characterized the interaction of vanadium with hTf (Kiss et al., 2008; Thompson et al., 1999; Thompson and Orvig, 2006; Jakusch et al., 2011; Kiss and Odani, 2007; Willsky et al., 2001). The complex has been isolated from blood or serum and examples are shown in Table 3. Serum transferrin samples including apo-hTf was dissolved in Tris–HCl containing $NaHCO_3$ because of the binding of the carbonate to the protein. The vanadium compound was dissolved in water and oxidation was prevented by the ascorbic acid added to the sample. The study was also conducted without bicarbonate, and then apo-hTf was dissolved in buffer such as Tris–HCl and nitrogen gas was bubbled through the solution to remove dissolved carbon dioxide completely. After the preparation, sample tubes were sealed under a nitrogen gas atmosphere (Nagaoka et al., 2002).

2.5.2. Human milk

For this specific example, the pretreatment used did not require harsh conditions or additives. In fact, any changes in the form of additives or other disturbance of the system may lead to flawed results. In this case, lipids were eliminated from these samples after cooling and solidification (Coni et al., 1996; McKinstry et al., 1999).

2.5.3. Human hair

Hair sample preparation is normally conducted after being rinsed with distilled water and dried at 50 °C. After pulverization under liquid nitrogen the hair powder was passed through a nylon screen to obtain small hair powder particles (μm). The sieved powder was then blended for 8h using a rocking mixer. The obtained homogenized powder was kept in pre-cleaned borosilicate bottles at −20 °C and in the dark (Yoshinaga et al., 1997).

In summary, for separation, speciation and quantitation purposes, sample preparation is adjusted to the nature of the matrix. Different procedures can be followed depending on the nature of the sample and depending on whether the sample came from an environmental matrix, or a biological matrix or a clinical matrix. In the following section we will describe the HPLC step.

3. HPLC: The methods reported for separation, speciation and quantitation of vanadium

3.1. Speciation

IUPAC recommends that the term *chemical species* is used to describe a specific form of an entity (Templeton et al., 2000; Bernhard et al., 1986). This entity can be an element with a specific formula, or in a specific electronic or oxidation state with a specific defined isotopic composition, and/or a complex or molecular structure. The concepts of speciation highlighted by IUPAC therefore involved changes to this species (Templeton et al., 2000; Bernhard et al., 1986) and as such are varied from that used by the classical solutions chemists (Pettersson et al., 1983, 1985a,b) who are focused more on the composition and formula of matter. This definition does highlight the importance of considering chemistry, particularly when some forms (that is species) can interconvert under mild conditions whereas others do not (Crans et al., 2004). It is therefore the complex vanadium chemistry that makes the issue of speciation so important for vanadium scientists and IUPAC has established a commission that has reported several definitions of the term (Templeton et al., 2000; Bernhard et al., 1986). For most studies, the literature use of the term *speciation* is used when the forms of interest are stable and can withstand the nature of the pre-treatment conditions. However, in the case of speciation of vanadium(V) compounds the situation is generally more complicated, because some of these species are labile and will interconvert under the pretreatment conditions (Kiss et al., 2008; Crans et al., 2004, 1990). In summary, the term speciation of a component, can be defined as the distribution of this component among defined chemical species in a system however, speciation must consider the mode of HPLC used, because the speciation observed will reflect the original sample composition of stable and labile species.

Since vanadium frequently exists in many different forms in various matrices which have different properties and biological activities (Crans and Schelble, 1990; Crans et al., 1990; Wittenkeller et al., 1991), it is important to know which form is present in particular systems (Aureliano and Crans, 2009; Crans et al., 2013; De Cremer et al., 2002; Rehder, 2003; Ortega and Metals, 2002). For example, the simple colorless vanadate salt species, readily convert to other vanadium(V) species in aqueous solution, and such species would interconvert and change during a pre-treatment step. In contrast, decavanadate is very stable and converts much slower to other vanadium(V) species, so this species may survive some of the pre-treatment conditions. The fact is that applications of HPLC methods often involve complexation of the vanadium with a chelator, and as such the speciation is being altered during the separation of the vanadium compounds with the more labile forms of the vanadium which complex to ligands, whereas the other forms remain intact and are isolated as such. In fact speciation by HPLC in general describes the metal ion speciation of chelated vanadium determining the total amount of vanadium in the various matrices, which with the proper consideration can be used to provide information on the speciation in original samples.

3.2. The possible HPLC modes

HPLC is often the analytical technique employed to identify and/or quantify chemical species in an environmental, biological or clinical sample (Chen and Owens, 2008; Pettersson et al., 1983, 1985a,b; Templeton et al., 2000). In general, speciation analysis of trace metals in different matrices yields their presence in different oxidation states, protonated forms, poly-

Table 1 A summary of the most known applications, within environmental matrices, of measuring vanadium using HPLC.

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Heavy crude petroleums	Size-exclusion (SEC)	Pyridine: Water	EPR	Vanadyl-porphyrins	Reynolds et al. (1987), Fish and
Boscan, Cerro Negro, Wilmington and Prudhoe Bay	ODS column	(4:1 v/v)	GFAA	No LOD	Komlenic (1984)
	4.6 × 250 mm			Quantities were respectively: 307 ppm 175 ppm 13.8 ppm 5.8 ppm	
Heavy crude oil (Boscan)	Size-exclusion (SEC)	Hexane: Benzene and ACN: MeOH	UV–Visible	Vanadium-Porphyrins	Ysambertt et al. (1995)
	C18 column 4.6 mm × 25 cm		GFAA	No LOD Quantities of 1150 ppm	
Oilsand coke (Alberta, Canada)	Anion exchange	3% Acetonitrile, 2M EDTA, and 80 mM of Ammonium bicarbonate (pH 6)	ICP-MS	V(IV, V)- EDTA	Li and Le (2007)
	Strong anion exchange column 3 µm, 4.1 × 50 mm			LOD of 0.7 µg/L V(IV) 1.0 µg/L V(V) Quantities of 1090 µg/L V(IV) 926 µg/L V(V)	
Crude oil (Castilla crude oil)	Size-exclusion (SEC)	Toluene: isopropanol: water	UV–Visible at	Vanadium-porphyrins[b]	Mogollon et al. (1997)
	Polystyrene/divinylbenzene column 0.0075 × 0.30 m		254 nm	No LOD Quantities of 1686 µg/g	
Arabian heavy crude oil	Reversed phase (RP)	Different eluents were tried (Polarity): Cyclohexane, benzene/cyclohexane (1:4 and 3:7), benzene, benzene/ethyl acetate (1:1) and methanol	UV–Visible at 405 nm	Vanadyl- porphyrins	Ali et al. (1993)
	C18 column 5µm 0.46 × 25 cm			No LOD Quantities of 189 ppm in residue 710 ppm in Asphaltenes	
Oil shale samples of the drill cores (Julia creek shale, Australia)	Reversed phase (RP)	Methanol	UV–Visible	Vanadium-Porphyrin[b]	Ekstrom et al. (1983)
	C18 column Bondapack semi preparative		(405–546 nm)	No LOD Quantities of 104 µg/g Deep 27 µg/g Shallow	
Crude oil (Castilla, Colombia)	Size-exclusion (SEC) Polystyrene/divinylbenzene column 1.0 × 45 cm	Methylene chloride	UV at 254 nm	Vanadium-porphyrins[b] No LOD	Mogolloń et al. (1998)

(*continued on next page*)

Table 1 (*continued*)

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Organic-rich dark siliceous mudstone (northern Japan)	Reversed phase (RP)	Acetonitrile: H_2O: Pyridine: Acetic acid (90:10:0.5:0.5 v/v/v/v)	APCI–MS	Quantities of 32.4 μg/12 mg VO- porhyrins[a]	Kashiyama et al. (2007)
	C18 column 5μm 4.6 × 250 mm		ELSD		
Organic-rich dark siliceous mudstone (northern Japan)	Reversed phase (RP)	Acetonitrile: Pyridine: Acetic acid	APCI–MS	VO- porhyrins[a]	Kashiyama et al. (2007)
	C18 column 5μm 4.6 × 250 mm	(100:0.5:0.5 v/v/v)	ELSD		
Organic-rich dark siliceous mudstone (northern Japan)	Normal phase (NP)	*n*-Hexane: Acetone: *N*,*N*-dimethyl formamide: Acetic acid: Pyridine (95:3:1:0.5:0.5, v/v/v/v/v)	APCI- MS	VO- porhyrins[a]	Kashiyama et al. (2007)
	C18 column 5μm 4.6 × 250 mm				
Organic-rich dark siliceous mudstone (northern Japan)	Normal phase (NP)	*n*-Hexane: DCM: *N*,*N*-dimethyl formamide: Acetic acid: Pyridine	APCI- MS	VO- porhyrins[a]	Kashiyama et al. (2007)
	C18 column 5μm 4.6 × 250 mm	(88:10:1:0.5:0.5, v/v/v/v/v),			
Sea water (Japan)	Reversed Phase (RP)	Acetonitrile: Water (55:45 v/v) containing 0.01 M	Absorbance detector at 593 nm	V(V)- Nitro-PAPS	Chen et al. (1999)
	Ion pair (IP)	Acetic acid, 0.01 M (TBABr), and 10^{-4} M EDTA		LOD of 0.017 μg/dm^3	
	ERC-ODS 1282 column 5 μm 6 × 250 mm			Quantities of 1.3 ng/cm^3	
River water (Japan)	Reversed phase (RP)	1:1 (v/v) mixture of Acetonitrile: acetate buffer (0.02 M, pH 3.5), 0.05 M potassium nitrate	Electrochemical (EC)	V(V)-PBHA	Nagaosa and Kimata (1996)
	C18 ODS column 5 μm 4.6 × 150 mm			LOD of 1.0 ng/mL	
				Quantities of 1.77 ± 0.04 ng/mL	
Mineral and synthetic samples (China)	Reversed Phase (RP)	Methanol: Water (55/45, v/v) containing 0.1% tartaric acid and 10 mM pH 3.5 acetate buffer	UV–Visible at 568 nm	V(V)- TADAP	Wang et al. (1997)
	C18 ODS column 5 μm 4.6 × 150 mm			LOD of 0.16 ng/mL	
				Quantities of 2.00 ng/mL	
Sea water	Reversed Phase (RP)	Methanol: Water (67:33, v/v) containing 1.25 · 10^{-3} M HQ and 0.02 M chloroacetate (pH 3.5)	Spectrophotometric detection at 400 nm	V(V)- HQ	Ohashi et al. (1991), Ryan and Meaney (1992)
	C18 column 5μm 4.0 mm × 25 cm			LOD of 0.5–50 μg/L	
				Quantities of 1.40 ± 0.09 μg/L	

Table 1 (*continued*)

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Natural waters	Reversed Phase (RP) ODS column 7 μm 4.0 × 125 mm	Aqueous acetonitrile solution buffered at pH 7.5 with sodium acetate and EDTA	Spectrophotometric detector at 550 nm	V(V)- QADAP LOD of 320 pg/25 mL Quantities of Rain 1.3 ng/25 mL Sea 17.2 ng/25 mL	Miura (1990)
Lake water	Reversed Phase (RP) Rapid analytical column 1.8 μm 4.6 × 10 mm	Acetonitrile: Water (50:50, v/v) containing phosphate buffer (pH 4.5) and 0.01 M of citrate	On-line enrichment technique	Vanadium- (5-Br-QADEAP)[b] LOD of 1.6 ng/L Quantities of 0.153 μg/L	Li et al. (2007)
Water	Reversed Phase (RP) C18 ODS column 1μm 4.0 × 250 mm	Methanol: THF: Water (20:15:65, v/v/v) containing 0.05 M lithium sulfate and 0.04 M acetate buffer (pH 5.5)	Spectrophotometric detection at 570 nm	V(V)- 2-(2-TADAP) LOD of 0.5 ppb Quantities of Tap 0.63 ± 0.015 ng/mL Spring 1.30 ± 0.02 ng/mL	Liu et al. (1992)
River water	Reversed Phase (RP) Cosmosil 5 C8-MS column 4.6 × 100 mm	Aqueous acetonitrile solution containing 5.0×10^{-3} M acetate buffer (pH 3.5) 1.0×10^{-4} M TBABr and 1.0×10^{-3} M EDTA	Spectrophotometric detector at 595 nm	V(V)- QAI- H_2O_2 LOD of 2.0 pg/mL Quantities of 2.34 ng/mL	Zhang et al. (2011)
Natural water	Reversed- phase (RP) Ion pair(IP) C18 column 5μm, 10μm 4.0 × 250 mm	Water and methanol mixture (0–10% of methanol) buffered with formic acid. (pH 3.0–5.5) and 1.0×10^{-3} M EDTA	On-line enrichment technique	V(V)-Plasmo Corinth B LOD of 30 μg/L Quantities of 0 μg/L no detection	Sarzanini et al. (1993), Sacchero et al. (1991)
Drinking water	Reversed- phase (RP) Ion pair (IP) C18 column 3.9 × 300 mm	Acetonitrile: Water (55:45 v/v) containing (0.01 M of acetic acid, 0.01 M TBABr and 10^{-4} M EDTA)	ICP-OES	V(V)-Nitro-PAPS LOD of 0.055 μg/ml Quantities of 0 μg/ml no detection	Pimrote et al. (2012)
Wines	Reversed- phase (RP) Ion pair (IP) C18 column 3.9 × 300 mm	Acetonitrile: Water (55:45 v/v) containing (0.01 M of acetic acid, 0.01 M TBABr, and 10^{-4} M EDTA)	ICP-OES	V(V)-Nitro-PAPS LOD of 0.055 μg/mL Quantities of 0 μg/mL no detection	Pimrote et al. (2012)
Lake water (Durham, US)	Reversed- phase (RP) Ion pair (IP) C18 column 1.7μm 2.1 ×	EDTA(18 mM), TBA (0.5 mM), phosphoric acid (20 mM), 4% Methanol	ICP-SFMS	V(IV/V) Species	Kilibarda et al. (2013)

(*continued on next page*)

Table 1 (*continued*)

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
	250 mm			LOD of 0.008 µg/L V(IV) 0.013 µg/L V(V) Quantities of 1 µg/L V(V)	
Synthetic water	Normal phase (NP) S5 Nitrile column 5µm 4.6 × 250 mm	5.9. 10^{-4} M solution of BPHA in chloroform (stabilized with amylene)	UV–Visible at 360 nm	V(V)- BPHA LOD of 2.1 ng/mL Quantities of 44.5 µg/L	Gracia and Bagur, (1996)
A mineral processing (GRD Minproc)	Anion exchange Dionex-AG5 anion exchange column	EDTA (200 mM), Sodium carbonate(500 mM)	ICP-OES	V(IV/(V)- EDTA LOD of 0.14 mg/L V(IV) 0.20 mg/L V(V) Quantities of 207.5 mg/L	Hu and Coetzee (2007)
A mineral processing (GRD Minproc)	Cation exchange Ion pack CG10 cation exchange column	H_2SO_4(120 mM), Sodium carbonate(500 mM)	ICP-OES	V(IV/(V) species LOD of 40 µg/L V(IV) 30 µg/L V(V) Quantities of 2.26 mg/L V(V) V(IV) below LOD	Hu and Coetzee (2007)
Mineral natural water	Anion exchange Dionex-Ion pack AG5 column 4.0 × 50 mm	4 mM carbonate buffer and 5 mM Na_2 EDTA	ICP-MS	V(IV/(V)-EDTA LOD of 0.16 µg/L V(IV) 0.025 µg/L V(V) Quantities of 0.57 µg/L V(IV) 37.34 µg/L V(V)	Aureli et al. (2008)
Sea water	Reversed- phase (RP) Lichrosorb RP-18 column 7µm 4.0 × 250 mm	Methanol: Water (65:35 v/v) 5×10^{-5} M of 5- Br-PAPS 1×10^{-1} M Lithium Chloride $1x10^{-2}$ M sodium acetate buffer (pH 3.50)	Spectrophotometric detection at 575 nm	V(V)-5-Br-PAPS LOD of 0.026 µg/L Quantities of 1.37 ± 0.15 µg/L	Shijo et al. (1996)
River water (Japan)	Reversed phase (RP) Merk Lichrosorbb RP-18 column 4.0 × 125 mm	Methanol: Water (65:35) 1×10^{-1} M 5- Br-PAPS 1×10^{-1} M LiCl 1×10^{-4} M CyDTA Acetate buffer (pH 4.00)	UV–Visible at 589 nm	V(V)-5-Br-PAPS LOD of 35 ng/L Quantities of 2.10 ± 0.13 µg/L	Uehara et al. (1989)
Water samples (Tap water and spring water)	Reversed phase (RP) C18 column 7µm 3.3 × 150 mm	THF: Methanol: Water (22:5:68 v/v/v), 4.68×10^{-4} M TBABr, 5% 0.1 M Sodium acetic acid (pH 3.3)	UV–Visible at 590 nm	V(V)-5-Br-PADAP LOD of 0.15 ppb 0 µg/L no detection owing to the presence of vanadium as V(IV). This latter does not form a complex with 5-Br-PADAP	Oszwałdowski (1995)

Table 1 (*continued*)

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Water samples (Tap water and spring water)	Reversed phase (RP) C18 column 7μm 3.3 × 150 mm	THF: Methanol: Water (22:5:68 v/v/v), 4.68×10^{-4} M TBABr, 5% 0.1 M Sodium acetic acid (pH 3.3)	UV–Visible at 590 nm	V(V)-H_2O_2-5-Br-PADAP LOD of 0.15 ppb 0 μg/L no detection owing to the presence of vanadium as V(IV). This latter does not form a complex with 5-Br-PADAP in the presence of H_2O_2	Oszwałdowski (1995)
Synthetic samples	Reversed Phase (RP) FLC-ODS column 3μm 4.6 × 50 mm	Acetonitrile: Water (58/42, v/v) containing 5.0×10^{-3} M sodium acetate, 1.0×10^{-4} M EDTA and 1.0×10^{-4} M PBS	Spectrophotometric detection at 448 nm	V(V)-PBS LOD of 6.00 $x10^{-9}$ mol/L Quantities of 2.22×10^{-6} mol/L	Kanbayashi et al. (1987)
Polluted soil, Aspergillus (Isfahan refinery, Iran)	Reversed phase (RP) Nova Pack C18 column 3.9 × 150 mm	Methanol: Acetone: Buffer (15:5:1 v/v/v)	UV–Visible at 254 nm	VOOEP LOD was not stated Quantities of 0.96 mg/L	Salehizadeh et al. (2007)
Farmland soil (Kaohsiung, Taiwan)	Reversed phase (RP) C8 column 3μm 3.0 × 30 mm	EDTA (5 mM), TBAP (0.5 mM) in 4% Methanol (pH 6.85)	DRC-ICP-MS	V(IV/V)-EDTA LOD of 0.06 ng/mL Quantities of 46.8 ± 0.5 V(IV) 26.0 ± 0.9 V(V) μg/g	Kuo and Jiang (2007)
Rocks (Japan)	Reversed phase (RP) C18 column 5μm 4.6 × 250 mm	Acetonitrile: Water with acetic acid and pyridine	APCI-MS ELSD	VO- Porphyrins[a] Both LOD and quantities were not stated as the study was mainly qualitative	Kashiyama et al. (2007)
Rocks (Japan)	Normal phase (NP) C18 column 5μm 4.6 × 250 mm	*n*-hexane: Acetone: N,N-Dimethylformaimde: Acetic acid: Pyridine (95:3:3:0.5:0.5, v:v:v:v:v)	APCI-MS	VO- Porphyrins Both LOD and quantities were not stated as the study was mainly qualitative	Kashiyama et al. (2007)
Phosphate rock (South Africa, Morocco, Jordan)	Ion- exchange C18 column 14 μm 3.9 × 150 mm	Methanol: Water (32:68 v/v) (pH 7) TBABr (3 mM), acetic acide (5 mM), citrate (5 mM)	Detection by absorbance at 540 nm	V(V)-PAR-H_2O_2 LOD of 0.09 ng/mL Quantities of 18.06 S.Africa 90.71 Morocco 123.2 Jordan (μg/g)	Vachirapatama et al. (2002)
NPK fertilizers: Nitrogen, phosphorus and potassium fertilizer	Reversed Phase (RP)	Methanol: Water (32:68 v/v) (pH 7) TBABr (3mM), acetic acide(5 mM), citrate(5 mM)	Detection by absorbance at 540 nm	V(V)-PAR-H_2O_2	Vachirapatama et al. (2002)

(*continued on next page*)

Table 1 (*continued*)

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
(Norway, Thailand, Australia	Ion pair (IP) C18 column 14 μm 3.9 × 150 mm			LOD of 0.09 ng/mL Quantities of 37.13 Norway 181.2 Thailand 7.482 Australia (μg/g)	
Pond sediment (Japan)	Reversed Phase (RP) Ion pair (IP) ODS column 5 μm 6.0 × 250 mm	Acetonitrile: Water (55:45 v/v) containing 0.01 M Acetic acid, 0.01 M TBABr, and 10^{-4} M EDTA	Spectrophotometric detector at 593 nm	V(V)- Nitro-PAPS LOD of 17 ppt Quantities of 247 ± 8 μg/g	Chen et al. (1999)
Vanadium slag	Reversed phase (RP) Aminobounded column 10 μm 4.6 × 250 mm	Methanol: water (25:75, v/v) containing 0.01 M of Li_2SO_4 and HAc-NaAc buffer (pH 5.0)	Spectrophotometric detector at 545 nm	V(V)- TAR LOD of 0.03 ng/20 μL Quantities of 0.05–5.0 ppm	Changshan et al. (1989)
Geologic samples	Reversed- phase (RP) Rapid analytical column 1.8 μm 4.6 × 10 mm	Acetonitrile: Water (50:50, v/v) containing phosphate buffer(pH 4.5) and 0.01 M of citrate	On-line enrichment technique	Vanadium- (5-Br-QADEAP)[b] LOD of 1.6 ng/L Quantities of 22 μg/g	Li et al. (2007)
Coal fly ash	Reversed Phase (RP) Ion pair (IP) ODS column 7 μm 4 × 125 mm	Aqueous acetonitrile solution buffered at pH 7.5 with sodium acetate and EDTA.	Spectrophotometric detector 550 nm	V(V)-QADAP LOD of 320 pg/25 mL Quantities of 92.7 ng/25 ml	Miura (1990)
Sediment from a lake in Venezuela (Maracaibo)	Anion- exchange AS-9 Anion dionex	1.87 mM Ammonium phosphate–1.87 mM diammonium phosphate	ICP-MS	V(IV,V)-EDTA LOD of 59.1 μg/L V(IV) 113.1 μg/L V(V) Quantities of 0.4–25.8 μg/g V(IV) 1.4–9.2 μg/g V (V)	Colina et al. (2005)
Sediment from a lake in Venezuela (Maracaibo)	Reversed Phase (RP) C18 column 4.6 × 15 mm	0.06 M ammonium acetate, 3% methanol, 0.1% 2-mercaptoethanol, 2 mM EDTA	ICP-MS	V(IV,V)-EDTA LOD of 59.1 μg/L V(IV) 113.1 μg/L V(V) Quantities of 0.4–25.8 μg/g V(IV) 1.4–9.2 μg/g V (V)	Colina et al. (2005)
Sediment from a lake in Venezuela (Maracaibo)	Reversed Phase (RP) Ion pair (IP) C8 column 4.6 × 250 mm	5–10% acetonitrile, 0.05 M TBAOH, 2 mM EDTA	ICP-MS	V(IV,V)-EDTA LOD of 59.1 μg/L V(IV) 113.1 μg/L V(V) Quantities of 0.4–25.8 μg/g V(IV) 1.4–9.2 μg/g V (V)	Colina et al. (2005)

Table 1 (*continued*)

Type of the environmental sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Micelle-solubilized complexes[c]	Reversed Phase (RP) Ion pair (IP)	Methanol: Acetone: Water containing TBA^+ and acetate buffer (pH 3.0)	Spectrophotometric detection (572–600 nm)	V(V)- 3,5-diBr-PADAP-Triton X-100	Yuan and Wang (1989)
Synthetic samples[b]	ODS column 5.0 × 250 mm (SEC) C8 column 5 μm 4.0 × 250 mm	THF: Chloroform (60:40 v/v)	UV at 254 nm	LOD of 1.1–3.6 μg/L Quantities of 30 μg/L V(V) V(V)- 8-hydroxy quinoline LOD of 0.5 ng/20 μL Difficulties in measuring vanadium because of the large solvent peak	Lajunen et al. (1984)
Used vanadium-containing catalyst[c]	Reversed phase (RP) C18 column 3.9 × 300 mm	Methanol: Water (30:70 v/v), acetate buffer (8.0×10^{-3} M, pH 6.0) and PAR (3.0×10^{-4} M).	UV–Visible at 540 nm	V(IV/V)- PAR LOD of 0.1 ng/100 μL V(IV) 0.2 ng/100 μL V(V) Quantities of 117 ± 4 ng/100 μL V(IV) 9.85 ± 0.32 ng/100 μL V(IV)	JaneáTsai (1994)

LOD: Limit of detection.
ODS: Octadecylsilyl.
ppm: Parts per million mg/kg.
ppb: Parts per billions μg/L.
ppt: Parts per trillions.
[a] This study was mainly concerned about the structure of the vanadium complex, and therefore the results were qualitative and not quantities.
[b] In the case where the speciation study is conducted to analyze the total amount of vanadium, species are presented with no oxidation state.
[c] These are not purely environmental samples, and were added to the table for comparison purposes (eluent, chelating reagents and detector).

merized forms or ligand complexes. The compounds are separated on the basis of the retention mechanism (Robards et al., 1991; Ellis and Roberts, 1997; Pozdniakova, 1998) which depends on the relative hydrophobicity of the specific vanadium species (Michalke, 2002). For vanadium, most studies detect the total amount of the dissolved vanadium(IV) and vanadium(V). The most common HPLC methods for separation and characterization studies include reversed phase chromatography (RP), normal phase (NP), ion exchange (IE) and size exclusion (SEC) (Kazakevich and Lobrutto, 2007). Among RP known modes are ion-pairing (IP) and micellar (Kazakevich and Lobrutto, 2007; B'Hymer and Caruso, 2004).

From a procedural point of view, most of the separation and quantitation methods use pre-column reactions of metal ions, including vanadium, with organic ligands to form stable organometallic and coordination complexes that can be separated by HPLC (Heumann, 2004; Ure and Davidson, 2008). Examples of known chelating reagents include 5-Br-PAPS (2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)-phenol), 5-Br-PADAP (2-(5-bromo-2-pyridylazo)-5-diethylaminophenol), H_2SA_2Ten (bis(salicylaldehyde)tetramethylethylenediimine), TADAP (2-(2-Thiazolylazo)-5-diethylaminophenol), HQ (8-hydroxyquinoline), nitro-PAPS (2-(5-Nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]-phenol) and others. In all of these cases, the quantitation of the vanadium complexes was performed by HPLC and spectrophotometrically detected using Ultra violet-visible (UV–Vis) spectra of the vanadium chelate. The pre-column complexations are generally used to concentrate the vanadium in various matrices with low detection limits. For instance, the quantitation and characterization of vanadium in a river water sample was conducted using *N*-phenylbenzohydroxamic acid (PBHA) in acidic solution (Nagaosa and Kimata, 1996). The ligand-vanadium complex was separated on a C18 column with a mobile phase that consists of 1:1 (v/v) mixture of acetonitrile-acetate as a buffer (Nagaosa and Kimata, 1996).

3.3. Separation, speciation and quantitation of vanadium compounds using HPLC

During the last few years HPLC techniques have developed in versatility for measuring vanadium and vanadium compounds due to new columns and detection techniques (Heumann, 2004). Among the different modes of RP-HPLC, IP has been most widely used and is gradually gaining momentum for the determination of vanadium in a variety of samples (Chen et al., 1999; Ma et al., 1997; Pimrote et al., 2012). Other modes such as IE involving anion exchange and cation exchange (Chen and Owens, 2008), SEC (Caumette, 2010) and micellar (Mirza et al., 2009) have also been used albeit less frequently for speciation of vanadium among other metal ions. Below are descriptions of the HPLC technique different modes for the separation and characterization of known vanadium compounds within different matrices.

3.3.1. Examples of measurements of vanadium levels from environmental matrices

Most studies determining vanadium compounds in environmental matrices have included samples from oil, water, soil, fertilizers and coal. The most common vanadium compound separated from crude oil samples is the vanadium porphyrin

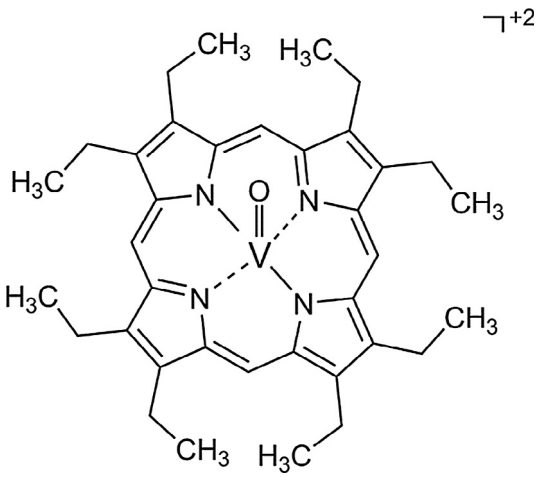

Fig. 7 The structure for vanadium(IV)-tetrapyrrole.

complex (see Table 1). Observation of other compounds include the vanadium-tetrapyrrazole complex and a range of complexes that form during the pre-treatment step where chelators are added to extract the vanadium from the sample. These samples are generally investigated with the objective of measuring vanadium content although because other metals ions are present in the samples, metal speciation is necessary to identify the signal for vanadium and carry out the calibration allowing for quantitation.

3.3.1.1. Determination of vanadium compounds in oil samples: Vanadium(IV)-porphyrin. The speciation of vanadium porphyrin compounds has been mainly reported using RP-HPLC (Table 1). For resolving a mixture of vanadium–porphyrin complexes from crude oil samples, a mobile phase of acetonitrile-acetone was used and detected by UV–Vis at 387 nm (Saitoh et al., 2001). The distribution and structure characterization of vanadium porphyrins in Yichang tube-transporting oil residue (China) had been reported in different fractions using RP-HPLC/UV–Vis and was supported by studies using mass spectrometry (MS) to characterize the type of vanadium porphyrins observed (Gao et al., 2012). Vanadium porphyrin compounds have as well been separated from selected crude oils harvested from Boscan, Beta, Morichal, Arabian Heavy, and Maya oil reserves using SEC-HPLC and RP-HPLC. The crude oils were separated into porphyrin and non-porphyrin fractions using methanol and inductively coupled plasma (ICP) and direct current plasma (DCP) atomic emission spectroscopy (AES) as detection methods (Biggs et al., 1985). Other samples from crude oils were studied and

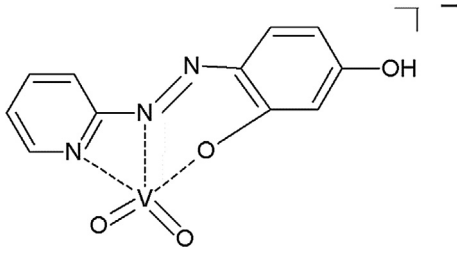

Fig. 8 The structure for vanadium(V)-pyridylazo (PAR).

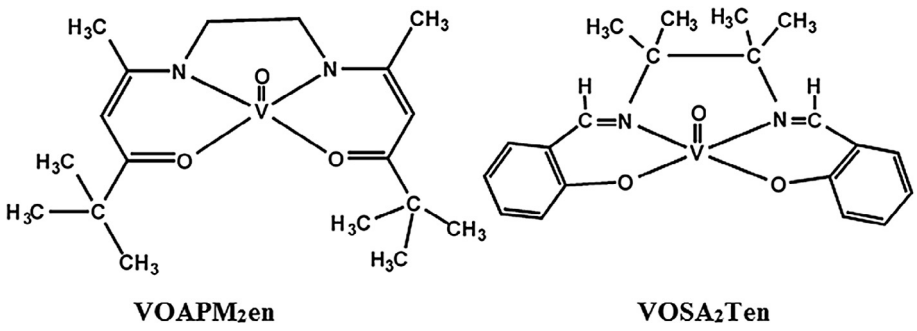

Fig. 9 The structures for the $VOSA_2Ten$ and $VOAPM_2en$ used for measuring vanadium content and speciation.

vanadium porphyrin complexes have been separated from Boscan, Cerro Negro, Wilmington and Prudhoe Bay. The method used in this study is SEC-HPLC, the separation solvent was a mixture of water and pyridine and the samples were detected by Electronic Paramagnetic Resonance (EPR) spectroscopy (Reynolds et al., 1987). Another study has investigated the separation of vanadium porphyrin compounds from crude oil (Castilla, Colombia) using HPLC and the biocatalytic modification method. The experiment was conducted using aqueous buffers and ternary systems of toluene, isopropanol, and water. It has been found that chloroperoxidase (CPO) was the best reagent of CPO-mediated reactions on the release of vanadium porphyrin complexes. The detector utilized in this case was UV–Vis at 254 nm (Mogolloń et al., 1998).

Other work has demonstrated that the separation and characterization of vanadium porphyrin complexes is also possible using NP and SEC-HPLC using a mobile phase that consists of a mixture of THF and xylene and detected by inductively coupled plasma mass spectrometry (ICPMS) (Caumette, 2010). Vanadium(IV) porphyrins were separated from fuel oil and crude oil samples using NP-HPLC on an aminopropyl column documenting that this method could be used to separate out the vanadium species. The detection of these vanadium complexes was performed with UV–Vis at a range of 553–573 nm (Xu and Lesage, 1992). The isolation of vanadium porphyrins from a heavy crude oil in Boscán crude oil sample was reported. The metalloporphyrins were extracted using first methanol and then acetonitrile and analyzed using the SEC-HPLC for both fractions. In this work hexane/benzene and dimethylformamide/benzene mixtures were used to separate the vanadium porphyrin compounds. The detection method in this work was UV–Vis and GFAA (Ysambertt et al., 1995).

The HPLC procedures described above were also used to characterize many vanadium porphyrin complexes from a range of other oil samples. In fact, the crude oil from Saudi Arabian American Oil Co. was investigated. Sample prepara-

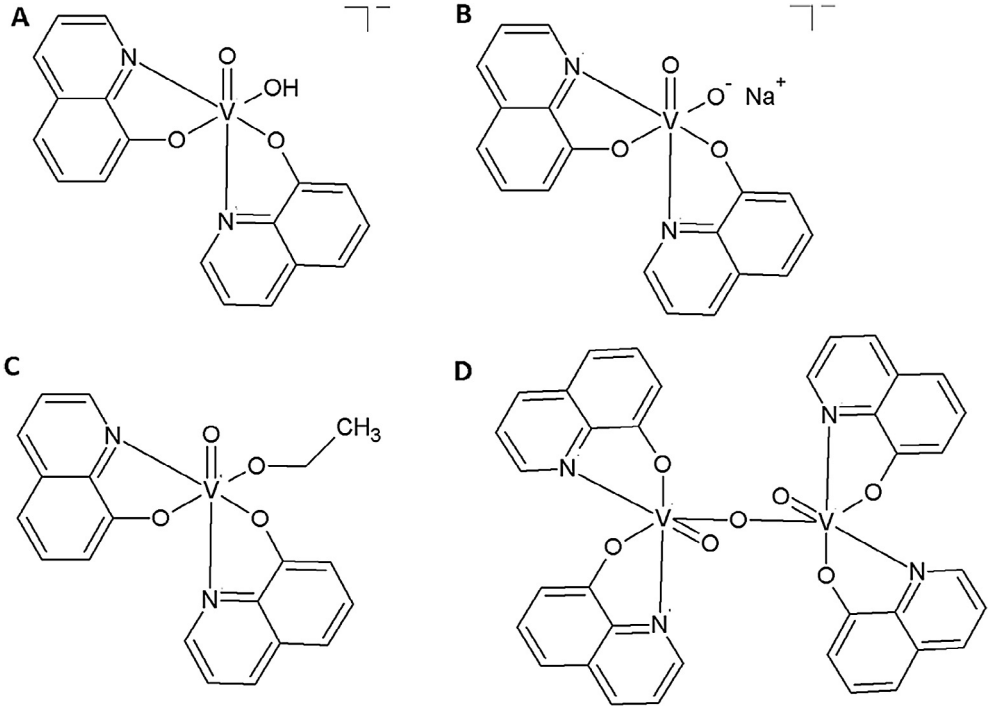

Fig. 10 Structures for the Vanadium- HQ. The complex hydroxobis(8-hydroxyquinolinato)oxovanadium(V) (A). Accordingly, it is possible to prepare salts (B), esters (C) and dimer anhydrides (D).

tion was conducted using distillation and elution. The analysis was performed by RP-HPLC and the detection by UV–Vis spectroscopy (Ali et al., 1993). From the drill cores at Julia Creek in Australia, samples were extracted with chloroform, filtered and analyzed for vanadium and metals such as Ni, Fe, Cu and Cr. UV–Vis spectra at 405–546 nm of the identified vanadium porphyrins were obtained (Ekstrom et al., 1983).

Another study has presented the separation of vanadyl-oxide tetraphenyl-porphyrin (oxidovanadium(IV)-porphyrin), VOTPP, from heavy oil (Canada) using extractions by solvent mixture of THF and methanol after addition of aqueous NaCl solution. The identification of VOTPP-compounds was performed using UV–Vis at 549–700 nm and RP-HPLC analysis (Ouled Ameur and Husein, 2012). A Japanese study has focused on characterizing vanadium porphyrins from an organic-rich dark siliceous mudstone in northern Japan (Kashiyama et al., 2007). The procedure used involved a pre-concentration phase and the resulting sample was dissolved using the appropriate eluent prior to the HPLC analysis. In this study both RP and NP-HPLC methods have been used and different eluents procedures were selected leading to detection of VO porphyrin complexes by various methods such as atmospheric pressure chemical ionization mass spectrometry (APCI–MS) and evaporative light scattering detector (ELSD) (Kashiyama et al., 2007).

Vanadium is known to be the most abundant trace metal in oil samples, and it occurs predominantly as vanadium(IV) in the form of vanadyl porphyrins. As shown above, A variety of studies have demonstrated that the HPLC determination of vanadium in oil samples can be performed using a variety of HPLC modes, columns (ODS column 4.6 × 250 mm, C18 column 4.6 mm × 25 cm, Polystyrene/divinylbenzene column 0.0075 × 0.30 m, ...) mobile phases and detectors. Furthermore, the characterization and quantitation of vanadium in petroleum is known to provide geological information and identify geographic origin of a crude oil especially in the case of environmental catastrophes such us an oil spill (Amorim et al., 2007).

3.3.1.2. Determination of vanadium compounds in oil samples: Vanadium(IV)-tetrapyrrole. In addition to vanadium porphyrin complexes, vanadium tetrapyrrole complexes have been identified within oil samples such as in a crude oil and from a sediment extract from an oil-polluted area in the Guanabara Bay, Rio de Janeiro, Brazil (Duyck et al., 2011). RP-HPLC was used to separate vanadium as a tetrapyrrole complex and its structure is shown in Fig. 7 (Duyck et al., 2011). Elution started from pure methanol to a mixture of chloroform and methanol. The column used was C18, 5 μm 4.0 × 125 m m, and the detection was performed using ICP-MS of each signal. This method demonstrate that HPLC can be used for speciation studies when the vanadium compounds are sufficiently stable.

3.3.1.3. Determination of vanadium in oil samples using chelating agents for measurements. Using a variety of chelating reagents, pre-treatment can form complexes that can be determined from the labile forms of vanadium and can thus be characterized from oil samples. When the chelator 4-(2-pyridylazo) resorcinol (PAR) was used prior to HPLC analysis, vanadium (V)-PAR complexes were formed and their structure is shown in Fig. 8 (Vachirapatama et al., 2002). Therefore, from a crude oil sample both vanadium-porphyrins and complexes such as vanadium(V)-PAR will emerge. These complexes can then be quantified using RP-HPLC, the column was selected to be C18, 14 μm 3.9 × 150 mm, and the mobile phase was chosen in this type of matrix to be $(NH_4)_2HPO_4^-$, $NH_4H_2PO_4$ and methanol, and vanadium-PAR complexes were characterized by the UV–Vis at 510 nm (Vachirapatama et al., 2002). Although the complexes are stable thermodynamically and have high formation constants, the vanadium(V)-PAR complex is labile and since the complex can hydrolyze, this system is prone to re-proportionation depending on the specific reaction conditions (Vachirapatama et al., 2002). Other complexes such as oxovanadium(IV)-(H_2SA_2Ten) complex was characterized in crude petroleum oil samples (Pakistan). The pre-complexation reagent in this case was H_2SA_2Ten, and the oxo-vanadium(IV) complex was separated and identified using NP-HPLC and chloroform or chloroform-l,2-dichloroethane and acetonitrile as eluent. The column was chosen to be silica gel 100, 5 μm 4.6 × 200 mm, and the detector was UV–Vis at 300 nm (Khuhawar et al., 1995). The structures of this vanadium complex and that of H_2APM_2en (bis(acetylpivalylme thane)ethylenediimine) is shown in Fig. 9.

Other ligands, such as H_2APM_2en and HQ were used for identification of the labile forms of vanadium from crude oil samples. The vanadium complexes were formed prior to RP-HPLC analysis. The solvent elution was performed by using methanol, acetonitrile and water. The columns were chosen to be respectively C18, 5 μm 4.6 × 150 mm and Rad-Pak short column, 5 μm 4.6 mm × 10 cm. And complexes were detected with UV–Vis at 260 and 370 nm (Khuhawar and Lanjwani, 1996; Salar Amoli and Porgam, 2006) (see Fig. 10).

In summary, the characterization of vanadium from oil samples using the different HPLC techniques do not follow one sole procedure. As described above, various types and modes of HPLC can be used, many eluents and chelating reagents can be chosen, and a variety of columns and detectors can be utilized. The domain in which researchers use vanadium separation, speciation, and quantitation for the HPLC analysis is therefore very rich and thus they have a wide variety of options to choose from during their studies of the environmental (oil) sample. However, as described above during the speciation section, the researchers must be careful in interpretation of their results, because when chelating agents are used, the identification of such complexes by HPLC methods is evidence that the vanadium complex in the original samples did not survive the pre-treatment step. Although one would presume that this would make such original complexes labile, considering the harshness of some pre-treatment procedures, even the stable vanadium-porphyrin complexes may partially succumb to decomposition during the pre-treatment step.

3.3.2. Determination of vanadium compounds in water samples

Many studies addressing the presence of vanadium metal ions in water samples have been conducted, including critical analytical aspect of the sample preparation such as sample clean up, pre-concentration and different phases of the speciation method (Nagaosa and Kimata, 1996; Chen et al., 1999; Uehara et al., 1989). Speciation of vanadium compounds is well understood in aqueous solution, and is recognized to be strongly dependent on pH, concentration and other parameters (Kiss et al., 2008; Crans et al., 2013; Chasteen, 1983;

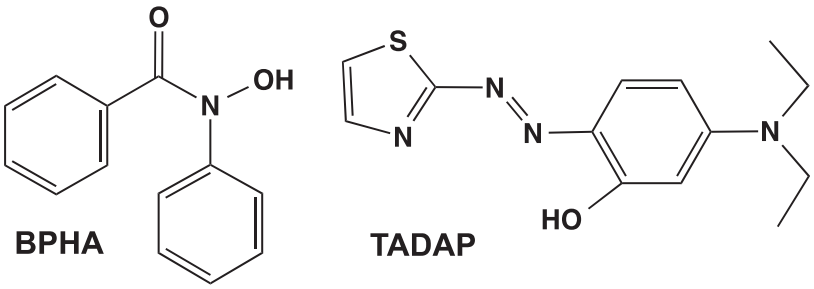

Fig. 11 Structure for the chelators BPHA and TADAP used for vanadium identification.

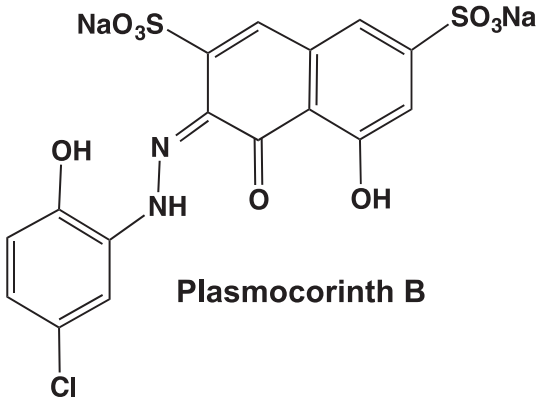

Fig. 12 The structure of Plasmocorinth B.

Crans et al., 2004; Crans et al., 1990; Pettersson et al., 1983, 1985a,b). However, the biological concentration ranges that vanadium generally occurs is very low and in order to be detected a more concentrated sample is needed. This comes with changes in speciation equilibria (see Figure 1 A-D), and interpretation of what is in the original sample to be consistent with the observations needs to be adjusted appropriately. In this section results are presented from various water samples such as sea, river, natural, synthetic, drinking, mineral, rain, liquor and lake waters. Due to the low concentrations of the vanadium systems, many studies characterizing vanadium in water samples have been done using chelating reagents to allow determination of vanadium(V) and vanadium(IV) concentrations.

3.3.2.1. Determination of vanadium compounds in water samples using IP-RP-HPLC and PAPS chelators. In aqueous solution the chelating reagent 2-(5-Nitro-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]-phenol abbreviated nitro-PAPS was used. This ligand forms very strong complexes with vanadium and particularly vanadium(V) which is the most likely form of vanadium in aqueous solutions at low concentration and near the neutral pH range (Makino et al., 1988). The separation of vanadium-nitro-PAPS was conducted using RP-HPLC in the ion pair mode (IP-RP-HPLC) which is a very common method for analysis of vanadium in water. Upon complexation the vanadium is much more hydrophobic, and the eluent was acetonitrile and water, containing acetic acid, tetrabutylammonium bromide (TBABr) and EDTA (Chen et al., 1999). The column was ERC-ODS 1282, 5 μm, 6.0 × 250 mm, and detection in this case was performed by UV–Vis at 593 nm. The results of this study suggest that vanadium in sea water can be successfully determined using this method (Chen et al., 1999).

Samples of drinking waters and wines were investigated using IP-RP-HPLC and Nitro-PAPS chelates. The column used was C18, 3.9 × 300 mm, and the detection was performed using ICP-OES (optical emission spectroscopy) (Pimrote et al., 2012). A similar ligand (5-Br-PAPS) was used for the determination of vanadium in sea water. The vanadium complex was extracted this time with trioctylmethylammonium chloride in xylene and back-extracted into sodium perchlorate solution. The column was chosen to be Lichrosorb RP-18 column 7μm, 4.0 × 250 mm. The detection was performed using UV–Vis spectroscopy at 575 nm (Shijo et al., 1996).

Other examples of vanadium characterization, separation and speciation using the HPLC method and within other water samples are described in details in Table 1.

3.3.2.2. Determination of vanadium compounds in water samples using IE-HPLC and EDTA chelators. Similarly to oil samples, studies performed within water samples relied most of the time on the concept of pre-complexation prior to vanadium HPLC separation and characterization of these samples. In fact, the speciation of vanadium(III), (IV), and (V) was developed by using HPLC. The vanadium-EDTA complexes were separated on an anion exchange column (IE) with an eluent containing EDTA, acetonitrile, and ammonium bicarbonate at pH 6. The method was applied to coke pore water samples. Separation, speciation and quantitation studies have shown that in these samples, vanadium(IV) and vanadium(V) were identified to be the major species (Li and Le, 2007).

Another study has shown the applicability of the HPLC based on the anion exchange mode to the simultaneous speciation and determination of vanadium(IV) and vanadium(V) as EDTA complexes in aqueous samples by using EDTA and $NaHCO_3$ in the eluent. The column utilized was Dionex-AG5 anion exchange, and the detector in this case was ICP-OES. The speciation results were excellent within mineral processing matrices (Hu and Coetzee, 2007).

3.3.2.3. Determination of vanadium compounds in water samples using HPLC BPHA and other chelators. NP-HPLC was used for the determination of vanadium with N-benzoyl-N-phenylhydroxylamine (BPHA) shown in Fig. 11. The vanadium(V)-BPHA complex was identified using a mobile phase of BPHA in chloroform after stabilization with amylene. The column used in this case was chosen to be S5 Nitrile 5μm, 4.6 × 250 mm. And UV–Vis detection at 360 nm was used for quantitation of the complex and the procedure was applied for the analysis of synthetic water samples (Gracia and Bagur, 1996).

In a similar way, the PBHA (N-phenylbenzohydroxamic acid) chelator was used to determine vanadium concentration in a river water sample where RP-HPLC was employed (Nagaosa and Kimata, 1996). The measurements were conducted after pre-complexation of vanadium with PBHA. In this case, the column utilized was C18 ODS 5 μm, 4.6 × 150 mm, and the electrochemical detection was performed at -0.60 V vs Ag/AgCl (Nagaosa and Kimata, 1996).

Vanadium(V)-TADAP (2-(2-thiazolylazo)-5-diethylamino phenol) complexes have been used for measurements of vanadium in a reference mineral and known synthetic samples (Wang et al., 1997). The structure of TADAP is in Fig. 11. RP-HPLC was employed to observe the separation of the vanadium-TADAP complex from the other metal–TADAP complexes (Wang et al., 1997). A second group reported investigations of the vanadium(V)-TADAP complex (Liu et al., 1992). This study showed this ligand could also chelate the vanadium prior to the submission for analysis by RP-HPLC. The pH of the mobile phase (methanol-THF containing lithium sulfate and acetate buffer) was chosen to be 5.5. The column was chosen to be C18 ODS 1μm, 4.0 × 250 mm, and the detection was performed spectrophotometrically at 570 nm. This method was applied to the identification of trace amounts of vanadium(V) in water samples (Liu et al., 1992).

3.3.2.4. Determination of vanadium compounds in water samples using Plasmocorinth B chelators and other chelators of similar structure. To identify vanadium trace levels in natural waters, a different type of complex was used. It is vanadium(V)-Plasmocorinth B where the ligand is a disulfonated azo-dye shown in Fig. 12. In this case, IP-RP-HPLC was used to measure vanadium(V) as a speciation complex with Plasmocorinth B. An on-line enrichment procedure was developed following the optimization of the eluent varying pH, ligand concentration, ionic strength and organic chelator (Sarzanini et al., 1993). RP-HPLC was also used for measuring the presences of the vanadium(V) species. In this case, HQ was used as a chelating reagent. Identification of vanadium(V) in sea water was performed by the speciation of vanadium(V)-HQ complexes using a mobile phase of methanol and water containing HQ and chloroacetate at a pH of 3.5 The column used was C18 ODS, 5 μm 4.0 mm × 25 cm and the complex was detected by a spectrophotometer at 400 nm (Ohashi et al., 1991). RP-HPLC was also used for the speciation of the vanadium(V) species after being complexed with 2-(8-quinolylazo)-5-(dimethylamino)-phenol. Tetraalkylammonium salts (TAASs) were added to an aqueous organic mobile phase and specifically TBABr permits rapid separation and sensitive spectrophotometric detection of the vanadium(V) chelate with 2-(8-quinolylazo)-5-(dimethylamino)-phenol, making it possible to determine trace vanadium(V). This methodology was used to identify vanadium in natural waters (Miura, 1990).

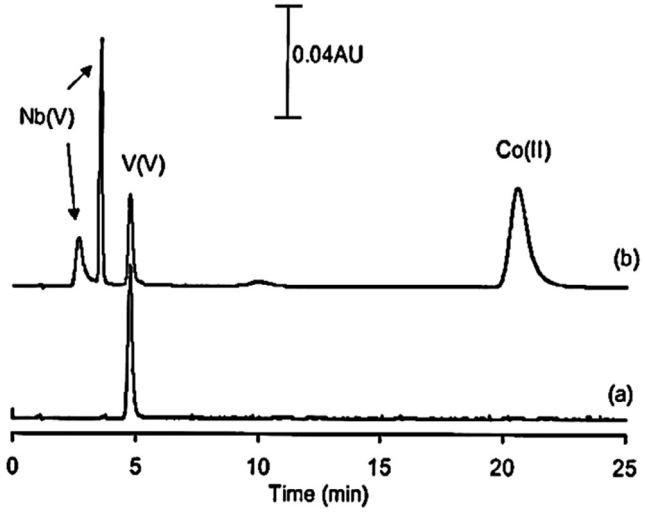

Fig. 13 Chromatograms of a) V(V)-PAR-H_2O_2 (0.178 g mL^{-1}). (b) mixtures of Nb(V)-PAR (2 mg mL^{-1}), V(V)-PAR-H_2O_2 (0.2 mg mL^{-1}) and Co(II)-PAR (2 mg mL^{-1}). Mobile phase was methanol-water (32:68 v/v) containing 5 mM acetate, 3 mM TBABr and 5 mM citrate buffer at pH 7 for use of analysis of NKP fertilizers. The diagram was reproduced with permission from references (Vachirapatama et al., 2002).

In another study, HPLC along with an on-line enrichment technique was used to determine vanadium species. The HPLC separation and species characterization was performed after the vanadium was complexed with the 5-Br-QADEAP (2-[2-(5-bromoquinolinylazo)]-5-diethylaminophenol) reagent. A mixture of acetonitrile and water containing a mixture of phosphate and citrate buffer (pH 4.5) was used as the mobile phase. The column utilized was Rapid analytical 1.8 μm, 4.6 × 10 mm This method was successfully applied to the determination of vanadium in several water samples (Li et al., 2007).

HPLC separation, speciation and quantitation studies of vanadium in water have mostly been done by separating vanadium coordination complexes that formed during a pretreatment step. Only few studies have reported the specifics regarding the vanadium species (IV and V) that are determined in the form in which they are present in the sample. The vanadium speciation in this case was based on chromatographic separation of vanadium(IV) and vanadium(V) in acidic medium (Hu and Coetzee, 2007). The two vanadium species were chromatographically separated using HPLC-cation exchange method. The eluent was mainly a solution of H_2SO_4. The column was chosen to be an ion pack CG10 cation exchange column. The detection was performed using ICP-OES and the developed method has been successfully applied to the determination of vanadium(IV) and vanadium(V) in synthetic and minerals processing samples (Hu and Coetzee, 2007). Another study has shown that the determination of vanadium(V) species was possible using tetradentate schiff-base ligands, as all N,N′-*o*-phenylenebis(salicylaldimine), PBS, derivatives are known to have a high selectivity towards few metal ions including vanadium. For instance, the known ligand was found to be suitable for the speciation of vanadium. The methodology used was RP-HPLC and the detection was spectrophotometric. The determination of the vanadium(V)-4,4-di-N,N-diethyl-PBS complex has been accomplished without the addition of a reagent to the eluent (Kanbayashi et al., 1987).

In summary, the studies determining the presence of vanadium in water samples generally face the challenge of low vanadium levels, which needs concentration before the vanadium can be detected. Although speciation is well understood in aqueous solution, and it is recognized that vanadium(IV) readily convert to vanadium(V) in aqueous solution in the presence of oxygen and at pH above 5, studies using HPLC detection described in this section focused on the measurement of total vanadium. The observed speciation of metal complexes

allows detection of the vanadium complex, which therefore correspond to the total amount of vanadium present in the sample. Because of the low concentration of vanadium, it is concluded that the vanadium is present as vanadate at pH above 5 in the presence of oxygen.

3.3.3. Determination of vanadium compounds in other environmental samples

HPLC has as well been used for measuring vanadium levels and speciation in environmental samples other than oil and water samples. For example, another vanadium porphyrin compound extracted from polluted soil from Iran has been identified using HPLC with methylene chloride as solvent. The compound is vanadium oxide octaethyl porphyrin (VOOEP). The organic methylene chloride phase was separated from the aqueous phase by centrifugation. The aqueous phase was then examined for the presence of vanadium compounds using HPLC. A mixture of methanol, acetone and buffer has been used as a mobile phase, and the sample was separated via a Nova Pack C18 column 3.9 × 150 mm, and detected by UV–Vis at 254 nm (Salehizadeh et al., 2007). In a similar study, the HPLC separation procedure has been used for the speciation of vanadium(IV) and (V) complexes in soil samples. The vanadium species were separated on a RP-HPLC column (C8 column 3μm, 3.0 × 30 mm). The mobile phase consisted of EDTA and TBAP in methanol at pH 6.85. The method has been applied on farmland soil and vanadium metal ions were successfully identified by dynamic reaction cell (DRC)-ICP-MS (Kuo and Jiang, 2007). In another study, the RP-HPLC was used to separate vanadium(V)-4-(2-thiazolylazo) resorcinol compounds using an amino-bounded column 10 μm, 4.6 × 250 mm. The mobile phase was a mixture of methanol and water containing Li_2SO_4 and acetic acid-sodium acetate (HAc-NaAc) buffer at pH 5.0. The method was applied to the determination of vanadium in vanadium slag (Changshan et al., 1989).

Other environmental samples highlighted in this work include phosphate rocks and NPK fertilizers (Nitrogen, phosphorus and potassium) (Vachirapatama et al., 2002); coal fly ash (Miura, 1990), and pond sediments (Chen et al., 1999). The RP-HPLC method was used for characterizing and identifying vanadium in their complexed forms (vanadium-PAR and vanadium(V)-PAR-H_2O_2) within samples of phosphate rocks as well as NPK fertilizers. The pH in this study was 7.0 (Vachirapatama et al., 2002) (Fig. 13). The same method has been used for coal fly ash samples. The ligand used this time was 2-(8-quinolylazo)-5-(dimethylamino)-phenol (QADAP) and it was possible to determine and characterize the vanadium (V) complex (Miura, 1990) using an ODS column 7 μm, 4 × 125 mm and a spectrophotometric detector at 550 nm. The separation of vanadium-Nitro-PAPS was conducted using RP-HPLC (ODS column, 5 μm, 6.0 × 250 mm) and a spectrophotometric detector at 593 nm. The mixture utilized was acetonitrile and water containing acetic acid, TBABr and EDTA. In this study metal ions were measured in pond sediments (Chen et al., 1999).

RP-HPLC (Rapid analytical column 1.8 μm, 4.6 × 10 mm) along with an on-line enrichment technique was as well been used in the determination of vanadium metal ions in geologic samples. The pH of the mobile phase was set to 4.5, and vanadium-5-Br-QADEAP complex was identified (Li et al., 2007). The occurrence of vanadium(V) in a variety of other environmental samples was investigated and RP-HPLC in combination with spectrophotometric detection was used (Zhang et al., 2011). Table 1 below summarizes most of the known examples of studies using HPLC for determination, separation and speciation of vanadium compounds in environmental matrices.

In summary, the understanding of the role of vanadium and vanadium species in terms of availability, toxicity, mobility and persistence within environmental matrices is of great interest. As a specific and sensitive analytical method, HPLC has been used for measuring vanadium levels and speciation in a variety of environmental samples. And as in the case of the previous studies the speciation is generally based on the complexation of the vanadium during the pre-treatment phase to render the vanadium observable by complexation and concentration. However, such modifications have the potential to change the original speciation. These analyses limit the original speciation studies to distinguishing existence of vanadium(IV) from vanadium(V) in the original samples.

3.3.4. Examples of vanadium compounds speciation from biological matrices

Studies determining vanadium metal ions by HPLC analysis within a wide range of biological matrices are the second most frequent method employed after analysis of environmental matrices. Many HPLC methods and modes have been used for the separation, speciation and quantitation of different vanadium species in these matrices. This work presents examples of these HPLC studies using different methods to prepare the samples, different chelating reagents, different mobile phases, different columns and different detectors. The examples highlighted will be organized according to the type of biological sample because most of the methods used to prepare the sample for analysis are those that have been described previously in this document.

3.3.4.1. Determination of vanadium compounds from mussel and fish samples. Before the HPLC method can be applied, the samples were prepared based on decomposition, dissolution and filtration. The pH was adjusted to 2.5, and vanadium(V) present in the mussel sample was complexed with PBHA reagent (Nagaosa and Kimata, 1996). The study used a C18 ODS column (Octadecylsilyl) 5 μm, 4.6 × 150 mm and an electrochemical detection method and the chromatogram shows several signals that are attributed to different metal ions including the signal caused by V(V)-PBHA complex.

In a similar study, using mussel and fish muscle samples, the separation, speciation and quantitation of the vanadium (IV)-EDTA and vanadium(V)-EDTA complexes were conducted using a variety of HPLC modes. RP, IP-RP and anion exchange, along with ICP-MS detection. These mussel and fish muscle samples were collected from lake Maracaibo in Venezuela (Colina et al., 2005). This study demonstrated the fact that the oxidation state of the vanadium was only stable if EDTA is present in the eluent. In the absence of EDTA only vanadium(IV) was observed. In an IP-RP-HPLC chromatographic column, the presence of TBA^+ offers dynamic ion-exchange sites because an adsorption equilibrium of TBA is established between the eluent and the stationary phase favoring the most stable form of the vanadium. This study showed

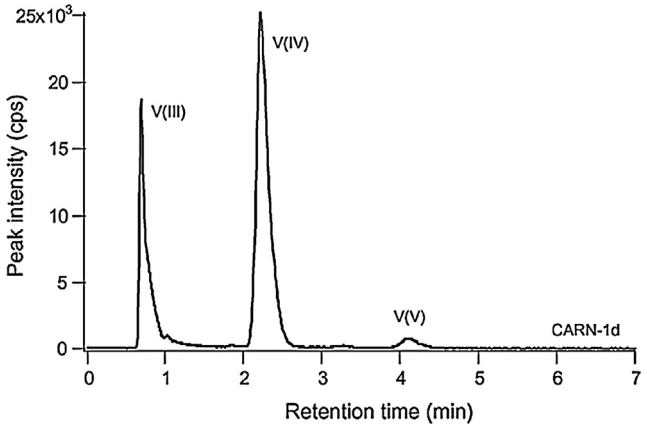

Fig. 14 The chromatogram from the HPLC-ICP-MS analysis of V(III), V(IV) and V(V) using EDTA as chelator and ICP-MS detection. The mobile phase consisted of 2 mM EDTA, 3% acetonitrile, and 80 mM NH_4HCO_3, pH 6.0. The diagram was reproduced with permission from reference (Li and Le, 2007).

that the detection and separation of vanadium(IV) and (V) species was possible using the HPLC anion exchange mode, and using $(NH_4)_2HPO_4$, $NH_4H_2PO_4$ and EDTA as eluent. However application of this method based on anion exchange did not yield results providing a good separation and speciation of vanadium compounds in the above mussel and fish samples (Colina et al., 2005).

3.3.4.2. Determination of vanadium compounds from tunicate and toadstool samples. Studies focused on vanadium characterization within biological samples have been done in tunicates. In fact, a large number of tunicates is known to concentrate vanadium ions, and thus this biological system may be one in which speciation carried out using the available methods will only describe oxidation state, because the concentration in the original samples are likely to be beyond simple monomeric forms of vanadium species. For example, *Ciona intestinalis* is known to accumulate vanadium to hundreds of millimolar concentrations directly from sea water thus going against an enormous concentration gradient (McLeod et al., 1975; Maclntyre, 1970). Other species of marine tunicates are known to accumulate oxygen-sensitive vanadium ions in blood cells and maintaining high levels of vanadium(III). Using X-ray absorption spectroscopy (XAS) on extracts, the main complexes found were tris(catecholate)- type vanadium(IV) complexes and bis(catecholate)-type oxovanadium(IV) complexes. The same XAS analysis have also shown the presence of a minor vanadium(III) component (Ryan et al., 1996). More recent studies have shown that some of these measurements were compromised by the extraction procedures and the vanadium may be located in different cells than the catecholates (Crans et al., 2004; Michibata et al., 2003; Odate and Pawlik, 2007; Takemoto et al., 2003; Ueki et al., 1770; Sugumaran and Robinson, 2012).

A recent study has investigated the vanadium–binding proteins in a variety of planktonic tunicates from the Cabo Frio region in the southeast part of Brazil (Kütter et al., 2014). These investigations were performed using multidimensional techniques such as SEC-UV–Vis-ICP-MS, as well as AE-UV–Vis-ICP-MS (Kütter et al., 2014). Another study has focused on the usage of IP-HPLC for the isolation and purification of the tunichromes from two tunicates: *Molgula manhattensis* and *Styela plicata*. These tunichromes contain polyphenolic polypeptides that are chelating agents of metals, particularly vanadium and iron (Liu, 2013). The presence of vanadium in tunicates has been the most studied among any biological samples (Frank et al., 1995; Hawkins et al., 1980; Kime-Hunt et al., 1991).

Other examples such as Amanita toadstools are also investigated and studies were performed toward finding the oxidation state of vanadium is present in the sample. These studies provided fuel regarding the biological role of vanadium in these biological matrices (Frausto da Silva, 1989).

3.3.4.3. Determination of vanadium compounds from plant samples. The characterization, quantitation and speciation of vanadium within plant samples has been mostly performed using chelators prior to using HPLC method. PAR has been one of the most famous reagent used for these samples. The vanadium(V)-PAR complex was used to measure the presence of vanadium in rice and flour samples (Peng et al., 2005). PAR chelating agent and acetate buffer were added, and the solution was diluted and solids removed by centrifugation. A sample from the resulting solution was injected to the RP-HPLC column (ODS, 4.0 × 150 mm) and the detection was done by UV–Vis at 525 nm (Peng et al., 2005). Using Japanese plant material from Chlorella and pepperbush, RP-HPLC was found to be highly effective to measure and separate the vanadium(V) complex. PAR was as well used as a chelating reagent, and the vanadium-PAR complex was separated using RP-HPLC (C18 column 5μm, 4.6 × 150 mm) with a mixture of methanol and phosphate buffer at pH 5.5, 1,2-cyclohexanediamine-N,N,N′,N′-tetraacetic acid and TBABr in water as mobile phase and detected at 555 nm by UV–Vis (Yamada and Hattori, 1986). RP-HPLC was applied to document the vanadium uptake by chinese green mustard and tomato plants (Vachirapatama et al., 2005). In this sample the ion pair mode (IP) was utilized and the levels of vanadium was measured using PAR and hydrogen peroxide as pre-column complexing agents where the mobile phase contains methanol, acetic acid, citric acid and (TBABr) at pH 7. The column utilized was Nova Pack C18, 4 μm 3.9 × 150 mm and the detection was performed by UV–Vis at 540 nm (Vachirapatama et al., 2005).

Other examples of plant samples such as tea leaves, rice and *Persea Americana* were investigated, and vanadium metal ions were identified. The separation, speciation and quantitation of a vanadium(V)-2-(2-thienylazo)-5-diethylamino phenol complex was performed using RP-HPLC on tea leaves and rice samples. The pH was chosen to be 5.5, the column was C18 ODS column 1μm, 4.0 × 250 mm and detection was performed spectrophotometrically at 570 nm (Liu et al., 1992). In another study, the RP-HPLC separation procedure, and the species were separated by RP (C8 column 3 μm, 3.0 × 30 mm) with a mobile phase consisting of EDTA and TBAP in methanol at pH 6.85 and detection was done using DRC-ICPMS, this procedure applied successfully to leaves of *Persea americana* (Kuo and Jiang, 2007).

3.3.4.4. Determination of vanadium compounds from samples of bacterial cultures. The speciation of vanadium(III), (IV), and (V) was developed by using HPLC (PRPx100 strong anion exchange column 3 μm, 4.1 × 50 mm). Vanadium-EDTA com-

Table 2 A summary of the most known applications, within biological matrices, of measuring vanadium using HPLC.

Type of the biological sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Marine mussel tissues	Reversed phase (RP)	Methanol: Water (9:1 v/v)	UV–Visible at 408 nm	Vanadyl porphyrin	Rivaro (1997)
	LiChrospher RP-8 column 5 μm 4.6 × 250 mm		ICP-AES	LOD of 5.0 ng/g Quantities of 3790 ± 180 ng/g (in the digestive glands)	
Mussel homogenate (Japan)	Reversed phase (RP)	Acetonitrile-acetate buffer	Electrochemical (EC)	V(V)- PBHA	Nagaosa and Kimata (1996)
	C18 ODS column 5 μm 4.6 × 150 mm	(0.02 M, pH 3.5) 1:1 (v/v), 0.05 M potassium nitrate		LOD of 1.0 ng/mL Quantities of 0.44 ± 0.01 μg/g	
Fish muscle tissue from Maracaibo lake in Venezuela (*Polymesoda artacta*)	Reversed Phase (RP) Ion pair (IP)	5–10% acetonitrile, 0.05 M TBAOH, 2 mM EDTA	ICP-MS	V(IV, V)- EDTA	Colina et al. (2005)
	C18 column 4.6 × 250 mm			LOD of 59.1 μg/L V(IV) 113.1 μg/L V(V) Quantities of 0.92 ± 0.08 μg/g V (IV)	
Mussel from Maracaibo lake in Venezuela (*Polymesoda artacta*)	Reversed Phase (RP) Ion pair (IP)	1.87 mM ammonium phosphate–1.87 mM diammonium phosphate, 2 mM EDTA	ICP-MS	V(IV, V)- EDTA	Colina et al. (2005)
	C18 column 4.6 × 250 mm			LOD of 59.1 μg/L V(IV) 113.1 μg/L V(V) Quantities of 1.52 ± 0.16 μg/g V (IV)	
Rice	Reversed phase (RP)	Methanol: THF: Water (60:5:35 v/v/v), 0.2 M LiCl, PAR (5×10^{-4} M) acetate buffer, pH 4.9	UV–Visible at 525 nm	V(V)-PAR	Peng et al. (2005)
	ODS column 4.0 × 150 mm			LOD of 3.5 ng/mL Quantities of 0.105 μg/g	
Flour	Reversed phase (RP)	Methanol: THF: Water (60:5:35 v/v/v), 0.2 M LiCl, PAR (5×10^{-4} M) acetate buffer, pH 4.9	UV–Visible at 525 nm	V(V)-PAR	Peng et al. (2005)
	ODS column 4.0 × 150 mm			LOD of 3.5 ng/mL Quantities of 0.180 μg/g	
Plant material (Chlorella and pepperbush)	Reversed phase (RP)	Methanol: Water (60:40, v/v) containing (phosphate buffer (pH 5.5) 1,2-cyclohexane diamine-N,N,N′,N′-tetraacetic acid and tetrabutylammonium bromide)	Spectrophotometer at 555 nm	Vanadium-PAR[a] LOD of 17 pg Quantities of 0.40 μg/g Chlorella 0.52 μg/g pepperbush	Yamada and Hattori (1986)
	C18 column 5μm 4.6 × 150 mm				
Shewanella putrefaciens CN32 bacterial cultures	Anion exchange	3% Acetonitrile, 2 mM EDTA, and 80 mM of Ammonium bicarbonate (pH 6)	ICP-MS	V(III, IV, V)- EDTA	Li and Le (2007)
	PRPx100 strong anion exchange column 3 μm 4.1 × 50 mm			LOD of 0.6 μg/L V(III) 0.7 μg/L V(IV) 1.0 μg/L V(V)	

(*continued on next page*)

Table 2 (*continued*)

Type of the biological sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
				Quantities of more than 90% of V (IV) were present after few days of incubation	
Leaves of *Persea Americana*	Reversed phase (RP) C8 column 3 µm 3.0 × 30 mm	In 4% Methanol, 5 mM EDTA, 0.5 mM TBAP (pH 6.85)	DRC-ICPMS	V(IV, V)-EDTA LOD of 0.06 ng/mL Vtot	Kuo and Jiang (2007)
				Quantities of 0.31 ± 0.01 µg/g V (IV) 0.69 ± 0.01 µg/g V(V)	
Ascidian *Ciona intestinalis* (Woods Hole, USA)	Reversed Phase (RP) C18 column 5 µm 25 mm	35% Methanol, 65 mM ammoniumphosphate	Dual absorbance	Vanadium LOD of 0.03 nmol	Stacey and Driedzic (2010)
				Quantities of 0.026–0.290 µmol/g	
Ascidian Tunicate *Styela plicata* *Molgula manhattensis*	Reversed Phase (RP) C8 column	Water-acetonitrile (0.1%)	Absorbance at 280 nm	Vanadium- polyphenolic polypeptides[a]	Liu (2013)
Tea leaves	Reversed Phase (RP) C18 ODS column 1µm 4.0 × 250 mm	Methanol: THF: Water (20:15:65, v/v/v) containing 0.05 M lithium sulfate and 0.04 M acetate buffer (pH 5.5)	Spectrophotometric detection at 570 nm	V(V)- 2-(2-TADAP) LOD of 0.5 ppb	Liu et al. (1992)
				Quantities of 670 ± 17 ppb	
Rice	Reversed Phase (RP) C18 ODS column 1µm 4.0 × 250 mm	Methanol: THF: Water (20:15:65, v/v/v) containing 0.05 M lithium sulfate and 0.04 M acetate buffer (pH 5.5)	Spectrophotometric detection at 570 nm	V(V)- 2-(2- TADAP) LOD of 0.5 ppb Quantities of 137 ± 1.7 ppb	Liu et al. (1992)
Clam tissue (*cittereas*p.)	Normal Phase (NP) Superspher Si 60 column 4.0 × 244 mm	Methanol: Trichloromethane (3:97 v/v) containing 9.4×10^{-4} M PBHA	UV–Visible at 430 nm	V(V)- PBHA LOD of 1.8 µg/dm^3	Bagur et al. (1994)
				Quantities of 2.24 ± 0.12 µg/g	
Chinese green mustard (*B. campestris* ssp. *chinensis* var. *parachinensis*)	Reversed phase (RP) Ion pair(IP) A Nova Pack C18 column 4 µm 3.9 × 150 mm	20% Methanol, 5 mM acetic acid, 5 mM citric acid, l0 Mm TBABr at pH 7	UV–Visible at 540 nm	V(V)-PAR- H_2O_2 LOD of 3.1 ng/kg	Vachirapatama et al. (2005)
				Quantities of 2.10 mg/g leaf 0.91 mg/g stem 25.93 mg/g root	
Tomato plant (*Lycopersicon esculentum*)	Reversed phase (RP) Ion pair (IP) A Nova Pack C18 column 4 µm 3.9 × 150 mm	20% Methanol, 5 mM acetic acid, 5 mM citric acid, l0 Mm TBABr at pH 7	UV–Visible detector at 540 nm	V(V)-PAR- H_2O_2 LOD of 3.1 ng/kg	Vachirapatama et al. (2005)
				Quantities of 0.093 mg/kg fruit 2.18 mg/kg root	

[a] This study was mainly concerned about the structure of the vanadium complex, and therefore the results were qualitative and not quantitative.

plexes were separated with an eluent containing EDTA, acetonitrile, and ammonium bicarbonate at pH 6 and species were detected by ICP-MS. The chromatogram shown in Fig. 14 illustrates that although the vanadium-EDTA complexes are not as stable as the vanadium(IV)-porphyrin complexes, they still are able to be separated on a HPLC column. The method was applied to *Shewanella putrefaciens* CN32 bacterial cultures incubated with vanadium(V). The speciation analysis has shown that vanadium(III)-EDTA complex was detected to be the major complex in the bacterial cultures (Li and Le, 2007). This work represents a real success for the HPLC method, because not only is metal speciation demonstrated but also vanadium speciation is measured.

In summary, the investigation of HPLC studies on the vanadium content in biological matrices required separation for elucidating the speciation and quantitation in biological matrices. The studies show that many modes of HPLC can be used even though the RP-HPLC is used most often. Studies have shown that a variety of eluents, chelating reagents and detectors can be utilized to characterize vanadium species in biological samples. However, it is important to recognize that in most of these biological matrices, separation, speciation and quantitation of vanadium species present in biological samples is indirect.

Species were identified after sample pre-treatment involving a complexation reaction prior to the analysis by the HPLC technique. Furthermore, in aqueous environment the speciation is pH dependent and the type of vanadium species present within the biological matrix are found to be defined in terms of oxidation state, but could not otherwise be defined based on the treatments used for the studies (Kiss et al., 2008; Crans et al., 2013; Chasteen, 1983; Crans et al., 2004, 1990; Pettersson et al., 1983, 1985a,b). For the examples of biological matrices where pH is acidic, there are different vanadium species depending on the oxidation state and the concentration range of the vanadium. When the pH is close to neutral pH if the samples are in contact with oxygen, vanadium(V) is the most stable species (Crans et al., 2013; Pettersson et al., 1983, 1985a,b). However, in the absence of contact with oxygen, three vanadium species (III, IV, V) can co-exist in the biological sample because vanadium is known to be able to shuttle between oxidation state. Table 2 summarizes most of the known examples of vanadium compounds obtained through HPLC speciation in biological matrices.

3.3.5. Examples of separation, speciation and quantitation of vanadium compounds from clinical matrices

During the last few decades, efforts has been directed toward developing and improving speciation methods and their application in biomedical samples with the objective of development of new drugs (Caroli, 1996). Applications of vanadium compounds have increasingly been investigated since they have antidiabetic, antioxidant stress, anti-trypanosomal and anticancer effects (Kiss et al., 2008; Willsky et al., 2013; Aureliano and Crans, 2009; Crans et al., 2013; Jakusch et al., 2011; Tracey et al., 2007; Sanna et al., 2014; Ueki et al., 2012; Benítez et al., 2011). When vanadium is absorbed by the organism through food and inhalation, it distributes among tissues and various organs such as liver, kidney and bones (Nielsen, 1995). For example, the osteogenic effects of vanadium compounds have been reviewed and vanadium compounds effects on the hard tissue and bone related cells are detailed (Etcheverry et al., 2012). Insight into the mechanism of anti-trypanosomal action of vanadium compounds are investigated and gel electrophoresis studies document their DNA interaction on plasmid DNA (Benítez et al., 2011). The distribution of vanadium in serum, packed cells and homogenates of tissues was investigated by liquid chromatography using both SEC and IE-HPLC. Clinical samples in this case were primarily kidney, bone, spleen and liver (De Cremer et al., 2002). Previous work in the field of vanadium species characterization has focused on the presence of vanadium metal ions in serum samples and tissue extracts (Sabbioni et al., 1978; Chasteen et al., 1986). Other studies had focused on bio-speciation of vanadium(IV) antidiabetic compounds in blood and cell samples (Tahán et al., 1994). However, HPLC applications that utilize clinical samples are limited in the literature. In this regard, only few recent studies have reported HPLC speciation results as per vanadium metal ions presence in human liver samples (Nischwitz et al., 2013); human serum samples (Nagaoka et al., 2002), human milk samples (Coni et al., 1996); human hair samples (Yuxia et al., 1993); and human urine samples (Tomlinson et al., 1994).

3.3.5.1. Determination of vanadium compounds from solid clinical samples. A new methodology was developed for the characterization of vanadium in its major anionic form in a liver cell model after exposure to bis(maltolato)oxovanadium (IV) BMOV, Fig. 15 (Nischwitz et al., 2013). This was necessary because some of the products that BMOV convert to are not as kinetically stable as the vanadium(IV) complex. SEC-HPLC (TSK 3000 PW X L column 8.0 × 300 mm) was used for this study with combined elemental and molecular MS detection (ICP-MS) of the vanadium complexes indicative of the speciation (Nischwitz et al., 2013).

Human hair is another clinical sample that was used to investigate the vanadium level (Table 3). The determination of vanadium(V) was conducted using RP-HPLC with a C8 column 5 μm, 4.6 × 150 mm and MBTAR (4-(6-methyl-2-benzothiazolyazo) resorcinol) as a chelating reagent. The detection was performed with UV–Visible at 560 nm (Yuxia et al., 1993).

3.3.5.2. Determination of vanadium compounds from liquid clinical samples cultures. Blood was a clinical sample investigated and it was shown that vanadium is bound to serum transferrin (Tf). Tf is a glycoprotein which has two metal-binding sites and the binding patterns of V(III), V(IV), and V(V) to human serum Tf (hTf) was described using HPLC (TSK gel BioAssist Q column 4.6 × 50 mm) where the mobile phase was Tris–HCl (pH 7.4) and ammonium acetate and detection by ICP-MS (Nagaoka et al., 2002, 2004; Jakusch et al., 2009). Fig. 16 shows an example of vanadium detection within an hTf sample. The determination of vanadium in human serum was also measured using electrothermal atomic absorption spectroscopy (AAS). Using this technique, the median concentration of vanadium in 108 persons was found to be 50 ng/L. This report was in good agreement with previously reported results by other techniques such as neutron activation analysis and radiochemical neutron activation analysis (NAA) (Heinemann and Vogt, 1996; Byrne and Versieck, 1990).

Table 3 A summary of the most known applications, within clinical matrices, of measuring vanadium using HPLC.

Type of the clinical sample	Type of HPLC (type of column)	Mobile phase	Detector	Vanadium compounds (LOD and quantities)	Reference
Human liver	Size exclusion TSK 3000 PW X L column 8.0 × 300 mm	Tris-HCl buffer (up to 50 mM, pH 7.4) and 10 mM ammonium acetate (pH 7.4)	ICP-MS	BMOV[a] Vanadium-citrate[a]	Nischwitz et al. (2013)
Human serum transferrin (hTf)	Anion Exchange TSK gel BioAssist Q column 4.6 × 50 mm	50 mM Tris–HCl (pH 7.4) and 0.25M ammonium acetate	UV at 280 nm HR-ICP-MS	V(III, IV, V)- hTf Quantities of 2.0 ppb V(IV) 0.15 ppb V(V)	Nagaoka et al. (2002)
Human serum	Anion Exchange TSK gel BioAssist Q column 4.6 × 50 mm	Bicarbonate	HR-ICP-MS	V(III, IV)-hTf Quantities of 140 ppb Vtotal	Nagaoka et al. (2004)
Human serum	Anion exchange Mono Q (HR 5/5) column	25.0 mM HCO_3^- pH 7.4	HR-ICP-MS	$V^{IV}O$–mal- hTf[a] $V^{IV}O$–dhp- hTf[a]	Jakusch et al. (2009)
Human milk	Size exclusion TSK 3000 PW X L column	0.1 M HEPES and 0.3 M NaCl	ICP-MS	Vanadium species[a]	Coni et al. (1996)
Human hair	Reversed phase (RP) C8 column 5 μm 4.6 × 150 mm	Methanol: Water (75: 25 v/v) containing (15 mM TBA Br, 10 mM HAc-NaAc buffer at pH 6)	UV–Vis at 560 nm	V(V)- MBTAR LOD of 0.08–0.2 μg/ml Quantities of 0 μg/ml	Yuxia et al. (1993)
Human hair	Reversed phase (RP) C18 column 7μm 3.3 × 150 mm	THF: Methanol: Water (22:5:68 v/v/v), 4.68×10^{-4} M TBr, 5% 0.1 M Sodium acetic acid (pH 3.3)	Detector at 590 nm	V(V)– 5-Br-PADAP LOD of 0.15 ppb 0 μg/L no detection owing to the presence of vanadium as V(IV). This latter does not form a complex with 5-Br-PADAP	Oszwałdowski (1995)
Human hair	Reversed phase (RP) C18 column 7μm 3.3 × 150 mm	THF: Methanol: Water (22:5:68 v/v/v), 4.68×10^{-4} M TBr, 5% 0.1 M Sodium acetic acid (pH 3.3)	Detector at 590 nm	V(V)–H_2O_2 5-Br-PADAP LOD of 0.15 ppb 0 μg/L no detection owing to the presence of vanadium as V(IV). This latter does not form a complex with 5-Br-PADAP	Oszwałdowski (1995)
Urine reference material (RM)	Mixed mode Silica based column 5 μm 4.6 × 250 mm	6 mM 2,6-pyridinecarboxylic acid, 8.6 mM LiOH	ICP-MS	Vanadium species LOD of 2.3 ppg V(IV) 0.48 ppg V(V) Quantities of 94.0 ±5.17 V(IV) 20.8 ± 1.37 V(V) ng/ml	Tomlinson et al. (1994)

[a] This study was mainly concerned about the characterization of vanadium complexes, and therefore the results were qualitative and not quantitative.

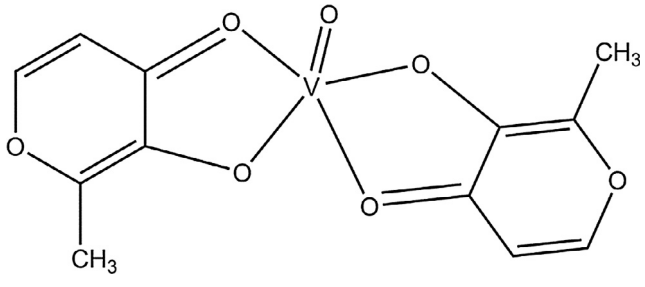

Fig. 15 The structural formula of BMOV (shown as the trans-isomer).

An investigation was undertaken by applying SEC-HPLC to the vanadium speciation in human milk (Table 3). The study results showed that experimental conditions must be optimized. The column utilized was a TSK 3000 PW X L column, and the detection was performed by ICP-MS (Coni et al., 1996).

A speciation study of vanadium(IV) and (V) in urine has been done using a mixed mode HPLC column (Silica based column 5μm, 4.6 × 250 mm) with ICP-MS. In this work, the effect of the pH of the mobile phase containing pyridinecarboxylic acid and LiOH and the ionic strength were studied (Tomlinson et al., 1994). A detailed study was carried out examining the metal speciation and quantification of human urine. The study used a combination of NMR spectroscopy, HPLC, gas chromatography MS (GC–MS), direct flow injection mass spectrometry (DFI/LC-MS/MS) and ICP-MS on a variety of human urine samples. The combination of analytical techniques has allowed the identification of metabolites (Bouatra et al., 2013). The determination and speciation of vanadium within urine samples has been performed using many other techniques such as electrothermal AAS (Ishida

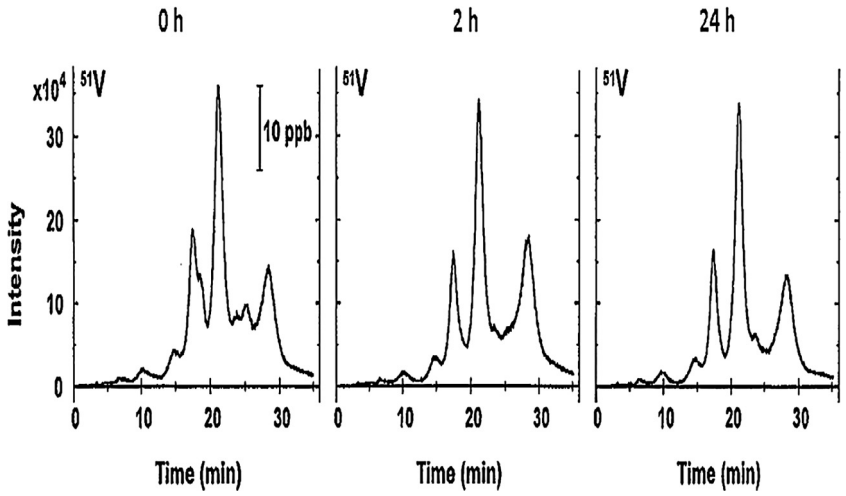

Fig. 16 A time –course in HPLC/HR-ICP-MS for the apo-hTf solution added a VCl_3 solution in the presence of bicarbonate. The V/hTf molar ratio was 1. Chromatogram are shown as a function of time. The diagram was reproduced with permission from reference (Nagaoka et al., 2002).

et al., 1989) and ICP-MS (Minnich et al., 1997). Furthermore, vanadium speciation determination by various separation methods within clinical matrices has been known. Examples of separation methods used for the identification of vanadium species include liquid chromatography (LC), ion chromatography (IC) and capillary electrophoresis (CE) (De Cremer et al., 2002; Chen and Owens, 2008).

As far as clinical matrices are concerned, it is recognized that many trace elements, including vanadium, are involved in anthropogenic activities resulting in human exposure. Therefore, more interest should be directed to characterization, speciation and quantitation of vanadium in these types of samples. As described above, studies that involves separating, characterizing and quantifying vanadium and vanadium species in clinical matrices using HPLC technique do not follow one unique method. In fact, a variety of HPLC modes, of eluent choices, of columns and of detectors have been used (Table 3).

4. Conclusion and perspectives

The speciation and analysis by HPLC of vanadium within various matrices is of great interest due to the presence of different forms of vanadium and their respective properties. However, the HPLC speciation procedures are complicated due to several factors. One of them is the possibility of redistribution of vanadium metal ions, particularly when the condition of the sample (matrix), such as acidity and redox potential, is changing during the analysis. Another critical factor is the relatively low abundance of the metal ion, as vanadium is known to be present with very low concentration in environmental, biological and clinical matrices. Most vanadium studies have used RP-HPLC methods in its various modes. Selected vanadium chelators were characterized using NP-HPLC or SEC-HPLC. Samples from different matrices have been investigated and attention was given to sample preparation, sample analysis and vanadium species' detection. However, it is important that the reader recognizes that the studies are generally investigating speciation of vanadium species by pre-treated samples distinguishing between different metal ions in the sample rather than the different forms of vanadium species in the original sample.

For an evaluation of the separation, speciation and quantitation results of the vanadium metal ion, it is very important to consider the oxidation state, the number and the type of ligands bound to the vanadium, the stability and lability of vanadium metal ion complexes. Most studies that used HPLC techniques to investigate the presence of vanadium species, have shown that the common oxidation states were found to be (IV) and (V) (Tables 1–3), yet vanadium(III) complexes were also found within a few matrices. From a stability point of view, a number of vanadium speciation studies have used pre-column reactions of vanadium with organic chelating reagents to form stable organic coordination complexes that can be separated and characterized by HPLC. Furthermore, redox potential and pH are important factors that determine the stability of vanadium compounds in various matrices. Lability is a physical characteristic that should be looked at carefully during separation, characterization and speciation studies. In fact, a number of vanadium complexes, although known by their high stability, undergo ligand exchange when administered to live animals or humans. Except for oxidation, most of the vanadium speciation studies that have used the HPLC technique did not discuss stability and lability of vanadium species. This does emphasize the need for future speciation studies in this area. Information on these factors would provide a better understanding of the chemistry and biochemistry of vanadium metal ions in environmental, biological and clinical matrices.

In summary, conducting separation, speciation and quantitation experiments for vanadium species are complicated by the existence of a number of oxidation states, by the variation in the coordination, stability and lability of vanadium complexes in general. Fortunately, the HPLC technique, which is a non-trivial technique due to its various methods and modes, has shown good results in characterizing, separating and speciation of vanadium compounds in a variety of matrices. This review was prepared to summarize the use of HPLC studies in separation, speciation and quantitation studies and to emphasize the observed metal speciation which can provide

information on the original speciation in the vanadium samples. The review therefore provides information on the interpretation of the data by pointing to the importance of vanadium coordination chemistry for understanding the biological and environmental processes that this metal is involved in.

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