# 7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring vanadium, its metabolites, and other biomarkers of exposure and effect to vanadium. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

Vanadium can be determined as the total metal, as well as in its different oxidation states (species). The various oxidation states of vanadium can interconvert between the oxidation state depending on conditions such as, oxidation-reduction potential, pH, and salinity. In natural waters, dissolved vanadium exists as vanadium(IV) or vanadium(V) and these species have different toxic properties; therefore, determination of the vanadium species present in a sample can be more important than the total vanadium content of the sample in order to best evaluate human exposure (Pyrzyńska and Wierzbicki 2004).

Analytical techniques for the determination of species of vanadium include standard atomic spectroscopic techniques and separations methods coupled with sensitive detectors. Separation methods include capillary electrophoresis (CE) and liquid chromatography (LC). Atomic spectroscopic methods used for the determination of vanadium include atomic absorption spectroscopy (AAS) with flame and graphite tube atomizers, inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), x-ray fluorescence spectrometry (XRF), and spectrophotometric methods (Chen and Owens 2008).

Sample preparation is one of the most important steps in the analysis of vanadium in biological and environmental samples. Direct analysis of vanadium species using atomic spectroscopic or separation techniques is generally not feasible due to the relatively low concentrations of vanadium found in samples as compared to other metals. In addition, the complexity of the matrices of biological and environmental samples can interfere with the determination of vanadium species, and it is often necessary to remove the matrices prior to vanadium analysis (Pyrzyńska and Wierzbicki 2004; Chen and Owens 2008).

The main methods for matrix removal are liquid-liquid extraction (LLE) and solid phase extraction (SPE). LLE is based on the distribution of the analyte between two immiscible solvents and involves the formation of an uncharged chemical species in the aqueous phase by chelation or ion-association of the vanadium ion, followed by extraction into an organic solvent. Example of complexing reagents (chelates) used to bind vanadium species include, vanadium(IV) with bis(salicylaldehyde) tetramethylethylene-diimine in a chloroform/water mixture, vanadium(V) with N-benzoyl-N-phenylhydroxylamine (BPHA) in a chloroform/water mixture, and vanadium(V) with 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropy-lamino)-phenol (5-Br-PAPS) in a xylene/water mixture. Each of these LLE steps was followed by separation using liquid chromatography with UV detection. Other complexing agents that have been studied include dibenzo-18-crown-6 and N-phenyl-(1,2-methanofullerene)-formohydroxamic acid (PMFFA) (Chen and Owens 2008; Pyrzyńska and Wierzbicki 2004).

SPE is based on the transfer of metal ions from an aqueous phase to the active sites of a solid phase. Compared to LLE, SPE is simpler and more convenient to automate. It also uses less solvent and requires fewer manipulations. Several ion-exchange resins, functionalized cellulose sorbents, and chelating resins have been studied for the selective preconcentration and separation of vanadium species. Cellulose sorbent with phosphonic acid exchange groups gives excellent enrichment of vanadium(IV) and vanadium(V) and can be simultaneously eluted using an ethylenediamine tetraacetic acid (EDTA) solution. Other solid phases used to separate and preconcentrate vanadium species include Sephadex DEAE A-25 with Eriochrome Cyanide R complexation, $C_{18}$ microcolumn or XAD-7 resin with complexation using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol or dithizone or 8-hydroxyquinoline (8-HQ), and Chelex 100 (Chen and Owens 2008; Pyrzyńska and Wierzbicki 2004).

Vanadium concentrations in biological and environmental samples are typically very low, and vanadium analysis requires powerful analytical methods. Analytical methods with sufficient sensitivity include neutron activation analysis (NAA), electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), ICP-MS, and some UV-vis spectrophotometric methods (Pyrzyńska and Wierzbicki 2004). ETAAS is routinely used for the determination of trace concentrations of vanadium. ICP-MS has better sensitivity than ETAAS; however, interference from the sample matrix can complicate the analysis. The species $^{16}O^{35}Cl^{+}$ and $^{34}S^{16}OH^{+}$ from the sample matrix can overlap with the most abundant isotope of vanadium at *m/z*=51 (Nixon et al. 2002; Pyrzyńska and Wierzbicki 2004).

Due to the low levels of vanadium that are typically found in biological and environmental samples, care must be exercised during sample handling in order to avoid contamination. Vanadium may be found in disposable steel needles, collection vials, storage containers, and chemicals and reagents (Kučera and Sabbioni 1998).

## 7.1 BIOLOGICAL MATERIALS

Methods for determination of vanadium in biological samples are summarized in Table 7-1.

NAA has been widely used to measure trace elements (including vanadium) in biological samples (Allen and Steinnes 1978; Lavi and Alfassi 1988; Martin and Chasteen 1988; Mousty et al. 1984). In NAA, the sample is bombarded with neutrons, and the element of interest is made radioactive. The amount of the element present in the sample is then determined by measurement of the radioactivity or radioactive decay products. When $^{51}V$ is bombarded with neutrons, it becomes $^{52}V$ (half-life 3.75 minutes and $\gamma$ emission of 1.433 MeV). The resultant $\gamma$ emission is detected with an efficient detector with high spectral resolution such as a well-type germanium detector combined with a multichannel analyzer. The concentration of vanadium is determined through its short-lived half-life of $^{52}V$ (Seiler 1995). Detection limits of low- to sub-ppb (µg/L) levels of vanadium in blood and urine samples have been obtained (Allen and Steinnes 1978; Lavi and Alfassi 1988; Mousty et al. 1984). The advantages of the NAA technique are its sensitivity and multi-elemental capability. The disadvantages of this technique include its high cost and the limited availability of nuclear facilities for NAA analysis (Seiler 1995).

Sabbioni et al. (1996) surveyed the literature for reports on vanadium determination in human blood, serum, and urine. Many analytical methods have been used to determine vanadium concentrations in blood, serum, and urine samples, including spectrography, colorimetry, catalytic reactions, XRF, particle induced x-ray emission (PIXE), ICP-AES, isotope dilution mass spectrometry (ID-MS), graphite furnace AAS (GF-AAS), and NAA. Only ID-MS, NAA, and GF-AAS can determine vanadium concentrations at levels of a few picograms (pg) of vanadium; GF-AAS and NAA are used most frequently (Kučera and Sabbioni 1998; Nixon et al. 2002; Sabbioni et al. 1996).

Nixon et al. (2002) reported the use of a Dynamic Reaction Cell™ ICP-MS (DRC-ICP-MS) for the analysis of vanadium in serum and urine. Generally, Zeeman graphite furnace atomic absorption spectrometry (ZGFAAS) and NAA are routinely used for the determination of vanadium in urine and serum. While ICP-MS as been routinely used to determine heavy metal concentrations in blood, serum,

## Table 7-1. Analytical Methods for Determining Vanadium in Biological Materials

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Blood/urine | Digest sample and evaporate; redissolve in acid; extract with MIBK; evaporate; redissolve in acid | NAA | ~1 µg/L (blood); 2–4 µg/L (urine) | No data | Allen and Steinnes 1978 |
| Urine | Wet ashing with $HNO_3$; chelation with cupferron; extraction into MIBK | GFAAS | 1 µg/L | 96–100% | Buchet et al. 1982 |
| Serum/urine | Digestion in $H_2SO_4/HClO_4/HNO_3$ add $KMnO_4$, sulfamic acid, and HCl; extract with BTA in benzene | ETAAS | 0.008 µg/L | 90.3% (serum); 90.8% (urine) | Ishida et al. 1989 |
| Serum | Coprecipitate sample with lead nitrate or bismuth nitrate; dry and irradiate | NAA | 0.7 µg/L | No data | Lavi and Alfassi 1988 |
| Blood | Microwave digestion with $HNO_3$ | ICP-MS | 0.0078 µg/L | No data | Lin et al. 2004 |
| Serum/urine | Dilution with 1% $HNO_3$ and addition of internal standard | DRC-ICP-MS | 0.028 µg/L | No data | Nixon et al. 2002 |
| Hair | Washing and drying of hair samples, followed by cryogenic grinding; powdered hair samples prepared as slurries in mixtures of $HNO_3$ and a slurry stabilizer | ETAAS | 0.28–0.34 µg/L | No data | Fernandes et al. 2007 |

BTA = N-benzoyl-N-(o-tolyl)hydroxylamine; DRC-ICP-MS = Dynamic Reaction Cell™ inductively coupled mass spectrometry; MIBK = methyl isobutyl ketone; NAA = neutron activation analysis

and urine, and ICP-MS quantitation is at least an order of magnitude better than ZGFAAS for elements such as arsenic, lead, selenium, and cadmium, interference from $^{16}O^{35}Cl^{+}$, which is produced in the argon plasma of the instrument, has limited the use of ICP-MS for the determination of vanadium. In this study it was found that with proper dynamic reaction cell conditions, $OCl^{+}$ interference can be eliminated. The detection limit for vanadium (0.028 µg/L) was also found to be superior to that of ZGFAAS (1.9 µg/L) (Nixon et al. 2002).

Fernandes et al. (2007) reported on a method to analyze hair samples using ETAAS. Samples were powdered using cryogenic grinding and hair slurries contained nitric acid, Triton X-100 (a nonionic surfactant), and water soluble tertiary amines. Limits of detection of 0.28 and 0.34 µg/L were reported using longitudinal heating and transversal heating graphite furnace atomizers, respectively.

## 7.2 ENVIRONMENTAL SAMPLES

Standard methods are available to measure vanadium concentrations in air, surfaces, water, soil, sediment, and plant and animal tissue (EPA 1983a, 1983b, 1983c, 1994a, 1994b, 1997a, 2003a; NIOSH 2003a, 2003b, 2003c, 2003d; OSHA 2002; USGS 1987, 1993, 1996, 1998, 2006, 2007). Atomic spectroscopic methods are generally used in these methods as well as ICP-MS and spectrophotometric methods. NIOSH Method 7504 (1994) and OSHA Method ID-185 (1991) can be used to measure vanadium oxides in air samples using XRF. Methods for determination of vanadium in environmental samples are summarized in Table 7-2.

## 7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of vanadium is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of vanadium.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean

## Table 7-2. Analytical Methods for Determining Vanadium in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Vanadium | | | | | |
| Air | Collect sample on MCE or PVC filter, followed by $HNO_3/HClO_4$ ashing | ICP-AES | 0.028 µg/filter | 98.3–103.2% (MCE) 102.5–108.3% (PVC) | NIOSH 2003a (Method 7300) |
| Air | Collect sample on MCE or PVC filter, followed by aqua regia ashing | ICP-AES | 0.028 µg/filter | 101.3–106.0% (MCE) 77.8–96.1% (PVC) | NIOSH 2003b (Method 7301) |
| Air | Collect sample on MCE filter, followed by hot block/$HCl/HNO_3$ digestion | ICP-AES | 0.003 µg/mL | No data | NIOSH 2003c (Method 7303) |
| Wipes | Wipe surface; ash wipe with $HNO_3/HClO_4$ | ICP-AES | 0.01 µg/wipe | No data | NIOSH 2003d (Method 9102) |
| Air, wipe, or bulk | Digestion of filters with $HNO_3$/ $H_2SO_4/H_2O_2$ | ICAP-AES | 1.9 µg | No data | OSHA 2002 (Method ID-125G) |
| Water | Acid solubilization | ICP-MS | 0.014 µg/L | 97–109.2% | EPA 1997a (EPA Method 200.10) |
| Water | Sample is mixed with $HNO_3$/HCl and heated | AVICP-AES | 0.2 µg/L | 93% | EPA 2003 (EPA Method 200.5) |
| Water | Acidified with $HNO_3$ | FAAS | 200 µg/L | 95–100% | EPA 1983a, 1983b (EPA Method 286.1) |
| Water | Acidified with $HNO_3$ | GFAAS | 4 µg/L | No data | EPA 1983a, 1983c (EPA Method 286.2) |
| Water | Filter and acidified samples | ICP-AES | 6 µg/L | No data | USGS 1987 (USGS Method I-1472-87) |
| Water | Filter and acidified samples | ICP-MS | 0.08 mg/L | 64–105% | USGS 1998 (USGS Method I-2477-92) |

## Table 7-2. Analytical Methods for Determining Vanadium in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Water | Treatment with an ammonium persulfate phosphoric acid reagent and gallic acid solution | Colorimetric | 1 µg/L | No data | USGS 1993 (USGS Method I-2880) |
| Water | Filter and acidified samples | ICP-OES | 5 µg/L | 98% | USGS 1998 (USGS Method I-4471-97) |
| Water (filtered) | Filtered (0.045 µm membrane); preserved with $HNO_3$ | ICP-MS | 0.05 µg/L | No data | USGS 2006 (USGS Method I-2020-05) |
| Water (unfiltered) | Preserved with $HNO_3$ followed by digestion | ICP-MS | 0.05 µg/L | No data | USGS 2006 (USGS Method I-4020-05) |
| Water/waste water/solid wastes | Digestion with nitric and hydrochloric acid | ICP-AES | 3 µg/L | 84–104% | EPA 1994a (EPA Method 200.7) |
| Water/wastes | Digestion with nitric and hydrochloric acid | ICP-MS | 2.5 µg/L | 74.9–113.4% | EPA 1994b (EPA Method 200.8) |
| Water/waste water/solid wastes | Acid digestion | ICP-AES | 5 µg/L | No data | EPA 2007 (EPA Method 6010 C) |
| Soil/sediment | Air-dried and sieved; digestion with $HNO_3$ using a closed-vessel microwave digestion procedure | ICP-MS | 0.01 µg/L | No data | USGS 2006 (USGS Method I-5020-05) |
| Animal tissue | Acid digestion | ICP-MS | 0.06 µg/g | 101% | USGS 1996 (USGS Method B-9001-95 [ICP-MS]) |
| Animal tissue | Acid digestion | ICP-AES | Not calculatable | 96% | USGS 1996 (USGS Method B-9001-95 [ICP-AES]) |

## Table 7-2. Analytical Methods for Determining Vanadium in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Biota | Digestion with $HNO_3$ using a closed-vessel microwave digestion procedure | ICP-MS | 0.01 µg/L | No data | USGS 2006 (USGS Method I-9020-05) |
| Vanadium oxides | | | | | |
| Air | Collect sample on PVC filter, dissolve filter in THF; redeposit on silver filter | XRD | 4–28 µg ($V_2O_5$), 5–62 µg ($V_2O_3$), 7–50.3 µg ($NH_4VO_3$) | No data | NIOSH 1994 (Method 7504) |
| Vanadium pentoxide | | | | | |
| Air | Collect sample on PVC filter, dissolve filter in THF; suspension is produced with the collected dust, which is transferred to silver membrane | XRD | 25 µg at 65 s | 163.4–190.2% (respirable dust); 85.9–91.1% (fine-respirable dust) | OSHA 1991 (Method ID-185) |

AVICP-AES = axially viewed inductively coupled plasma-atomic emission spectrometry; EPA = Environmental Protection Agency; FAAS = flame atomic absorption spectrometry; GFAAS : graphite furnace atomic absorption spectrometry; ICAP-AES = inductively coupled argon plasma-atomic emission spectroscopy; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; ICP-MS = inductively couples plasma-mass spectrometry; ICP-OES = inductively coupled plasma-optical emission spectroscopy; MCE = mixed cellulose ester; NIOSH = National Institute for Occupational Safety and Health; OSHA = Occupational Safety and Health Administration; PVC = polyvinyl chloride; THF = tetrahydrofuran; USGS = United States Geological Survey; XRD = X-ray diffraction

that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

### 7.3.1 Identification of Data Needs

**Methods for Determining Biomarkers of Exposure and Effect.** Sensitive and selective methods are available for the detection and quantitative measurement of vanadium after the sample matrix in which it is contained as been properly treated. Atomic spectroscopic methods used for the determination of vanadium include AAS with flame and graphite tube atomizers, ICP-OES, ICP-MS, XRF, and spectrophotometric methods (Chen and Owens 2008). No data needs are identified.

***Exposure.*** Methods exist to determine vanadium levels in environmental samples and human tissues. While several biomarkers of exposure have been indentified, none of them can be used to quantitatively determine exposure levels (Rydzynski 2001). Kučera et al. (1998) reported that blood and urinary vanadium levels are considered the most reliable indicators of occupational exposure to vanadium. No data needs are identified.

***Effect.*** No well-documented biomarkers of effect specific for vanadium have been report (Rydzynski 2001). The primary effects of exposure to vanadium dusts are coughing, wheezing, and other respiratory difficulties; however, these effects are not specific to vanadium and can be found following inhalation of many types of dusts (Rydzynski 2001). No data needs are identified.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** Methods for determining vanadium in water, air, and waste samples with adequate selectivity and sensitivity are well developed and undergoing constant improvement. No data needs are identified.

### 7.3.2 Ongoing Studies

No ongoing studies regarding methods for measuring vanadium in biological and environmental samples were located.