University of Alberta

Vanadium Speciation in Samples Relevant to the Athabasca Oil Sands Region

by

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Abstract

The most prevalent oxidation states of vanadium in nature are V(IV) and V(V). Toxicity of vanadium increases with increasing oxidation state. A high-performance-liquid-chromatography inductively-coupled-plasma mass-spectrometry method was optimized for the speciation of V(IV) and V(V). It was applied to the investigation of vanadium leaching from petroleum coke into oil sands process water. Both V(IV) and V(V) leached from the coke into the water but the V(IV) rapidly oxidized to V(V) in the absence of ethylenediaminetetraacetic acid (EDTA). A species preservation method using EDTA was developed that stabilized the species distribution and concentration for 56 days. The method was applied to a 7-day exposure study of V(IV) and V(V) on *Hyalella azteca*. Tissue extract contained V(IV), V(V), and an unknown vanadium species. V(IV) oxidized in the test water, so the animals were mostly exposed to V(V). Speciation provides essential insight and information on vanadium present in samples.

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Abbreviations

AAS	atomic absorption spectroscopy
ACN	acetonitrile
AOS	Athabasca Oil Sands
ARS	anion-exchange resin suspension
ATSDR	Agency for Toxic Substances and Disease Registry
CAB	chromazurol B (2,6-dichloro-4'-hydroxy-3,3'-dimethylfuchsone-5,5'-dicarboxylic acid, disodium salt)
CE	collision energy
CES	collision energy spread
CXA	N-cinnamoyl-N-2,3-xylylhydroxylamine
DIW	deionized water
DP	declustering potential
EDTA	ethylenediaminetetraacetic acid
EMS	enhanced mass spectra scan
EPA	Environmental Protection Agency
EPI	enhanced product ion
ESI-MS	electrospray ionization mass spectrometry
ESR	electron spin resonance
ETAAS	electrothermal atomic absorption spectroscopy
GF-AAS	graphite furnace atomic absorption spectroscopy
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high performance liquid chromatography
HPLC-ICP-MS	high performance liquid chromatography inductively coupled plasma mass spectrometry
i.p.	intraperitoneal injection
ICP-AES	inductively coupled plasma atomic emission spectroscopy

ICP-DRC-MS	inductively coupled plasma dynamic reaction cell mass spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
IS	ionspray voltage
LC	liquid chromatography
LC50	lethal concentration 50
LD50	lethal dose 50
LiOH	lithium hydroxide
LOD	limit of detection
LOQ	limit of quantitation
MeOH	methanol
MS	mass spectrometry
Na_2EDTA	ethylenediaminetetraacetic acid disodium salt
Na_4EDTA	ethylenediaminetetraacetic acid tetrasodium salt
NIDDM	non-insulin-dependent diabetes mellitus
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NOLC	no observed lethal concentration
OSHA	Occupational Safety and Health Administration
OSPW	oil sands process water
PEL	permissible exposure limit
PET	polyethylene terephthalate
REL	recommended exposure limit
RM	reference material
RT	room temperature
SAX	strong anion exchange
SCO	synthetic crude oil
SPE	solid phase extraction
SRM	standard reference material

TBAOH	tetrabutylammonium hydroxide
TBAP	tetrabutylammonium phosphate
TOC	total organic carbon
UV-Vis	Ultra-violet/visible
WHO	World Health Organization

Chapter 1. Introduction

1.1. VANADIUM BACKGROUND

The discovery of vanadium has all the intrigues of a science drama, from its first discovery in North America, to a letter lost at sea during a trans-Atlantic voyage, rescinded scientific achievement and its rediscovery on the old continent.

Vanadium was originally discovered by Manuel del Rio in Mexico in 1801. He first named it panchromium for the diversity of colours it exhibited but later renamed it erythronium due to the red compounds formed during its heating. The samples were sent to France with Alexander von Humbolt for further analysis at the *Institut de France*. Unfortunately, the accompanying explanatory letter describing del Rio's methods and conclusions was lost during a shipwreck [1].

Based on experiments performed by Collet-Descostils on the samples [2] and under the influence of von Humbolt, del Rio rescinded his claim on the discovery of a new element. The vanadium containing samples were believed to be impure chromium.

Decades later, in 1831, Swedish scientist Nils Gabriel Sefström was studying ores from the Taberg mine in Småland, Sweden. He rediscovered element 23, naming it after Vanadis, the nickname for Freya, the Norse goddess of beauty and fertility [3].

1.2. GENERAL USES AND OCCURRENCE OF VANADIUM

Since its first discovery, vanadium has had a multitude of uses. Europium-doped yttrium vanadate was used in the tubes of colour televisions [4]. Vanadium is an alloy additive in the manufacturing of tool steels and special steels, as it increases strength, and a catalyst in the synthesis of sulfuric acid [4-7].

The concentration of vanadium in the earth's crust is around 100 µg/g [6]. There are over 70 different vanadium containing minerals [8]. They are found in Canada (wakefieldite), Gabon (curienite, metavanuralite and vanuralite), Germany (pucherite), Kazakhstan (kazakhstanite), El Salvador (lyonsite and fingerite), Mexico (vanadinite) and the United States (carnotite) to name but a few [8]. Crude oil has a large vanadium concentration range, from 3 µg/g for crude from Qatar to 846 µg/g for Venezuelan crude [6, 9].

Certain animal species contain high vanadium concentrations. The most notable are the ascidians (sea squirts) where concentrations as high as 350 mM were found in the blood cells of *Ascidia gemmata* [10]. These concentrations are 10^7 times higher than the vanadium in the surrounding water [10] (32.7 nM and 36.4 nM for surface waters and deep waters of the Pacific, respectfully [11]).

In ascidians, the concentration varies from 25 to 9000 µg/g dry weight [12]. By comparison, in benthic invertebrates other than ascidians, the concentration in non-contaminated areas ranges from 0.2 to 4.5 µg/g dry weight [12]. Higher concentrations have been found in fan worms (*Pseudopotamilla occelata*) off the coast of Japan [13]. The concentration in whole body was 510 ± 330 µg/g dry

weight with lower concentrations in the trunk body (60 ± 25 µg/g) and higher concentrations in the branchial crown (5500 ± 1800 µg/g). Samples from a contaminated site can contain higher levels of vanadium. Feather duster tube worms *(Eudistylia vancouveri)* collected near a storm water drainage sewer of North Vancouver, British Columbia, Canada, contained 786.1 µg/g dry weight [14]. In Tuktoyaktuk Harbour, Northwest Territories, Canada, Arctic propeller clam (*Cyrtodaria kurriana*) tissue samples contained 5 times more vanadium than tissue from clams collected at a control site (McKinley Bay, 60 km away) [15]. The concentrations were 20.1 and 4.3 µg/g dry weight, respectively.

The mushroom fly agaric (*Amanita muscaria*) was first identified to contain high levels of vanadium (3.3 mg/kg) in 1931 [16]. Amavadin, the first vanadium compound isolated from plant material, is responsible for the elevated vanadium concentration [17, 18]. Samples collected in Finland and Sweden of *Amanita regalis*, a mushroom closely related to *A. muscaria*, had whole body vanadium concentrations ranging from 38 to 169 mg/kg dry weight [19]. Most species of mushrooms contain less than 2 mg/kg dry weight [20]. Hornwort (*Ceratophyllum demersum*), pondweed (*Potamogeton praelongus*), and yellow water lilly (*Nuphar advena*), collected from a pond and a lake in Connecticut, USA, had 0.44 ppm, 0.4 ppm and 3.8 ppm V dry weight, respectively [21]. Vanadium was not detected in 20-fold concentrated water.

1.3. MEDICINAL USES

Medicinal applications of vanadium have been numerous. In 1899, Lyonnet et al. [22] tested the effects of sodium metavanadate on 44 patients, including 3

diabetic patients, in Lyon, France. They noticed an increase in appetite for most of the patients, thus suggesting it be used as a treatment for loss of appetite. In 2 out of the 3 diabetic patients, there was a decrease in the sugar levels.

In 1979, Tolman et al. [23] observed the effects of sodium orthovanadate, sodium metavanadate, ammonium metavanadate and vanadyl sulfate on the metabolism of glucose in rat adipocytes, hepatocytes, diaphragm, and intestine. They found increased sugar utilization in the *in vitro* systems studied. Work on the insulin-mimetic properties of vanadium developed thereafter with the first Phase I clinical trial starting in 2000 for bis(ethylmaltolato)oxovanadium(IV) (BEOV) [24]. The Phase IIa trial completion was announced in 2008 [25].

The use of vanadium in the treatment of cancer has also been investigated. Vanadyl (IV) sulfate was used as a dietary supplement and reduced the incidence of cancer in female rats while stimulating their appetite [26]. METVAN (bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV)) showed antitumor activity in mice grafted with human breast cancer as well as delaying tumor progression [27]. Other potential medical applications of vanadium compounds include vanadocene dithiocarbamate as a spermicide, since it reduces sperm motility [28, 29], and oxovanadium(IV) porphyrins as anti-HIV agents due to anti-viral activity [30].

Concern has been raised about the long term effects of using vanadium compounds to treat patients [31]. Its toxicity and its potential accumulation in tissues, such as bone and kidney, are the main causes for concern [32]. Rats fed vanadyl sulphate in their drinking water for a year still had 10 to 60% of the

pre-withdrawal vanadium concentration in various organs (bone, kidney, testis, liver, pancreas and brain) 16 weeks after withdrawal [33]. In diabetic patients given vanadyl sulphate orally for 3 weeks, the vanadium concentration in their plasma 2 weeks after the end of treatment was 13% of the concentration observed during treatment [34]. The pre-treatment vanadium concentrations were not detectable. The debate regarding the use of vanadium compounds as drugs will continue until these issues have been resolved.

1.4. TOXICOLOGY

1.4.1. Human Toxicology

Occupational exposure arises mainly from boiler cleaning and maintenance, and industrial production and use of vanadium [4, 35]. Wyers [36] found that workers exposed to vanadium pentoxide dust showed signs of pale skin, a greenish-black tongue discoloration, cough, finger and arm tremors, and chest pains among possible symptoms. Cleaning would not remove the green-black substance on the tongue, possibly formed by the reduction from V(V) to V(III) by bacteria and ptyalin in the mouth. When exposure to vanadium ceased, the discolouration of the tongue disappeared within two to three days.

During an oil-to-coal conversion of a power plant in Massachusetts, USA, 74 boilermakers were exposed to levels of vanadium pentoxide fumes that ranged from 0.05 to 5.3 mg/m^3 [37]. The symptoms developed by more than 70% of the workers were a cough with mucus, a sore throat, and shortness of breath upon exertion. Other symptoms commonly experienced were chest pain, headaches,

runny nose, wheezing, tiredness, and a cough without mucus. The average time between the start of work and the first appearance of the symptoms was 7 days. Symptoms stabilized, improved or disappeared after work had stopped.

At a newly opened vanadium pentoxide refinery in Western Australia, Australia, four workers developed typical symptoms of acute vanadium poisoning [38]. All workers developed green tongue, wheezing, and shortness of breath, within either hours or days of exposure. One worker was exposed to a large amount of vanadium dust while shoveling ammonium vanadate for six hours. He developed a headache, epiphora (tears), dry mouth and a green discoloration of the tongue after 2 hours. Despite wearing industrial gloves, the skin of his fingers was green. The skin of his scrotum and upper legs were also green. Three days after exposure, new symptoms arose: wheezing, shortness of breath, and a cough with green mucus. The respiratory difficulties lasted about a month and he was asymptomatic after six weeks. A second employee working in the deammoniation shed developed green tongue on the first day. Over the course of two weeks, he had a cough, stuffy nose, sore throat, hoarse voice, as well as shortness of breath upon exertion. He improved once he was removed from that work environment. While a third worker shoveled dry vanadium pentoxide for two to three hours, his tongue became green. The other symptoms appeared the following morning.

A small study was performed on Japanese workers using vanadium pentoxide to dye metal surfaces yellow. Two workers had direct exposure to vanadium and 13 workers had indirect or no exposure to vanadium. The worker exposed to 0.1 mg/m^3 for less than 30 minutes daily had a green tongue [39]. His co-workers,

exposed to significantly lower levels, did not show any discolouration of the tongue.

Zenz and Berg [40] exposed nine healthy volunteers, aged 27 to 44, to vanadium pentoxide dust for 8 hours under constant temperature and humidity. They attempted to determine the response of the human respiratory system to vanadium pentoxide dust. During the first test, two volunteers were unintentionally exposed to 1 mg/m^3 V_2O_5 dust rather than the expected 0.5 mg/m^3. After five hours, sporadic coughing developed which later turned into 8 days of persistent coughing. No other symptoms developed. Three weeks later, the two volunteers were exposed for 5 minutes to a heavy cloud of vanadium pentoxide. Coughing developed within 16 hours and lasted about a week. Due to these high concentration exposures, the following tests used lower concentrations of V_2O_5 dust. Five volunteers exposed to 0.2 mg/m^3 for eight hours had a loose cough the next morning that stopped a week to 10 days later. The volunteers who breathed in 0.1 mg/m^3 for eight hours showed no symptoms until 24 hours later when mucus formed accompanied by slight coughing that lasted no more than 4 days. No further testing was performed due to the reaction of the initial volunteers upon reexposure. As all available volunteers had been exposed at least once to vanadium pentoxide dust, the researchers thought better of exposing them again. They were therefore unable to determine the effects of reexposure or the concentration of vanadium dust that would not cause a response.

Lewis [41] studied twenty-four workers who milled and handled vanadium in two different plants in Colorado and Ohio, USA. They had worked with vanadium for

2.5 years on average, with a minimum of six months. He compared them to a control group of forty-five men from the same towns as the exposed group with similar socio-economic status and work. The vanadium concentration in the air at the plants ranged from 0.1 to 0.9 mg/m^3 (as V_2O_5). The symptoms present in significantly higher levels in the exposed group than in the control group were coughing, sputum, wheezing, and eye, nose and throat irritation. Of the exposed workers, 37% had a green tongue. Lewis stated that “no evidence was found of chronic intoxication or injury attributable to vanadium exposure” [41].

Ingestion of vanadium has also been studied in humans. In a study conducted by Dimond et al. [42], healthy humans were fed tablets containing 25 mg of ammonium vanadyl tartrate with a meal one to 4 times a day for 45 to 94 days. The subjects ate normal diets and were ambulatory. The daily tolerable dosage ranged from 50 to 100 mg depending on the patient. Cramping and diarrhea limited the administration of higher doses to the patients. No other toxic effects were observed.

In another study by Cohen et al. [34], six subjects with non-insulin-dependent diabetes mellitus (NIDDM) were given a capsule containing 50 mg of vanadyl sulfate twice daily (100 mg/day) for 3 weeks. Side-effects included mild gastrointestinal symptoms: nausea, mild diarrhea and abdominal cramps.

1.4.2. Animal Toxicology

Studies have been performed to determine the toxicity of different vanadium compounds on animal models for over a century. In 1899, Laran [43] investigated the effects of vanadic acid on dogs. He found the lethal dose for samples injected

into the saphenous vein was 8 mg/kg. A dose of 1.5 mg/kg produces the first signs of intoxication. Lyonnet et al. [22] investigated the toxicity of sodium metavanadate on rabbits, dogs, guinea pigs, and frogs before using it on humans. The animals usually died with a violent dyspnea accompanied with either convulsions or hypothermia. In 1938, Daniel and Lillie [44] looked at vanadium poisoning in white rats. The rats were fed different concentrations of sodium metavanadate *ad libitum*. They found acute poisoning symptoms included intense distress, diarrhea, labored respiration, and convulsions followed by death.

After vanadium toxicity was suspected as the cause of a die-off of Canada geese at a Delaware refinery fly ash pond, Rattner et al. [45] looked into the effects of vanadium pentoxide and sodium metavanadate on mallard drakes (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*). For acute oral toxicity testing, they fed the mallard drakes gelatin capsules containing vanadium pentoxide or sodium metavanadate. Eight concentrations (10 to 700 mg/kg body weight) plus the control were tested with 4 animals per concentration. The birds were observed for 7 days. A similar test was performed on 14 male Canada geese testing only sodium metavanadate. The concentrations ranged from 18 to 151 mg/kg body weight. Chronic feeding studies were performed on mallard drakes. Sodium metavanadate was mixed into the food and given *ad libitum*. The concentration was increased weekly over a period of 10 weeks. They determined the lethal dose 50 (LD50) of vanadium pentoxide and sodium metavanadate to be 113 and 75.5 mg/kg, respectively, for the mallards. For the male Canada geese, the LD50 was 37.2 mg/kg for sodium metavanadate. For the chronic exposure, they observed an accumulation of vanadium in the liver and kidney.

White and Dieter [46] fed mallards vanadyl sulfate dissolved in propylene glycol mixed in with the feed *ad libitum* during a 12 week study. The control diet contained propylene glycol. The vanadium concentrations were 1, 10, and 100 ppm (wet weight). They tested the concentration of vanadium in various body parts (blood, brain, fat, kidney, liver and femur) and found the highest concentrations were in the bone (274 ± 47 ppb for males and 3327 ± 2208 ppb for the females, wet weight) and liver (657 ± 113 ppb, wet weight). They noticed that the females accumulated 5 times more vanadium than the males in the 100 ppm group, but the difference in accumulation was only in the femur. One hen from that group accumulated 11 times more than the males.

Llobet and Domingo [47] determined the LD50 (14 days) in mice and rats for sodium metavanadate and vanadyl sulfate pentahydrate given orally and i.p. (intraperitoneal injection). A single dose of vanadium was given and the effects were observed over 14 days. For each administration method, five doses were tested. The ranges are shown in Table 1.1. Each group contained 10 animals. The vanadium compounds were dissolved in 10 mM Tris-HCl-NaCl buffer. Control animals were given the buffer.

Table 1.1. Dose ranges for administered vanadium

Vanadium compound	$NaVO_3$ (mg/kg)		$VOSO_4 \cdot 5H_2O$ (mg/kg)	
	Oral administration	i.p. administration	Oral administration	i.p. administration
Rats	39-256	7-34	296-845	50-143
Mice	41-157	18-91	186-714	45-178

From Llobet and Domingo [47]

LD50 values are summarized in Table 1.2. In general, the toxicity of V(V) is greater than the toxicity of V(IV) for both rats and mice by both methods. For oral administration, most deaths occurred in the first 48 hours. No animals died after 7 days. For i.p. administration, most deaths occurred in the first 24 hours with no deaths after 48 hours. The effects of the highest concentration of both vanadium species included irregular breathing, increased cardiac rhythm, and ataxia. The rats suffered from diarrhea. Overall, there was a decrease in locomotor activity, paralysis of the hind legs, and a decreased sensitivity to pain for the first week after vanadium administration.

Table 1.2. Acute toxicity of vanadium compounds in rats and mice

Vanadium compound	LD50 (14 days) of $NaVO_3$ (mg/kg), (mg V/kg)		LD50 (14 days) of $VOSO_4 \cdot 5H_2O$ (mg/kg), (mg V/kg)	
	Oral administration	i.p. administration	Oral administration	i.p. administration
Rats	98.0, 41.0	18.4, 7.7	448.0, 90.3	74.1, 14.9
Mice	74.6, 31.2	35.9, 15.0	467.2, 94.2	113.0, 22.8

From Llobet and Domingo [47]

A more complete list of vanadium toxicity studies for oral exposure (acute, intermediate, and chronic) in mice and rats as well as intermediate exposure in humans can be found in the Toxicological Profile for Vanadium prepared by the Agency for Toxic Substances and Disease Registry (ATSDR) [48].

Toxicity studies have also been performed on aquatic organisms. Holdway and Sprague [49] used vanadium pentoxide to test chronic toxicity in American flagfish (*Jordanella floridae*). The effect on reproduction and second generation larvae, as well as on mortality of first generation larvae and older fish were determined. Up to the maximum concentration tested (1.5 mg/L), there were no harmful effects on the average daily egg production. There were, however, some "slow developers" in the second generation as well as a few fry with abnormal spine curvature in the two highest concentrations tested (0.48 and 1.5 mg/L). The lethal concentration 50 (LC50) (28 days) was 1.13 mg/L for the larvae while the LC50 (96 hours) for the adult fish was 11.2 mg/L.

Stendahl and Sprague continued this work by looking at the effect of water hardness and pH on the toxicity of vanadium to juvenile rainbow trout (*Salmo gairdneri R.*) [50], a fish native to the Athabasca river drainage basin [51]. They used vanadium pentoxide in the experiments. The water hardness tested was 30 to 355 mg/L. The pH range tested was 5.5 to 8.8. The LC50 (7 days) ranged from 1.9 to 6.0 mg/L. Hardness was determined not to be a major factor and the pH had a small consistent effect with pH 7.7 being the most toxic and pH 5.5 being the least toxic.

Exposure to vanadium, as sodium metavanadate, was tested on zebrafish (*Brachydanio rerio*) (LC50, 7 days, 2 to 3 mg V/L), guppies (P*oecilia reticulata*) (LC50, 7 days, 3.3 mg V/L) and daphnids (*Daphnia magna*) (LC50, 48h, 3.4 to 4.8 mg V/L) [52]. The effect of vanadium on the daphnids life-cycle was also investigated during a 23 days exposure test. The reproduction of the daphnids was not inhibited for concentrations up to 1.6 mg V/L. Perez-Benito [53] looked at the effect of sodium metavanadate on the lifespan of guppies (*Poecilia reticulata*) and he found the LC50 (7 days) was 3.84×10^{-5} M (2.0 mg/L).

1.4.3. Vanadium Guidelines

Guidelines exist to protect workers, the general population, and livestock from the possible hazards of exposure to vanadium. The World Health Organization (WHO) air quality guidelines for Europe indicate that 1 $\mu g/m^3$ averaged over 24 hours would likely not cause adverse health effects [54]. The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for workers in general industry is 0.5 mg/m^3 for dust (as V_2O_5) and 0.1 mg/m^3 for

fumes (as V_2O_5) [55]. The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) is 0.05 mg/m^3 for both vanadium dust and fumes (as V) [56].

Currently, there are no Canadian water quality guidelines that exist for the protection of aquatic life [57]. The Canadian water quality guideline for the protection of agriculture (irrigation and livestock) is set at 100 µg/L [57]. There are no WHO [58] or Environmental Protection Agency (EPA) drinking water guidelines although vanadium is listed in the EPA's Drinking Water Contaminant Candidate List 3 (CCL 3) [59].

1.5. VANADIUM SPECIES

Vanadium chemistry is rich and varied. It has many oxidation states: -1, 0, +2, +3, +4, +5 [4, 6]. The most common ones found in nature are +3, +4, and +5 [7, 35]. Vanadium-containing minerals reflect this range of oxidation states. Erlianite contains V(III), duttonite, V(IV), V(V) is found in navajoite, franciscanite has mixed oxidation states with V(III) and V(V), while both V(IV) and V(V) are present in hendersonite [6-8]. In natural water pH ranges, V(V) is the most mobile form and V(III) and V(IV) leaching from minerals will be oxidized to V(V) [7].

1.5.1. Vanadium(IV)

The vanadyl ion, VO^{2+}, dominates V(IV) chemistry [10]. Below pH 3, it is hydrated and forms $VO(H_2O)_5^{2+}$ which is air stable in acidic conditions [60]. As the pH is increased, oligomeric and polymeric species form, some of which are insoluble.

$VOOH^+$ and $(VOOH)_2^{2+}$ form above pH 4. At neutral pH, a precipitate of $VO(OH)_2$ forms. The precipitate slowly dissolves as the pH is increased above 11 to form $VO(OH)_3^-$ [7]. However, the presence of ligands containing oxygen, nitrogen or sulfur can prevent the formation of the polymeric precipitates [60].

V(IV) was found in the vanadocytes, vanadium containing blood cells, of *Ascidia gemmata* [61]. One of the most stable V(IV) complexes known is amavadin, the vanadium complex present in mushrooms [20, 62]. V(IV) is the main oxidation state in oil. It exists as mainly as a porphyrin [7]. Vanadyl porphyrins have been found in meteorites and Precambrian shale [63].

1.5.2. Vanadium(V)

The chemistry of V(V) is pH sensitive. VO_2^+ is the cation formed at low pH [7]. At near-neutral pH and for concentrations below 1 mM, V(V) is present mainly as $H_2VO_4^-$ [60, 64]. $H_2VO_4^-$ and HVO_4^{2-} dominate in freshwater and seawater [7]. Various oligomers form for V(V), especially at concentrations above 1 mM. From pH 3 to 6, decavanadate, $V_{10}O_{28}^{6-}$, is the main oligomer and forms yellow-orange solutions [60]. A dimer ($H_3V_2O_7^-$), a cyclic tetramer ($V_4O_{12}^{4-}$) and a cyclic pentamer ($V_5O_{15}^-$) are formed at pH 6 to 10 [7, 60]. The V(V) species range based on concentration and pH can be seen in Figure 1.1 [10].

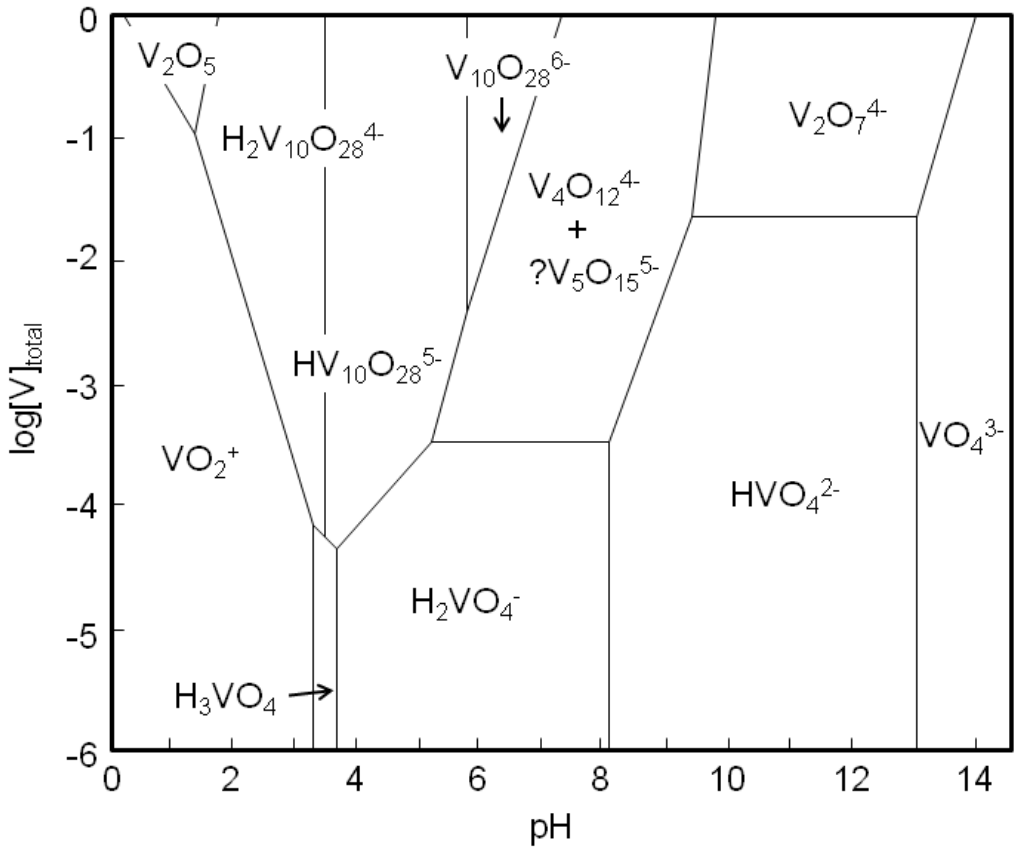

Figure 1.1. V(V) species distribution in water (Redrawn from Michibata and Kanamori [10], originally from Pope [65])

Several algae and lichen have been shown to have a V(V) containing enzyme, bromoperoxidase. *Xanthoria parietina*, a lichen found in the Netherlands [66], and the red algae *Corallina officinalis* [67] are such examples. The bacterium *Shewanella putrefaciens CN32* can reduce V(V) to both V(IV) and V(III) [68].

1.6. VANADIUM SPECIATION METHODS

The sample matrices for which vanadium speciation has been performed are diverse: from volcanic waters [69] to fish tissue [70]. The speciation methods are just as varied. Separation methods include liquid chromatography (LC) [71-73] and solid phase extraction (SPE) [74, 75]. The detection methods cover ultra-violet/visible absorption (UV-Vis) [71, 72], atomic absorption spectroscopy (AAS) [69, 74, 76, 77], and mass spectrometry (MS) [78-81]. Complexing agents are often used in the separation methods to prevent species interconversion. Ethylenediaminetetraacetic acid (EDTA) is commonly used for vanadium speciation [70, 71, 80, 82]. Others include 2,6-pyridinecarboxylic acid [73] and Chromazurol B [74].

Komarova et al. [71] showed that V(IV) and V(V) could be separated by ion chromatography as EDTA complexes. They used a UV detector with a limit of detection of 0.2 and 1.0 mg/L for V(IV) and V(V) respectively.

Jen et al. [72] separated V-EDTA complexes by liquid chromatography with a UV-detector. They noted that EDTA was required in the eluent to maintain the stability of the V(V)-EDTA complex. They applied their technique to a leachate sample from an oil-refining waste site. The results were comparable to those obtained by AAS.

Groups using SPE for vanadium speciation started publishing their work in the early 2000s. Vanadium was adsorbed on a column and then eluted using different mobile phases. Minelli et al. [69] looked at the speciation of vanadium in

Italian volcanic waters. The vanadium was trapped on a strong anion exchange (SAX) column loaded with EDTA. V(IV) was eluted with a solution of tetrabutylammonium hydroxide (TBAOH), isopropanol and Na_2EDTA with analysis performed by electrothermal atomic absorption spectroscopy (ETAAS). The total vanadium concentration was determined by ETAAS and the difference was attributed to V(V).

Nukatsuka et al. [74] developed an SPE-ETAAS method for speciation of vanadium in seawater samples. For the determination of vanadium, the water sample was mixed with an anion-exchange resin suspension (ARS). Chromazurol B (2,6-dichloro-4'-hydroxy-3,3'-dimethylfuchsone-5,5'-dicarboxylic acid, disodium salt, CAB) was added to form the V(IV)-CAB complex. The anion-exchange resin was then collected and subjected to several preparation steps before analysis by ETAAS. For V(V), a V(V)-CXA (N-cinnamoyl-N-2,3-xylylhydroxylamine) complex was formed in the ARS and analyzed the same way as the V(IV) complex. For the total analysis, V(V) was first reduced by ascorbic acid then the procedure for analysis of V(IV) was followed. They preferred using ETAAS versus inductively coupled plasma mass spectrometry (ICP-MS) as the instrumentation is less expensive; however, they performed the analysis of the samples in a clean room and used labour intensive sample preparation methods. The detection limit was 0.02 ng/mL for a sample of 40 mL.

Wang and Sañudo-Wilhelmy [75] used a Chelex 100 resin to adsorb V(IV) and V(V), then selectively eluted the vanadium species under a nitrogen atmosphere to avoid any interconversion. The eluents were dried, dissolved in HNO_3 and analyzed by graphite furnace-AAS (GF-AAS). They proceeded to use this

method on coastal seawater samples from the Peconic River Estuary and the Long Island Sound.

Other methods were also developed. De Cremer et al. [78] applied a size-exclusion method to separate bound V(V) from unbound V(V) in rat spleen homogenate. They used a Superose 12 HR 10/30 gel filtration column. The buffer consisted of 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 0.15 NaCl at a pH of 7.5. Building on work by De Cremer et al., Chéry et al. [79] developed a size-exclusion inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) method for analysis of vanadium species in human serum. The goal was to use it for analysis of vanadium complexes with insulin-like properties. Ytterbium (Y^{89}) was used as an internal standard. The detection limit was 40 ng/L. The use of a high salt concentration decreased the sensitivity of the instrument over the course of a few weeks but was reestablished with a simple cleaning procedure. The analysis time was 1 hour.

Mandiwana and Panichev [76] also used ETAAS for vanadium speciation in acacia plant leaves (*Acacia xathophloea*) and grass (*Chloris gayana* and *Digitaria eriantha*) collected near a vanadium mine in South Africa. V(V) was leached from the plant matter in 1 M $(NH_4)_2HPO_4$, then analyzed by ETAAS. V(IV) was determined by ashing the residue left after the extraction of V(V). The ash was dissolved in acid and diluted for analysis by AAS. Total vanadium was determined by the ashing of plant material. The detection limit for V(V) was 0.3 µg/L for a 10 mL sample or in the plant material, it was 0.02 µg/g. They also

expanded their work to the analysis of soil samples [77]. The V(V) extraction solution for plants was replaced with 0.1 M Na_2CO_3.

In the last few years, many methods using HPLC-ICP-MS for vanadium speciation have been developed. ICP-MS has been used for the analysis of a myriad of elements in different sample types. Mercury in single human hair strands [83, 84], arsenic in groundwater [85], nickel and vanadium in crude oils [86], trace elements (Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sr, Tl, V, Zn) in human milk [87], and transition elements (Sc, V, Cr, Mb, Fe, Co, Ni, Cu, Zn) in tomato leaves, oyster tissue and rocks [88] are but some examples. Multiple sample introduction systems can be coupled with an ICP-MS such as laser ablation [83], gas chromatography [89, 90], and liquid chromatography [91]. Table 1.3 summarizes the different HPLC-ICP-MS

Table 1.3. HPLC-ICP-MS methods for vanadium speciation

First author	Tomlinson [73]	Wann [80]	Liu [81]	Colina [70]	Kuo [92]	Chen [93]	Li [68]	Aureli [82]
Year	1994	1997	2002	2005	2007	2007	2007	2008
Column								
Type	mixed mode HPLC-CS5	silica based CRC8 reversed phase	silica based CRC8 reversed phase	HICHROM C-8	reversed phase C8	porous polymethacrylate resin	strong anion exchange	anion exchange
Dimensions (length x I.D. x particle size)	250 mm x 4 mm x 13 µm	30 mm x 3 mm x 3 µm	30 mm x 3 mm x 3 µm	150 mm x 4.6 mm	30 mm x 3 mm x 3 µm	150 mm x 4.6 mm x 10 µm	50 mm x 4.1 mm x 3 µm	50 mm x 4 mm
Injection volume (µL)	50	100	100	50	200	50	20	50
Mobile Phase								
[EDTA] (mM)	-	3	4	2.5	none, then 5	5	2	5
Other mobile phase components	6 mM 2,6-pyridine-carboxylic acid, 8.6 mM LiOH	12% MeOH 0.5 mM TBAP	10% MeOH 0.2 mM TBAP	0.06 M CH_3COONH_4, 10 mM TBAOH, 10 mM $(NH_4)_2HPO_4$	4% MeOH 0.5 mM TBAP	30 mM $(NH_4)_2HPO_4$	3% acetonitrile, 80 mM NH_4HCO_3	4 mM $NaHCO_3$/ Na_2CO_3
pH	3.6	6.5	6.0	6.0	6.85	8.0	6.0	7.1
Flow rate (mL/min)	1.5 then 3	1.2	1.2	1.2	1.0	1.0	1.0	1.2
Isocratic	yes	yes	yes	yes	no	yes	yes	yes
Run time (min)	18	4	3	10	8	15	5	6
Temperature (°C)	25	RT	RT	-	-	-	~22	23
Pre-column complexation	no	-	-	yes	-	no	yes	yes
Limit of Detection[a]								
V(IV) (µg/L)	2.3	0.025	0.007	59.1	0.06	0.5	0.7	0.16
V(V) (µg/L)	0.48	0.041	0.013	113.1	0.06	1	1	0.025
Samples Analyzed								
	Urine reference material (RM)	Seawater and river water RM	Estuarine and river water RM, tap and pond water	Sediment, mussel, fish muscle tissue	Soil, leaves (RM and real samples)	Waste water	Coke pore water, supernatant of bacteria incubated with V(V)	Bottled natural mineral water

a) Limits of detection were calculated as three times the standard deviation of the background from the calibration curve, except for Colina [70] who did not specify how it was calculated

Tomlinson et al. [73] performed their experiments with a metal free HPLC system which can be costly. The chelating agent, 2,6-pyridinecarboxylic acid, required the aid of LiOH in the dissolution of the complex. They did not perform any pre-column complexation and found that their standards degraded unless stored in the fridge immediately after preparation. The separation took 18 minutes.

Wann and Jiang [80] improved the sensitivity of their method by using ultrasonic nebulization. They also developed a method that only took 4 minutes to separate the vanadium species.

Liu and Jiang [81] improved upon that method by using ICP-DRC- MS thus reducing spectroscopic interferences. The sensitivity of the method was improved and the detection limit was lowered.

Colina et al. [70] used pre-column complexation with EDTA. They extracted the vanadium from lyophilized samples in a 2.5 mM EDTA solution. They also observed that in the absence of EDTA in the mobile phase, only the V(IV) peak was present. They found the use of acetonitrile caused peak tailing and preferred not to use any organic solvents in their mobile phase to improve their sensitivity. The detection limit is the highest for the LC methods presented. They were therefore unable to perform speciation on water samples collected from the same area as the sediment and tissue samples.

Kuo et al. [92] expanded the work done by Wann [80] and Liu [81] by applying the technique to the determination of vanadium and chromium in soil and plant material. The organic content of the mobile phase was reduced. They found that

in order to resolve the different species, EDTA must be present in the mobile phase.

Chen et al. [93] used a phosphate buffer which is known to cause clogging problems with some ICP-MS systems. The detection limit was higher than other methods. They were the first to publish electrospray ionization mass spectrometry data regarding the V-EDTA complexes (discussed in Section 2.3.).

The detection limit for the Li et al. [68] method was comparable to Chen's [93] but the separation was three times faster. The sample volume used was also the smallest of all the methods at 20 µL. V(III) speciation was also performed using this method and the detection limit was 0.6 µg/L. Excess Na_4EDTA was added to the samples and allowed to complex for 20 minutes.

Aureli et al. [82] used 25 mM EDTA and a 30 minute complexation time to stabilize the vanadium species between the time the water bottle was opened and the analysis was performed. The EDTA complexes were stable for one week when stored at room temperature.

1.7. ATHABASCA OIL SANDS

The Athabasca Oil Sands, located in Northern Alberta, Canada, have had many uses over the centuries. The first Nations used the tar to make their canoes waterproof [94]. More recently, the industrial world has used the resources from the area to run everything from power plants to cars.

The oil sands are unconventional oil deposits. In Alberta, they consist of three major deposits covering an area of 140 000 km^2: the Athabasca, Cold Lake, and Peace River oil sands [95] (Figure 1.2). Together, they contain 27 billion m^3 of crude bitumen reserves [96]. In 2010, the daily production of crude bitumen was 256 300 m^3 [96]. That year, 46.1 million m^3 or 290 million barrels of synthetic crude oil (SCO) were produced [96]. The oil sands are water-wet sand particles with different amounts of bitumen in the spaces (Figure 1.3) [97, 98]. The average composition of the oil sands are 8–14% bitumen, 3–5% water and 83–88% solids [99, 100]. The solids consist of sand, silt, and clay.

Figure 1.2. Alberta's oil sands areas. (from ERCB report, Figure 2.1, p. 2-1 [101])

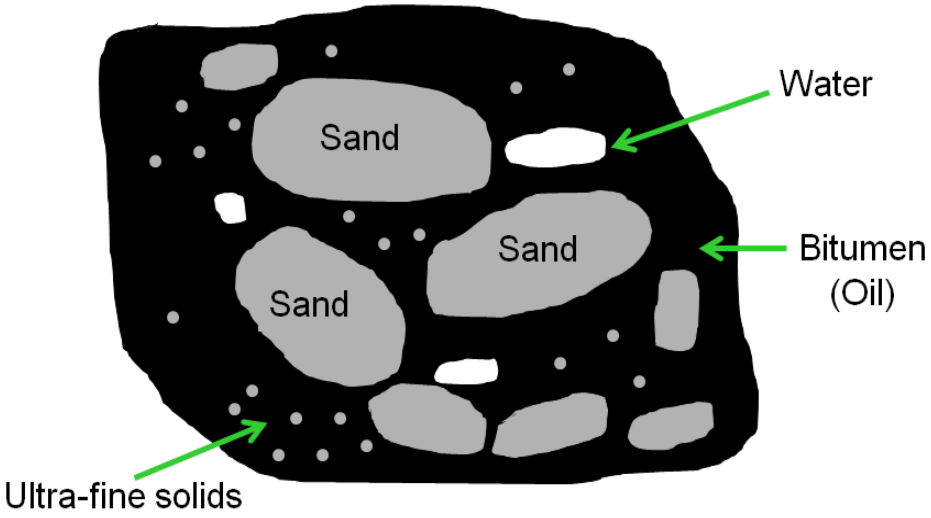

Figure 1.3. Schematic of the Alberta oil sands (Courtesy of Jacob Masliyah and Zhenghe Xu, adapted with permission from Figure 4.1, RSC report [90])

1.7.1. Mining, Extraction and Upgrading Processes

To go from oil sands ore to SCO, bitumen undergoes three major processes: mining, extraction and upgrading (Figure 1.4). The Clark hot water extraction process, the method used today for the extraction of bitumen from the oil, was first developed in 1923, specifically for the Athabasca Oil Sands (AOS) [94, 102-104]. In caustic hot water, clay particles in the water film in the ore structure swell causing the structure to disintegrate [98]. The recovery of the bitumen is 90% efficient at 85 °C and pH 8.5 [98].

1.7.1.1. Mining Process

Several technologies exist for the recovery of bitumen from the oil sands. The depth of the deposit determines which method is used. For deposits with 76 meters or less of overburden, open pit mining is feasible [100, 105]. The overburden is removed and the deposit is mined using shovels and trucks. The

oil sand ore is crushed by large crushers, transported via conveyor to a slurry preparation plant, mixed with hot water, sodium hydroxide, and steam and transferred via pipelines to the processing plant [100, 106]. As of 2009, open pit mining was used to produce 55% of the crude bitumen [100]. For bitumen recovery from deposits more than 150 m deep, in situ methods are required where steam and wells are used to produce a bitumen-water mixture that is pumped above ground. The recovery of the bitumen is 90% with open-pit mining while it is approximately 50% with in-situ methods [98, 100].

1.7.1.2. Extraction Process

The caustic slurry is aerated which produces a bitumen froth. In the primary separation vessels, the froth floats to the top and the sand settles to the bottom. The sand and other solids, now considered tailings, are removed and sent to the settling basins. The bitumen froth is collected for further treatment during the upgrading process. The slurry undergoes a secondary separation to collect any residual oil [98, 100, 106].

1.7.1.3. Upgrading Process

The bitumen undergoes several treatments (coking/cracking, catalytic conversion, distillation, and hydrotreating) to be upgraded to SCO. Primary upgrading breaks down the non-distillable molecules while secondary upgrading removes sulfur and nitrogen [100]. Coking is used for primary upgrading where the large hydrocarbons are cracked at temperatures ranging from 430 to 565 °C [100, 107]. The fluid coking process is used to produce Syncrude coke. Cracking occurs when feed is sprayed onto a bed of hot seed coke particles [107]. Lighter

hydrocarbons and coke are produced. Some of the coke is reused as the seed particles; the rest is stockpiled.

To produce 1 m^3 (6.3 barrels) of SCO, 11 tonnes of oil sands are required if the bitumen recovery rate is 90% [100]. The amount of water used for hydrotransport and bitumen recovery is 2.5 m^3 (16 barrels), though 80% of it is recycled [100].

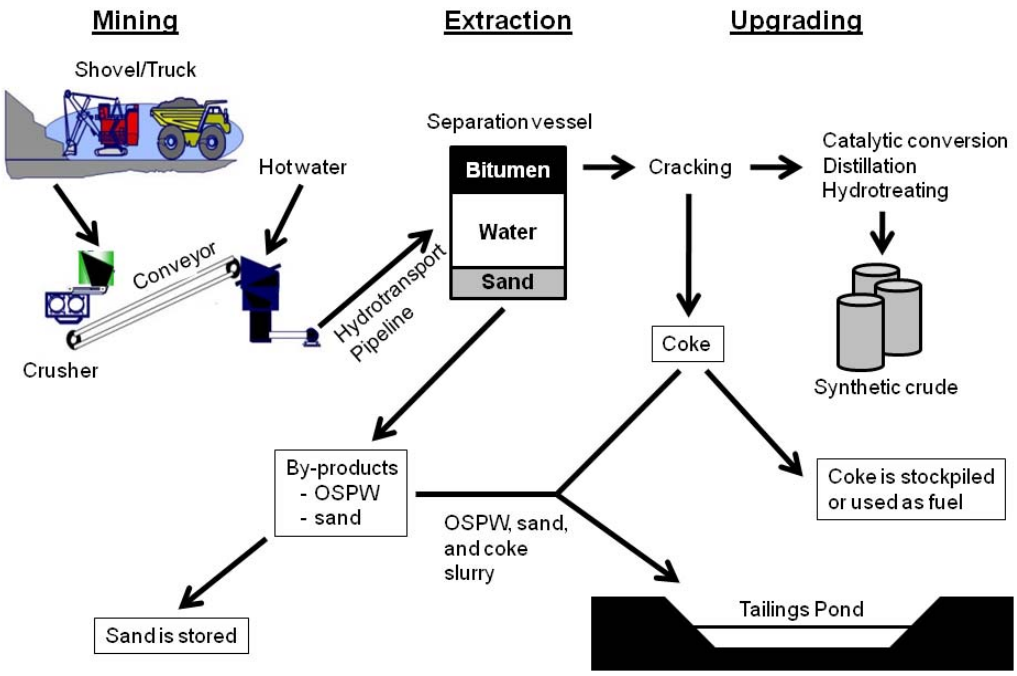

Figure 1.4. Schematic of mining, extraction, and upgrading (Courtesy of Jacob Masliyah and Zhenghe Xu, adapted with permission from Figure 4.5, RSC report [100])

1.7.1.4. Oil Sands Process Water

Oil sands process water (OSPW) is the water that results from the extraction process [108]. It is stored on-site in tailings ponds, also known as settling basins, due to a “zero discharge” policy [104, 107]. OSPW is a complex mixture of salts,

organic matter, and metals [104]. The major cations present are Na^+, K^+, Mg^{2+}, and Ca^{2+}. The major anions present are Cl^- and SO_4^{2-}. The water quality of the West In-pit lake at Syncrude from 1997 to 2007 is presented in Table 1.4.

Table 1.4. Concentration ranges for the water quality of OSPW from the West In-pit lake from 1997 to 2007

Variable	Concentration range (mg/L)
Na^+	7.4–1020
K^+	7–20.4
Mg^{2+}	5.5–11.7
Ca^{2+}	8.2–18
Cl^-	375–970
SO_4^{2-}	26–369
Naphthenic acids	51.4–80.1
Dissolved solids	1850–2930
Total suspended solids	200–2310

From Zubot [109]

1.7.1.5. Petroleum Coke

Petroleum coke is a byproduct of the cracking process [110]. It is rich in organic sulfur and contains trace levels of many metals (Ca, Fe, K, Ti, and V on a low mg/kg level, Mg, Ni, and Na on a high µg/kg concentration) [111]. The yearly production of coke is about 5 million tons with a potential of 1 billion m^3 over the lifetime of the operations [103]. Coke is currently used as fuel for the power plants in Fort McMurray [96] or is stockpiled for future use in land reclamation projects [95]. As of 2010, the inventory of coke was 68 million tonnes [96]. Syncrude coke has been described as a “fine sandy textured material with round smooth particles” [112]. In 2003, for every barrel of synthetic crude oil produced (42 US gallons or 159 L), approximately 23 kg of coke were produced, resulting in 2 million tons of coke produced annually [107].

1.7.2. Effect on the Biota

A few studies have been performed to determine the effect on the biota of the different by-products of the oil sands. Puttaswamy et al. [113] studied the toxicity of coke leachates on daphnids (*Ceriodaphnia dubia*). The coke leachates were found to be acutely toxic to the daphnids. Fedorak and Coy [103] looked at the effect of coke on methanogenic microorganisms. The results showed a reduction in the methane production rates. They concluded that coke was not biologically inert. Squires [105] tested the toxicity of coke and its leachates on *Chironomus tentans*, an invertebrate found in Northern Alberta wetlands. Based on a 10-day toxicity study of the leachates combined or not with coke, she determined that the physical properties of Suncor coke, not its leachate, negatively affected the *C. tentans* by affecting survival and growth. Syncrude coke leached more trace metals, but no toxic effects were observed, rather the growth was affected positively. She suggested that the metal concentrations were tolerable or the species present were not biologically active or available.

Siwik et al. [114] studied fathead minnows (*Pimephales promelas*), a fish species native to the area, in a 7-day growth and survival assay. The fish were exposed to tailings pond water from various ponds and a control (dechlorinated municipal water from Edmonton, Alberta, Canada). Overall, there was no statistical difference in the growth or survival rate between the different ponds and the control but two sites showed reduced survival. Peters et al. [115] exposed eggs from yellow perch (*Percha flavescens*), a native species, and Japanese medaka (*Oryziaz latipes*), a species commonly used in toxicity studies, to different concentrations of OSPW (0.16, 0.8, 4, 20, and 100%) from the Mildred Lake

settling basin. Egg fertilization for the yellow perch was 94 to 98% successful in dilute OSPW but did not occur in the 100% OSPW. They observed optic-cephalic abnormalities such as cyclopia, and spinal deformities such as dorsal curvatures in the yellow perch embryos. The medaka eggs were exposed to OSPW after fertilization. Developmental problems first occurred in the 100% OSPW treatment but were observed in the other treatments as the embryos showed circulatory distress.

Nakata et al. [116] investigated whether wheat (*Triticum aestivum*) and Northern tufted hairgrass (*Deschampsia caespitosa*), a grass native to the oil sands area, could grow on coke and how coke would affect them. Under greenhouse conditions, both plants grew on the coke but showed signs of stress (reduced transpiration, biomass and photosynthetic pigments). The stress was reduced in plants grown on coke capped with a peat-mineral mix. They were concerned with the accumulation of metals (Ni, Mo, and V) in the roots.

1.7.3. Vanadium and the Athabasca Oil Sands

The concentration of vanadium in the AOS is one of the highest in oil producing areas, the highest being in Venezuelan oils [7]. Vanadium-rich shales are usually the product of a marine environment [117]. The AOS come from marine deposits [118]. Vanadium is present most commonly as vanadyl (VO^{2+}) porphyrins but is also believed to bond with tetradentate ligands [63, 119-122]. The concentration of vanadium in the Athabasca river is 0.002 ± 0.003 mg/L [123].

Several groups have determined the vanadium concentration in the AOS. Jacobs and Filby [124] studied the concentration of vanadium in different components of the AOS by neutron activation analysis. The concentration in the bitumen for a ground sample and a non-ground sample was 144 ± 19 and 170 ± 5 µg/g respectively. The concentration in the asphaltenes was 630 ± 49 µg/g. The results for the asphaltenes agree with the work of Kotlyar et al. [125]. Using d.c. arc emission spectrometry, they found the vanadium concentration to be 640 ppm. Yang et al. [126] found a concentration of 1080 ppm in whole asphaltenes by ICP. Pourrezaei et al. [127] determined the concentration of vanadium in OSPW by ICP-MS to be 0.018 mg/L. Kessler and Hendry [112] looked at petroleum coke and determined the concentration ranged from 1134 to 1539 mg/kg. They performed an acid digestion followed by analysis by ICP-MS. Har [128] determined the concentration of vanadium in Syncrude fluid coke ash ranged from 1.83 ± 0.01 to 2.61 ± 0.01 % weight. The results were obtained by ashing the coke, followed by an acid digestion and analysis by ICP-AES. The concentration in the coke itself was 1518 ± 1 to 1947 ± 10 ppm.

The vanadium concentration is greatly reduced during the upgrading process from the initial concentration in the bitumen to the final concentration in the SCO (190 ppm and < 0.6 ppm, respectively) [100]. Vanadyl porphyrins are thermally stable which allows them to pass through the different extraction processes and into the upgrading process, thus concentrating the vanadium in by-products such as coke [129].

1.8. HYALELLA AZTECA

Hyalella azteca are omnivorous, non-cannibalistic epi-benthic freshwater shrimp used in toxicity studies as they are sensitive to metals and toxic substances [130-132] (Figure 1.5). As adults, they measure between 2 and 10 mm [133] and weigh from 0.8 to 2.8 mg wet weight [134]. They are widely distributed in freshwater systems across North and Central America, from Guatemala to Inuvik, Northwest Territories, Canada [131, 133, 135]. They are an important food supply for many fish [136]. They normally feed on dead animal and plant material or live plant material (e.g., filamentous green algae) [131].

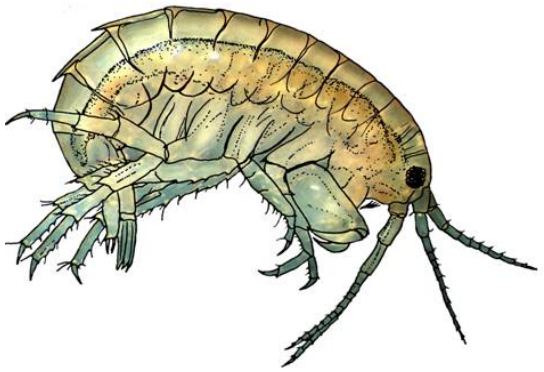

Figure 1.5. *Hyalella azteca* (courtesy of Warren Norwood, Environment Canada, reproduced with permission)

They have been used to study the acute toxicity of sixty-three metals and metalloids to help in classifying them for Canada's Domestic Substance List [137]. To determine the effects of fullerene (C_{60}) on aquatic organisms, *H. azteca* was one of several species studied (*Daphnia magna,* copepod, fathead minnow (*Pimephales promelas*), Japanese medaka (*Oryzias lapites*)) [138]. The maximum achievable concentration (7 ppm) of nano-C_{60} (clusters of fullerenes) did not cause observable toxic effects in water column exposure tests or when

mixed in with the food. Along with the midge *Chironomus dilutus*, they were used to establish which elements of U, Mo, As, and Ni, were responsible for change in the benthic community near a uranium mine in Northern Saskatchewan [130].

Using *H. azteca* in toxicity studies provides several advantages. They are larger than daphnids, providing more tissue for analysis [132]. To perform speciation on tissue using liquid chromatography, an extraction method is required. With more tissue available, a higher concentration of the chemical of interest can be obtained in the extract volume. Compared to another freshwater shrimp (*Gammarus fasciatus*) and a side-swimmer (*Crangonyx gracilis*)*, H. azteca* are easier to culture [132]. They can be fed Tetra-Min, commercially available fish food flakes, whereas daphnids require an algal food source [132].

A recently developed monitoring program for the Athabasca Oil Sands region, the Integrated Oil Sands Environment Monitoring Program, aims to determine what contaminants should be monitored, where and when the sampling should occur, and what methods are required for the sampling and testing [139]. The scope of the program includes monitoring the air, the water, and the biodiversity, both terrestrial and aquatic, of the region. *H. azteca* are currently being used in toxicity studies of sediment and water samples from the region as part of this monitoring program [140]. The toxicity studies involve samples from the Athabasca River and its tributaries, process ponds, ground water, and snow melt. Bioaccumulation and analysis of 45 metals were performed on the collected samples. The contribution of the detected metals to observed impacts on the ecosystem can therefore be assessed. Vanadium was one of the metals tested.

Speciation and bioaccumulation results for vanadium would indicate whether total analysis or speciation is required in the assessment of the region.

1.9. RATIONALE AND OBJECTIVES FOR THE RESEARCH

The objectives of this research are to:

1. *Optimize the HPLC separation method for vanadium*

   An HPLC separation method was previously developed in house [68]. Its optimization would allow for a separation better adapted to the samples of interest.

2. *Determine the speciation of vanadium in OSPW*

   Total vanadium concentrations ranging from 5 to 18 µg/L have been detected in OSPW [123, 127]. The next step is to determine the vanadium species present.

3. *Study the leaching of vanadium from coke into water*

   When coke and OSPW are mixed, chemicals can leach from the coke into the water or sorb to the coke. Previous work has shown that naphthenic acids adsorb from OSPW onto coke [109]. This phenomenon could be used as a treatment method to remove naphthenic acids from OSPW. However, vanadium could leach from coke into the water. Studying vanadium leaching would help determine the potential environmental impact of this treatment method.

4. *Develop a speciation preservation method for water samples containing vanadium*

   Speciation provides important chemical information as toxicity is related to chemical speciation. Maintaining the integrity of the speciation of a sample between collection time and analysis time is paramount to obtaining this key knowledge.

5. *Determine the vanadium concentration and speciation present in* Hyalella azteca *tissue samples and the water samples to which* H. azteca *were exposed*

   A collaborative project involves studies of uptake, potential toxicity, and speciation of vanadium in *H. azteca*, an aquatic organism native to Alberta. The overall objective is to determine the effect vanadium would have on organisms in the Athabasca Oil Sands region. The specific objective of this research is to determine vanadium speciation in the tissue of *H. azteca* and in the water to which *H. azteca* are exposed. There has been no study on vanadium speciation in this organism.

Chapter 2. Determination of Vanadium Species

Speciation analysis provides valuable chemical information from environmental and biological samples. In order to elucidate the speciation in the sample of interest, an appropriate analytical technique must be used. Many methods have been developed for vanadium speciation in biological and environmental samples (Section 1.6).

When analyzing new samples, previously existing methods can be optimized or new methods developed. Speciation of vanadium in OSPW had not been performed previously. The total vanadium concentration as determined by others [123, 127] ranged from 5 to 18 µg/L. Speciation would therefore require a technique with a low limit of detection (LOD). HPLC-ICP-MS would therefore be a viable technique as it had been shown to separate vanadium species quickly (under 10 minutes) with a low LOD (low to sub µg/L range).

The HPLC-ICP-MS method developed by Li et al. [68] had been applied to coke pore water collected from lysimeters, cylindrical tanks containing a top layer of soil and a bottom layer of coke, from the Athabasca Oil Sands. The sample matrix would therefore be expected to be similar to that obtained from mixing coke and OSPW. The method allowed for the simultaneous separation and determination of V(III), V(IV), and V(V) in liquid samples with low LODs, 0.6, 0.7, and 1.0 µg/L, respectively. Combined with a rapid separation (five minutes), it provided an excellent starting point for method optimization.

2.1. INSTRUMENTATION

2.1.1. HPLC-ICP-MS

Liquid chromatography, more specifically HPLC, was used in the study of vanadium speciation. The instrument used consisted of a PerkinElmer Series 200 HPLC system (PE Instruments, Shelton, Connecticut, USA) equipped with an autosampler and a column heater. The HPLC column outlet was connected to a PerkinElmer Elan 6100 DRC^{plus} ICP-MS (PE Sciex, Concord, Ontario, Canada) using a 38 cm long piece of PEEK tubing (1/16" O.D., 0.007" I.D., Supelco, Bellefonte, Pennsylvania, USA). The sample injection volume was 50 µL. The mobile phase flow rate was 1 mL/min (mobile phase composition, Section 2.4). The performance of the instrument was optimized daily using an atomic spectroscopy standard solution (Elan 6100 DRC Setup/Stab/Masscal Solution, PerkinElmer, Shelton, Connecticut, USA). The ICP-MS operating conditions are listed in Table 2.1.

Table 2.1. Elan 6100 DRC^{plus} ICP-MS operating conditions

Parameter	Setting
Nebulizer Gas Flow (NEB) (L/min)	0.91
Auxiliary Gas Flow (L/min)	1.50
Plasma Gas Flow (L/min)	15
Lens voltage (V)	10
ICP RF Power (W)	1350
Analog Stage Voltage (V)	-2100
Pulse Stage Voltage (V)	1400

Vanadium has two naturally occurring isotopes, V^{50} and V^{51}, with their abundance being 0.25% and 99.75% respectively [141]. Ti^{50} (5.4%) and Cr^{50} (4.3%) would cause isobaric interference with V^{50}. Given the abundance of V^{51} and the lack of elemental interferences, the m/z 51 was chosen for vanadium monitoring.

2.1.2. ESI-MS

ESI-MS was used to confirm the formation of the V-EDTA complexes. ESI-MS is used to analyze ionizable analytes in liquid samples and has been previously employed to explore the formation of metal-EDTA complexes for manganese, cobalt, copper, zinc, lead, iron, thorium and vanadium [68, 93, 142, 143].

The ESI-MS instrument used was an AB Sciex 5500 Qtrap (AB Sciex, Concord, Ontario, Canada). Analyst 1.5.1 (ABSciex, Concord, Ontario, Canada) was used to acquire and analyze the spectra. Spectral acquisition was performed in negative mode. The conditions used for the acquisition of each spectrum are listed below it. The declustering potential (DP), collision energy (CE), and ionspray voltage (IS) are the main conditions of interest. For the analysis of the V(IV) standard, a 200 µg/L solution in 1:1 methanol:water and 0.3% NH_4OH was prepared. The V(V) standards were analyzed with a 800 µg/L solution in 1:1 methanol:water and 0.45% NH_4OH. The solutions were infused with a 25 µL/min flow rate. An enhanced mass spectra (EMS) scan was performed to verify that the potential parent ion at the expected m/z was present. Once its presence was confirmed, an enhanced product ion (EPI) scan was completed to determine the daughter ions. An enhanced scan uses the ion trap to increase sensitivity.

2.2. PREPARATION OF VANADIUM STOCK SOLUTIONS

Vanadium stock solutions with a concentration of 0.5 g/L vanadium were prepared using the following procedure.

2.2.1. V(IV) from Vanadyl Sulfate Hydrate

The V(IV) standard was prepared by dissolving 0.50 g of vanadyl sulfate hydrate (Aldrich, St. Louis, Missouri, USA) in a 100 mL volumetric flask using deionized water (DIW). The solution was blue.

2.2.2. V(V) from Ammonium Metavanadate

The V(V) standard was prepared by dissolving 0.23 g of ammonium metavanadate (Aldrich, St. Louis, Missouri, USA) in 2 mL nitric acid (Fisher, Concord, Ontario, Canada) and 10 mL DIW in a 100 mL volumetric flask. The solution was sonicated until complete dissolution of the solid, then diluted to 100 mL with DIW. The solution was yellow.

2.2.3. V(V) from Vanadium Pentoxide

To explore other vanadium standards, a stock solution of vanadium pentoxide (Aldrich, St. Louis, Missouri, USA) was prepared as follows: 0.045 g of V_2O_5 were weighed and diluted in 25 mL DIW. The mixture was sonicated to ensure complete dissolution of the solid. The solution was orange, the characteristic colour of V_2O_5.

2.2.4. Vanadium-EDTA Complexes

V-EDTA complexes were formed by adding 0.215 g of EDTA acid (Aldrich, St. Louis, Missouri, USA) to 25 mL of the stock solution prepared above. Upon the addition of EDTA, the V(IV) turned a darker blue and formed a $[VOY]^{2-}$ complex, where Y represents deprotonated EDTA. The V(V) prepared with ammonium metavanadate turned a darker yellow and formed the $[VO_2Y]^{3-}$ complex. The mixtures were sonicated for 15 minutes, vortexed, and then allowed to stand for 30 minutes. The solution pH was adjusted to 6 using dilute ammonium hydroxide (Fisher Scientific, Concord, Ontario, Canada) and dilute nitric acid (Fisher Scientific, Concord, Ontario, Canada). Each solution was then diluted to 50 mL with DIW. The stock solutions were stored in the 4 °C fridge. Working standards were prepared from these stock solutions on analysis days.

Following addition of EDTA to the vanadium pentoxide solution, the solution became clear and yellow, the same colour as the ammonium metavanadate solution. The procedure for the preparation of the V-EDTA complex was followed: sonication, vortex mixing, standing, pH adjustment, and final dilution. The final solution was similar in colour to the $[VO_2Y]^{3-}$ standard prepared with ammonium metavanadate. This suggests that the V-EDTA complex formed is the same whether the initial V(V) standard is ammonium metavanadate or vanadium pentoxide. To confirm this hypothesis, ESI-MS analysis was performed on all the standards (Section 2.3).

2.2.5. Determining the Concentration of Vanadium in the V-EDTA Stock Solutions, Calibrated Against a Primary Standard

The V-EDTA stock solutions were standardized by direct ICP-MS analysis using a calibration curve prepared from a multi-element primary standard (Environmental Calibration Standard, Agilent Technologies, USA). All solutions were prepared in 1 % nitric acid (Fisher Scientific, Concord, Ontario, Canada). The calibration solutions and V-EDTA stock solutions were aspirated into the ICP-MS for 30 seconds, and triplicate signal intensities were integrated for 1 second each and averaged. The V-EDTA stock solutions were diluted to 10 µg/L. A NIST SRM 1643e (trace elements in water, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) was used to confirm the validity of the calibration curve. The purity of the stock solutions were determined by HPLC-ICP-MS analysis in a mobile phase of 3% acetonitrile (Fisher Scientific, Fair Lawn, New Jersey, USA), 2 mM EDTA (Aldrich, St. Louis, Missouri, USA), 80 mM ammonium bicarbonate (Fluka Analytical, St. Louis, Missouri, USA) and pH 6 by triplicate injection of a 100 µg/L stock solution. The separation was performed using a SAX PRP-X100 column (Hamilton, Reno, Nevada, USA).

2.3. ESI-MS ANALYSIS OF V-EDTA STANDARDS

ESI-MS was used to confirm the formation of the vanadium-EDTA complexes in the stock solutions as well as determine whether V_2O_5 had formed the same EDTA complex as ammonium metavanadate. Chen et al. [93] and Li [144] have previously used ESI-MS for the same purpose.

2.3.1. V(IV)-EDTA Complex

The structure of the $[VOY]^{2-}$ complex can be seen in Figure 2.1.

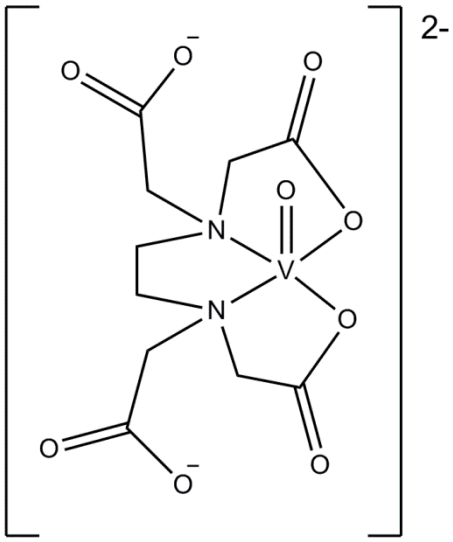

Figure 2.1. Structure for $[VOY]^{2-}$ (from Nelson and Shepherd [145])

An EMS scan of the blank solution (1:1 methanol:DIW, 0.3% NH_4OH) was performed (Figure 2.2), as well as a scan of the $[VOY]^{2-}$ standard (Figure 2.3). The background was noisy but the molecular ion, $[VOY+H]^-$ (m/z 356.3), does not appear in the blank, nor does the doubly charged molecular ion, $[VOY]^{2-}$ (m/z 177.9) (Figure 2.2). The EMS spectrum from the analysis of 200 µg/L $[VOY]^{2-}$ shows the presence of the expected ions at m/z 356.3 for $[VOY+H]^-$ and m/z 177.9 for $[VOY]^{2-}$ (Figure 2.3). In her work, Li [144] observed the $[VOY]^{2-}$ peak and concluded that the vanadium-EDTA complex had formed. She did not scan above m/z 300 so she did not observe the $[VOY+H]^-$ complex. Chen et al. [93] used ESI-MS to confirm the formation of a $[VOY]^{2-}$ complex. They observed a peak at m/z 356.2 that they assigned to $[VOEDTA-3H]^-$, which is equivalent to $[VOY+H]^-$.

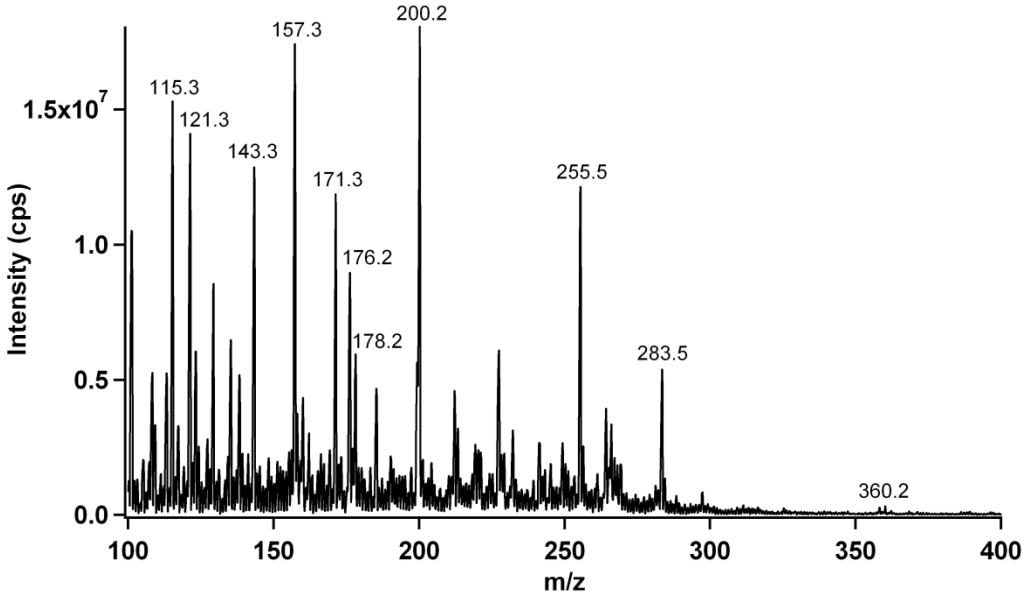

Figure 2.2. EMS spectrum of 1:1 methanol:DIW, 0.3% NH_4OH blank. DP = -50 V

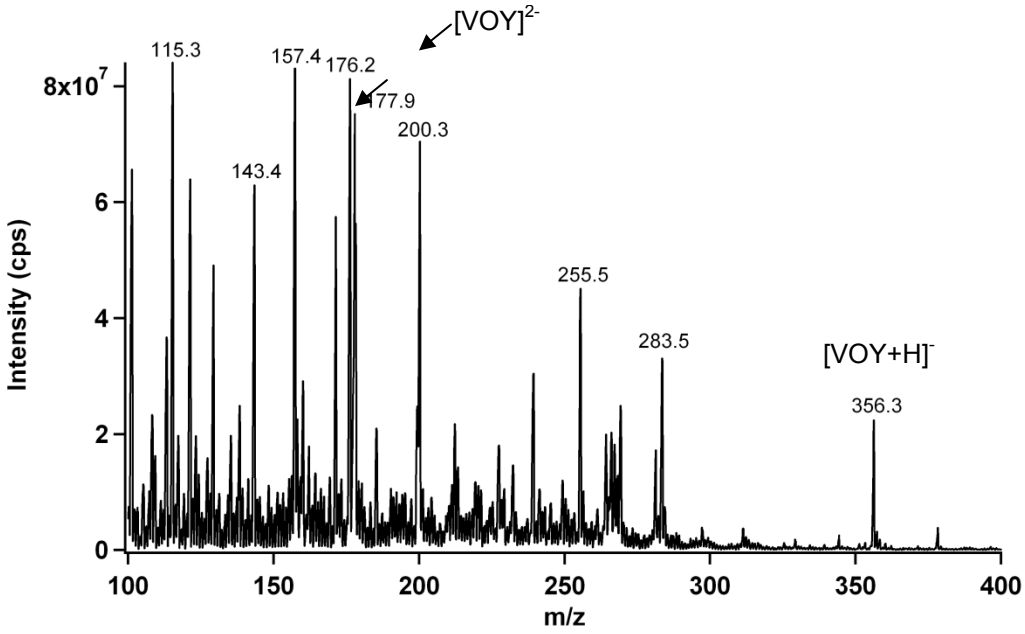

Figure 2.3. EMS spectrum of 200 µg/L $[VOY]^{2-}$. DP = -50 V

The fragmentation spectrum for $[VOY+H]^-$ was acquired using an EPI scan (Figure 2.4). The peaks at m/z 312.0 and 268.0 indicate the loss of two CO_2 molecules, respectively. The peak at m/z 196.9 would be from the loss of a nitrogen atom, which is consistent with the structure of the complex. To fully

interpret and understand the fragmentation pattern of the different V-EDTA complexes, experiments using high resolution MS-MS (to determine the chemical composition of the fragments) and isotopic labeling (to determine from where the different atoms are lost) would be required.

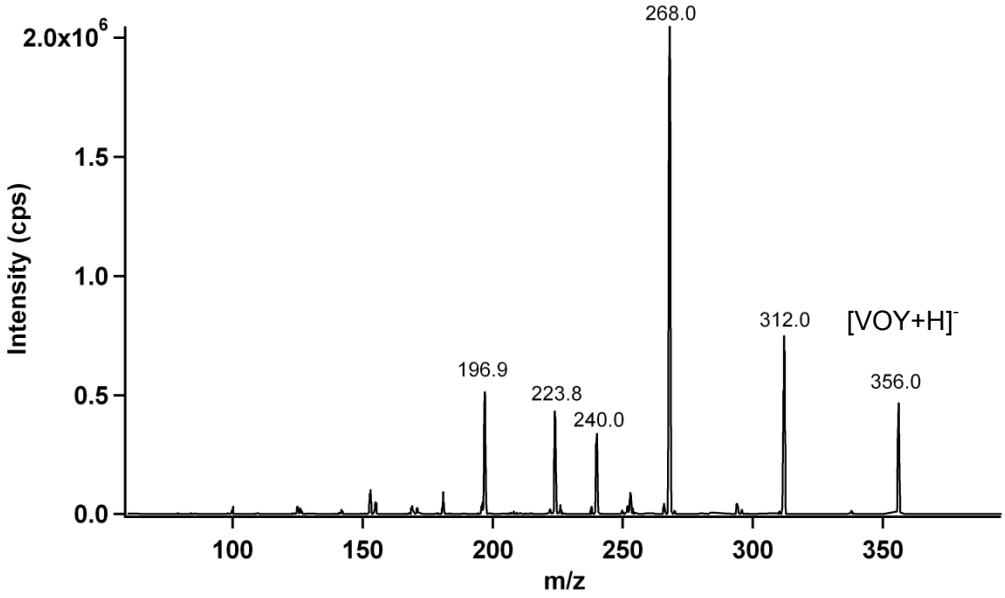

Figure 2.4. MS-MS spectrum of m/z 356.0 of 200 µg/L $[VOY+H]^-$. DP = -75 V, CE = -30 V.

2.3.2. V(V)-EDTA complexes

The structure of $[VO_2Y]^{3-}$ was determined by Crans et al. [146] by ^1H and ^{13}C NMR at pH 8.00 and 298 K (Figure 2.5).

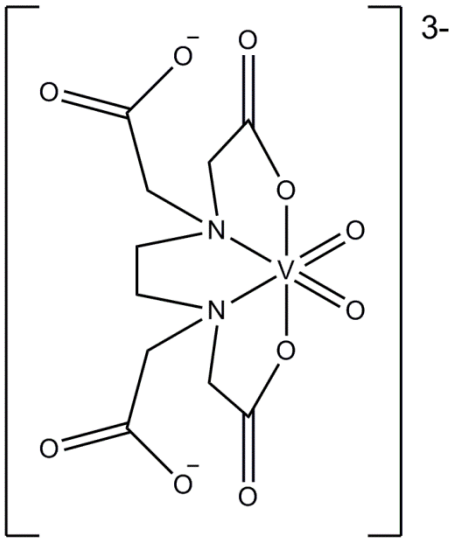

Figure 2.5. Structure of $[VO_2Y]^{3-}$ from Crans et al. [146]

MS scans of the $[VO_2Y]^-$ complex from ammonium metavanadate were acquired (Figures 2.6 and 2.7). For $[VO_2Y]^{3-}$, a peak would be expected at m/z 123.75. No peak was observed at m/z 123.75 (Figure 2.6). A peak at m/z 373.1 was observed which would correspond to $[VO_2Y+2H]^-$ (Figure 2.7). Li [144] did not observe a peak at m/z 123.75 and concluded that the complex fragmented in source. Chen et al. [93] found a peak at m/z 373.1 that they attributed to $[VO_2EDTA\text{-}2H]^-$, equivalent to $[VO_2Y+2H]^-$.

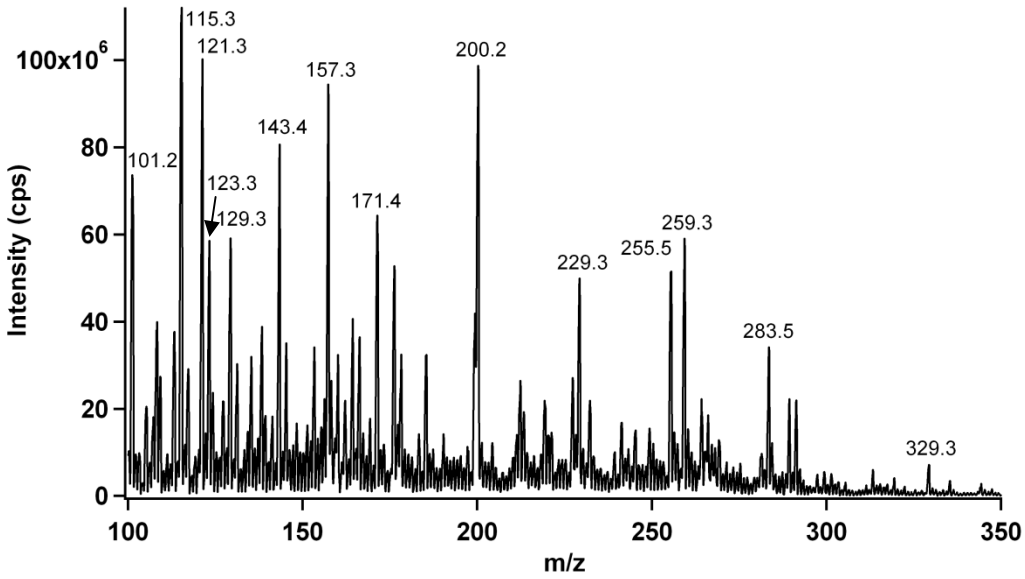

Figure 2.6. EMS spectrum of 200 µg/L $[VO_2Y]^{3-}$. DP = -40 V

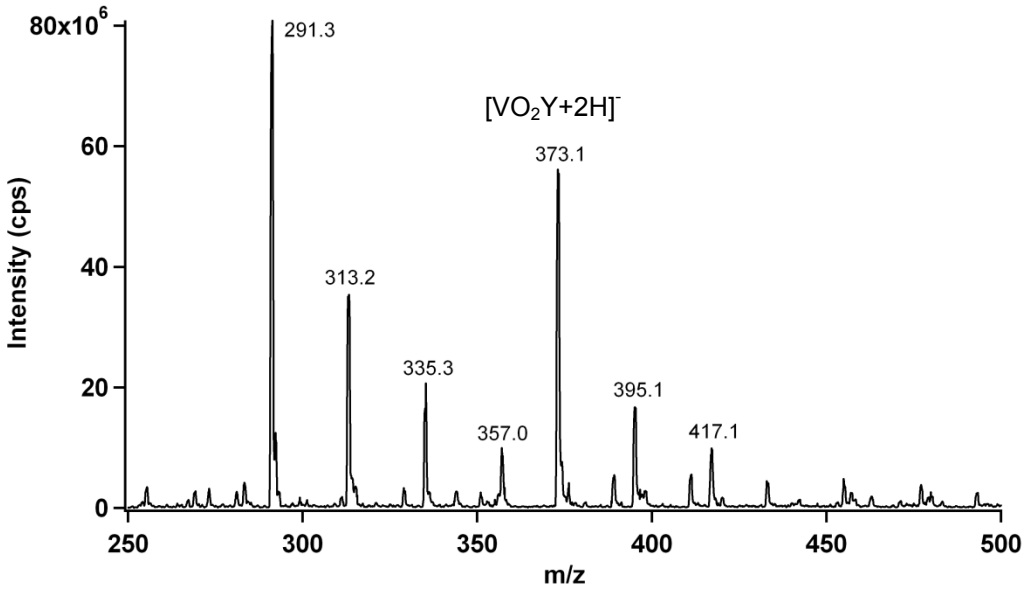

Figure 2.7. EMS spectrum of 800 µg/L $[VO_2Y]^{3-}$. DP = -60 V

The fragmentation spectrum for $[VO_2Y+2H]^-$ from ammonium metavanadate was acquired (Figure 2.8). The fragment at m/z 311.2 indicates the loss of CO_2 and H_2O from the parent ion. The m/z 281.0 fragment could be from the loss of NO.

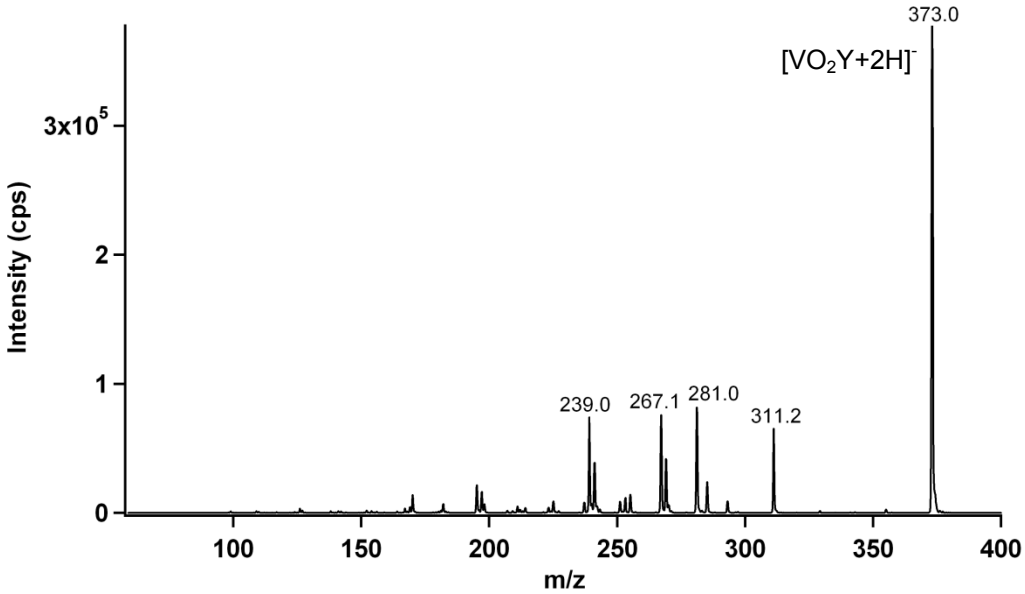

Figure 2.8. EPI spectrum of m/z 373 of 800 µg/L $[VO_2Y+2H]^-$ from ammonium metavanadate. DP = -80 V, CE = -20 V, CES = 10 V, IS = -4800 V.

The V(V)-EDTA complex formed with vanadium pentoxide was yellow. A mass spectrum (Figure 2.9) of this complex was acquired using the same conditions as the spectrum for the $[VO_2Y+2H]^-$ complex from ammonium metavanadate (Figure 2.6).

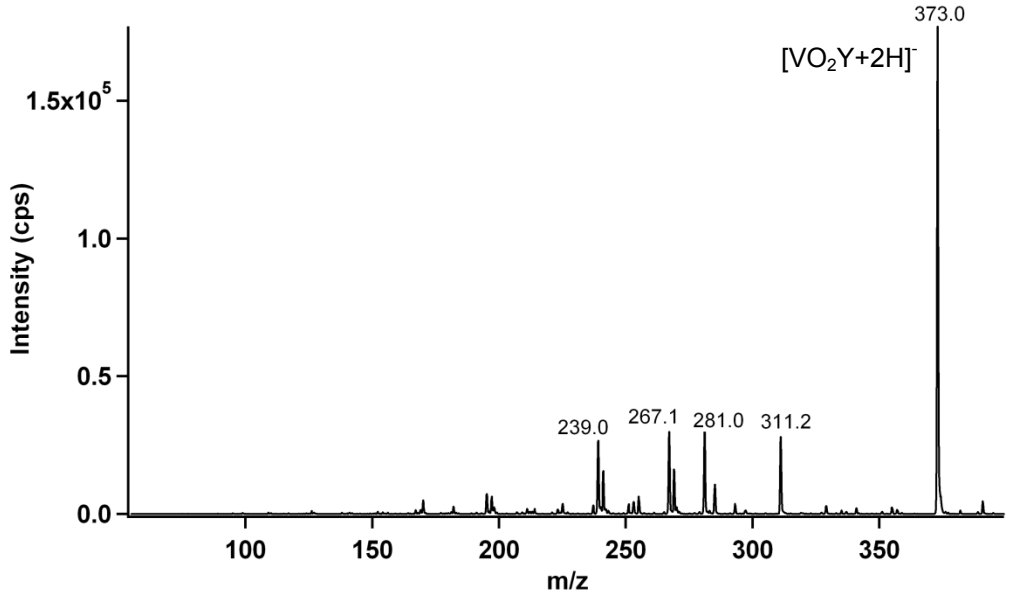

Figure 2.9. EPI spectrum of m/z 373 of 800 µg/L $[VO_2Y+2H]^-$ from V_2O_5. DP = -80 V, CE = -20 V, CES = 10 V, IS = -4800 V.

The fragmentation pattern of both V(V)-EDTA standards is the same with fragment peaks at m/z 239.0, 267.1, 281.0, and 311.2. The relative intensities of the fragment peaks are similar. This supports the assumption that the V-EDTA complex prepared with vanadium pentoxide converted to $[VO_2Y+2H]^-$.

To check if there was any V_2O_5-EDTA complex present in the solution, an MS-MS scan of the V_2O_5-EDTA parent ion (m/z 472) was acquired (Figure 2.10). The peak at m/z 471.8 is consistent with the molecular mass of V_2O_5-EDTA.

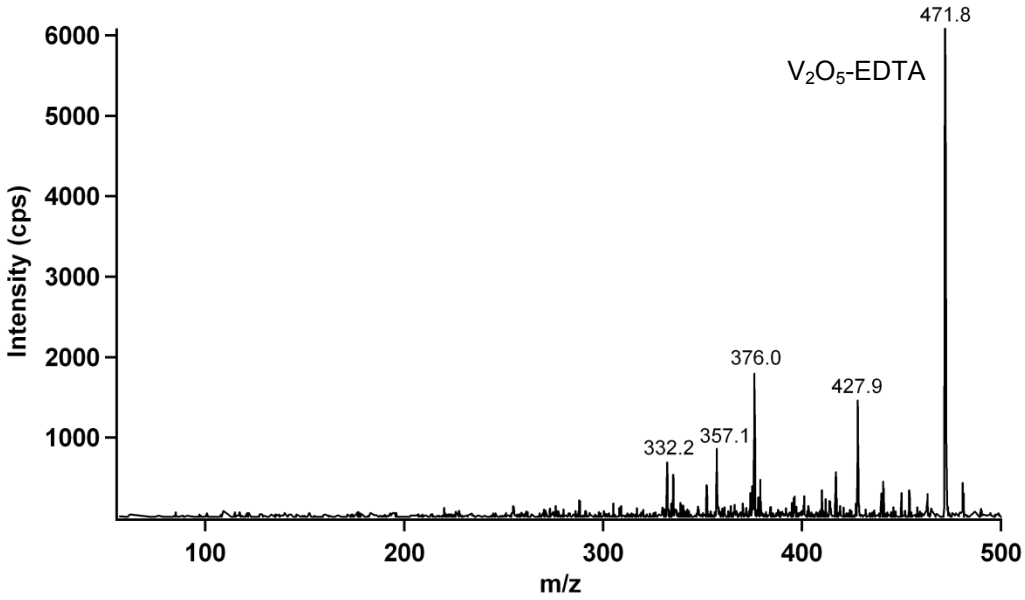

Figure 2.10. EPI spectrum of m/z 472 of 800 µg/L V_2O_5-EDTA. DP = -80 V, CE = -20 V, CES = 10 V, IS = -4800 V.

Although signal intensity in ESI-MS is dependent on the ease of ionization of analyte of interest and the intensities for different analytes cannot be directly compared, the large difference between the two ions suggests that $[VO_2Y+2H]^-$ is the main complex formed rather than V_2O_5-EDTA. The intensity of the parent ion peak (m/z 471.8) in Figure 2.10 is 29 times less intense than the parent ion peak (m/z 373) from Figure 2.7, 6086 cps and 177 037 cps respectively.

Ammonium metavanadate and vanadium pentoxide form the same $[VO_2Y]^{3-}$ complex with EDTA, but vanadium pentoxide is more toxic [147, 148], so ammonium metavanadate was used to prepare all V(V) standards for the

remaining experiments. For ease of reading, V(IV) will be used to designate the $[VOY]^{2-}$ complex, and V(V) for $[VO_2Y]^{3-}$, unless otherwise specified.

2.4. OPTIMIZING THE SEPARATION

The separation method used was initially based on the method described by Li et al. [68]. The column was a SAX PRP-X100 strong anion exchange column (50 mm x 4.1 mm x 5 µm). Different mobile phases were tested to provide a more rapid separation with sharper peaks. The acetonitrile concentration and EDTA concentration were varied (Table 2.2). The pH was maintained at 6 and the ammonium bicarbonate concentration was 80 mM for all the mobile phases tested. Triplicate runs of a 100 µg/L standard for each species of interest were used. The separations can be seen in Figures 2.11 and 2.12. The elution time of the peaks are shown in Table 2.3.

Table 2.2. Composition of different mobile phases tested

Mobile phase	[Acetonitrile] (%)	[EDTA] (mM)
1	4	15
2	4	2
3	3	2

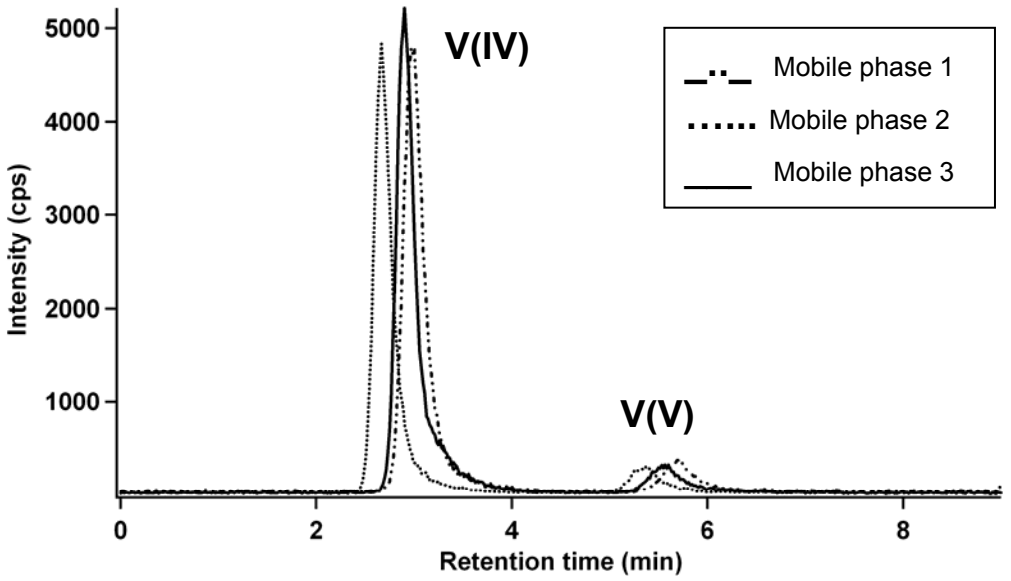

Figure 2.11. Separation of V(IV) and V(V) using different mobile phases

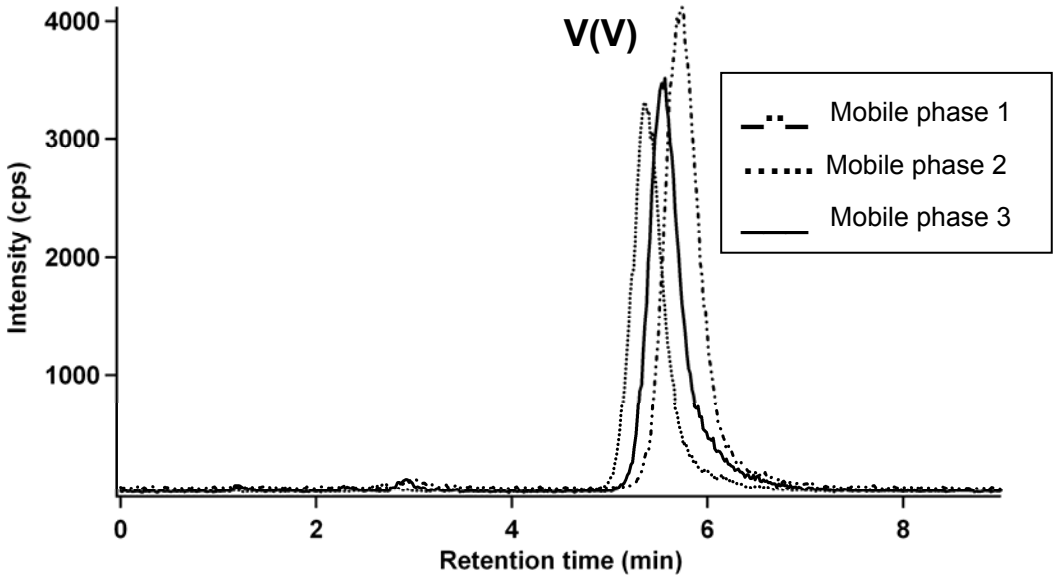

Figure 2.12. Separation of V(V) using different mobile phases

Table 2.3. Elution time for different vanadium species

Mobile phase	V(IV) (minutes)	V(V) (minutes)
1	2.99 ± 0.02	5.74 ± 0.01
2	2.677 ± 0.002	5.38 ± 0.02
3	2.914 ± 0.002	5.55 ± 0.03

The asymmetry ratio was calculated using Equation 2.1. The asymmetry ratios are shown in Table 2.4.

$$A_s = \frac{b}{a} \tag{2.1}$$

Where A_s is the asymmetric ratio, *a* is the distance between the peak apex and the front of the chromatographic peak at 10% peak height, *b* is the distance between the peak apex and the tail of the chromatographic peak at 10% peak height.

Table 2.4. Asymmetry ratio for different vanadium species

Mobile phase	V(IV)	V(V)
1	1.5 ± 0.2	1.3 ± 0.2
2	1.9 ± 0.1	1.4 ± 0.3
3	2.1 ± 0.4	1.7 ± 0.1

The mobile phases with a higher acetonitrile concentration (4%) provided more symmetrical peaks (Table 2.4). The mobile phases with 2 mM EDTA gave faster separations (Table 2.3). An important consideration when choosing a mobile

phase is whether or not the instrument will be operational afterwards. While mobile phase 1 gave the most symmetric peaks with a longer separation time and mobile phase 2 gave the fastest separation with good peak symmetry, both the mobile phases clogged the ICP-MS when they were used in overnight runs. The result was extensive cleaning of the instrument and loss of instrument time. For this reason, mobile phase 3 was chosen as the optimal mobile phase: 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

2.5. INTERFERENCES

Several polyatomic isobaric interferences exist for m/z 51 [88, 149]. The main interference of concern when dealing with OSPW is the $^{35}Cl^{16}O^{+}$ interference. The chloride concentration in OSPW is high. Between 1997 and 2007, the OSPW chloride concentration from the Syncrude West In-pit ranged from 375 mg/L to 970 mg/L [109]. When analyzing an OSPW sample by HPLC-ICP-MS, there was a peak at 0.8 minutes that was not consistent with the vanadium standards (Figure 2.13).

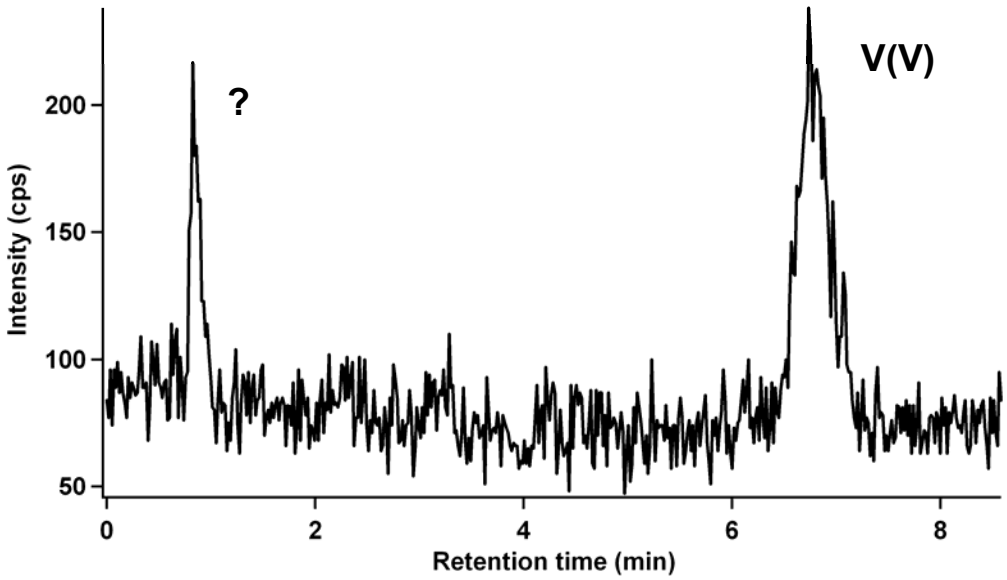

Figure 2.13. OSPW (1:2 dilution in DIW). The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

The assumption was that it was a ClO^+ peak. A solution containing 10 mg/L Cl^-, 10 µg/L V(IV), and 10 µg/L V(V) prepared in DIW was analyzed to determine the elution time of ClO^+. As seen in Figure 2.14, a peak elutes at 0.8 minutes, well separated from the V(IV) and V(V) peaks. The elution time for ClO^+ was the same as the unknown peak, thus confirming it was ClO^+.

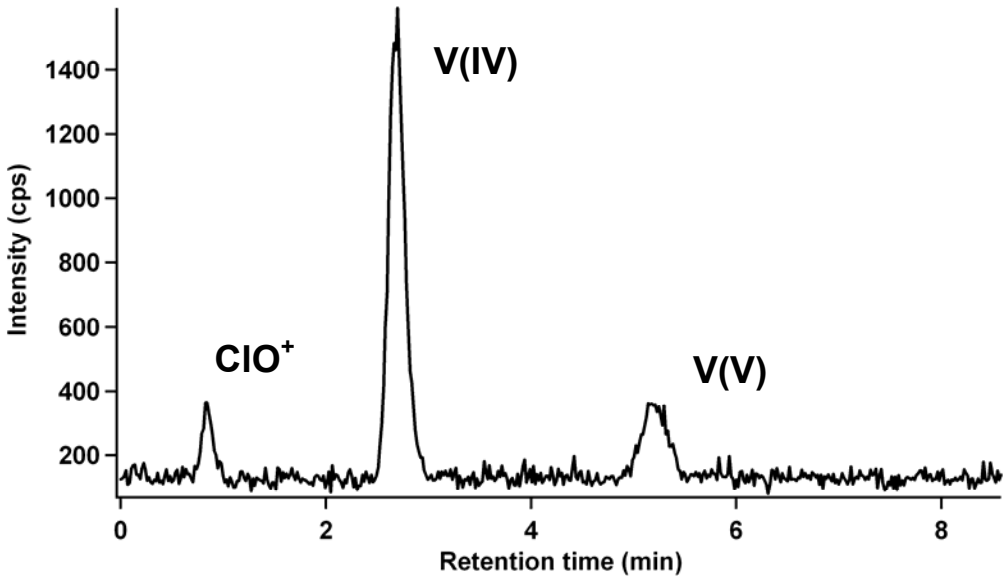

Figure 2.14. Solution of 10 mg/L Cl^-, 10 µg/L V(IV) and 10 µg/L V(V). The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

2.6. DETECTION LIMITS AND CALIBRATION

2.6.1. Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined using both peak area and peak height. For peak area, the standard deviation (σ) for replicate injections of a 1 µg/L standard was calculated. Equations 2.2 and 2.3 were then used to determine the LOD and LOQ, respectively.

$$LOD = \frac{3\sigma}{m} \quad (2.2)$$

Where LOD is the limit of detection, σ is the standard deviation of the 1 µg/L standard, m is the slope of the calibration curve.

$$LOQ = \frac{10\sigma}{m} \quad (2.3)$$

For the calculation based on peak height, a 1 µg/L standard was injected in triplicate for both V(IV) and V(V). The average baseline signal for the 30 seconds before the peak was calculated as well as its standard deviation (σ). The same was done for the 30 seconds after the peak. The maximum peak height was determined for the standard. Equations 2.4 and 2.5 were then used to determine the LOD and LOQ. The values obtained are in Table 2.5. The values from both methods are comparable to those obtained by other HPLC-ICP-MS methods from 0.01 to 2 µg/L for both V(IV) and V(V) [68, 82, 92, 93].

$$LOD = \frac{3\sigma\ x\ concentration}{peak\ height - average\ baseline\ signal} \quad (2.4)$$

Where LOD is the limit of detection, σ is the standard deviation of the average baseline signal, concentration is the concentration of the analyte of interest present in the standard being analyzed, peak height is the signal intensity due to the analyte of interest, average baseline signal is the average of the signal caused by the baseline.

$$LOQ = \frac{10\sigma\ x\ concentration}{peak\ height - average\ signal} \quad (2.5)$$

Table 2.5. Limit of detection and limit of quantitation for V(IV) and V(V)

Method	Peak Area		Peak Height	
Species	LOD (µg/L)	LOQ (µg/L)	LOD (µg/L)	LOQ (µg/L)
V(IV)	0.5	1.8	0.3	1.0
V(V)	0.4	1.3	0.4	1.4

To show that low concentrations of vanadium can be seen and separated, a sample containing 0.5 µg/L of V(IV) and 0.5 µg/L of V(V) was run (Figure 2.15).

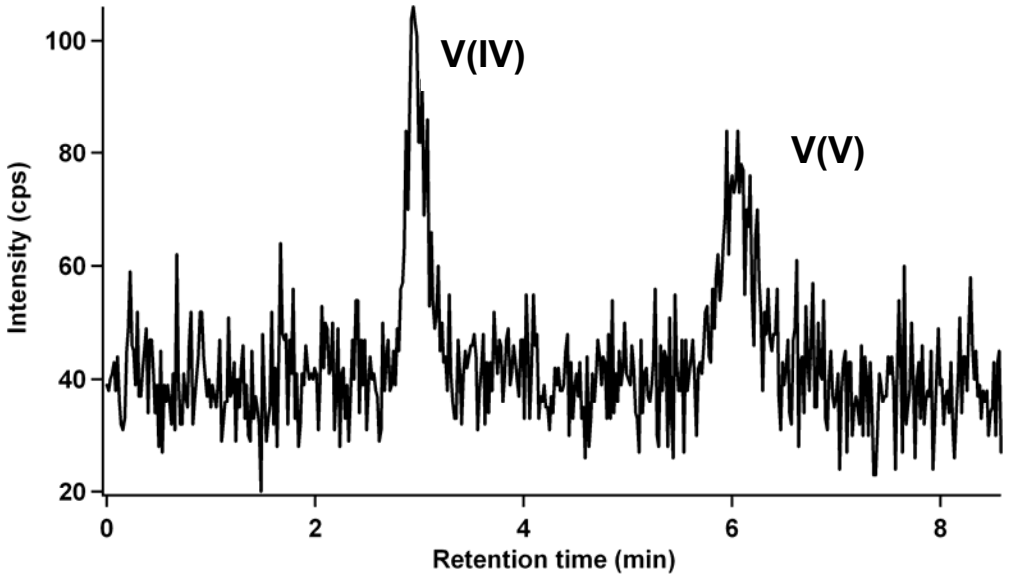

Figure 2.15. Separation of 0.5 µg/L of V(IV) and 0.5 µg/L of V(V). The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

2.6.2. Calibration Curves

Calibration curves were constructed with the V(IV) and V(V) standards. The nominal concentrations of the standards were 0.5, 1.0, 2.5, 5, 10, 25, 50, 75, and 100 µg/L. As can be seen in Figures 2.16 and 2.17, the curves were linear from 0 to 100 µg/L with R^2 values greater than 0.999.

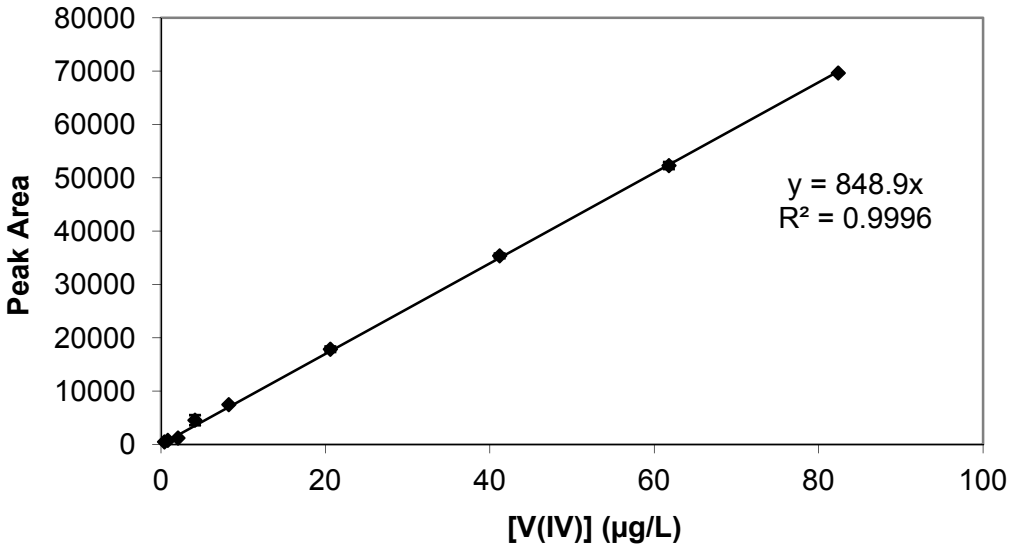

Figure 2.16. Calibration curve for V(IV). Standards with concentrations of 0.4, 0.8, 2.1, 4.1, 8.2, 20.6, 41.2, 61.8 and 82.4 µg/L were used. The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6. Error bars are 1 standard deviation.

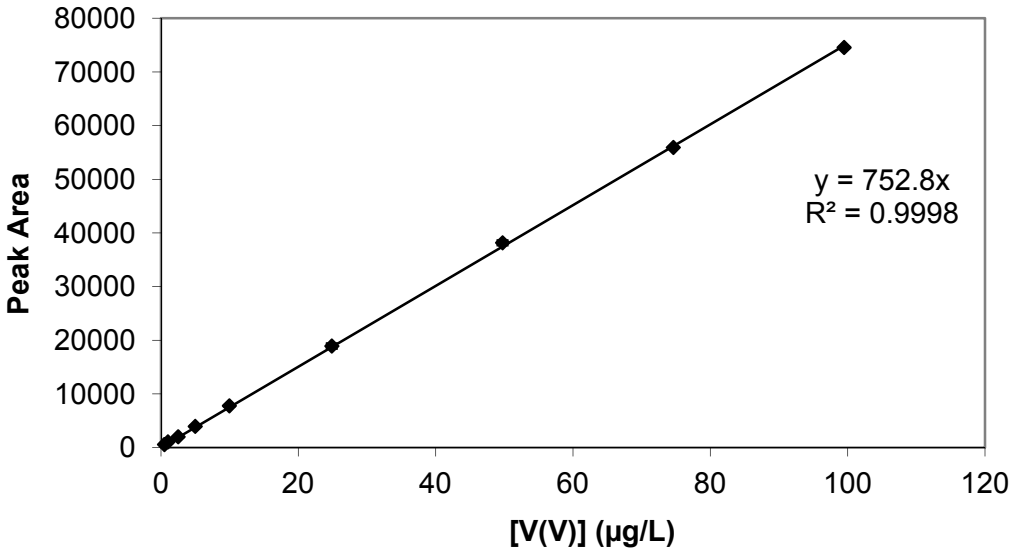

Figure 2.17. Calibration curve for V(V). Standards with concentrations of 0.5, 1.0, 2.5, 5.0, 9.9, 24.9, 49.7, 74.6 and 99.5 µg/L were used. The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6. Error bars are 1 standard deviation.

2.7. CONCLUSION

The optimized method for the separation of V(IV) and V(V) by HPLC-ICP-MS consisted of a SAX PRP-X100 column with a mobile phase made of 3% acetonitrile, 2 mM EDTA, and 80 mM ammonium bicarbonate at a pH of 6. The separation took 8 minutes and 35 seconds. The main interference of concern, ClO^+, eluted before the vanadium standards and was well resolved from the other peaks. The limits of detection were low, 0.3 µg/L and 0.4 µg/L for V(IV) and V(V) respectively. The calibration curves were linear over 2 orders of magnitude.

Chapter 3. Oil Sands Process Water, Petroleum Coke, and Vanadium

3.1. BACKGROUND

OSPW and coke are byproducts of the extraction and upgrading of bitumen from oil sands. They are produced in large quantities (Section 1.7.1) and have been the subject of many studies to determine their properties [109, 127, 150]. As part of the tailings management, coke and OSPW are transported as slurry to settling basins. The interaction between the two has been used as a method to reduce the toxicity of OSPW by reducing the concentration of naphthenic acids [109, 127]. Establishing the fate of vanadium in this process is important to determine whether this method produces undesired effects.

3.2. OVERVIEW OF THE EXPERIMENTS

Using the HPLC-ICP-MS method for vanadium speciation developed in Chapter 2, OSPW was analyzed. Further experiments were performed to determine if oxidation or reduction of vanadium occurred in OSPW, if there was a way to prevent it, and how quickly it happened. Having a method that would stop the oxidation or reduction from occurring would allow samples from leaching studies to be collected at different times and their speciation remain stable until analysis time.

Once a method was found that prevented chemical speciation changes, it was applied to the study of vanadium leaching from coke into water (OSPW and

DIW). The concentration of the preserving agent was optimized. Different coke:water ratios were tested to determine what type of vanadium leached and at what concentration.

3.3. SPECIATION OF VANADIUM IN OSPW

Total analysis of vanadium in OSPW has already been performed. Pourrezaei et al. analyzed OSPW collected from the West In-pit tailings pond at Syncrude [127, 151]. ICP-MS analysis on acid digested and filtered samples gave a total vanadium content in the OSPW of 18 µg/L for the sample collected in October 2009 and 13 µg/L for the sample collected in January 2010. MacKinnon [150] determined the concentration of dissolved vanadium in the Mildred Lake tailings pond by ICP-AES. The samples taken from different depths in the pond over the course of the ice-free period (April to November) of 1980 had concentrations ranged from 1 to 180 µg/L.

The OSPW used for vanadium speciation in this study was collected from the West In-pit lake in March 2009 and January 2010. Excess EDTA (Sigma-Aldrich, St. Louis, Missouri, USA) was added to a homogenized sample to reach a concentration of approximately 3.6 mM EDTA. The sample was shaken, vortexed and filtered through a 0.45 µm membrane, diluted in DIW and analyzed using HPLC-ICP-MS with external standard calibration.

Standard addition was also used to quantify the vanadium species present in OSPW. To determine the vanadium concentration in OSPW, a 1:2 dilution was

performed and the solution spiked with 1, 5, or 10 µg/L of each vanadium species (Figure 3.1).

The concentration of dissolved vanadium ranged from 2.8 to 9.8 µg/L. The concentration is lower than the total analysis from similar OSPW samples as the analysis was performed on acid digested samples and therefore included the vanadium from the suspended matter, not just the dissolved vanadium.

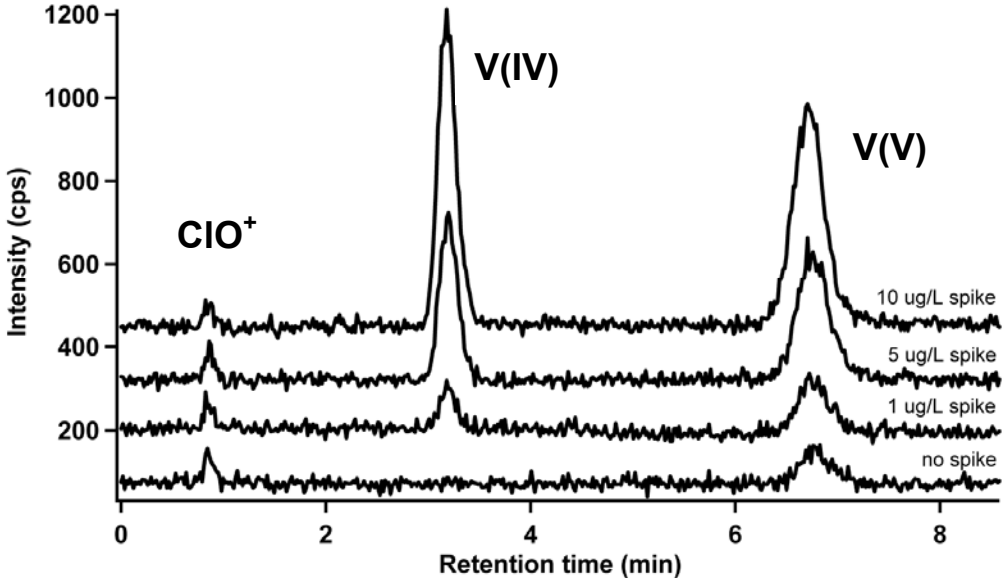

Figure 3.1. Chromatograms from standard addition analysis of OSPW by HPLC-ICP-MS. OSPW was spiked with different concentrations of V(IV) and V(V) standards (1, 5, and 10 µg/L). The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

Li et al. [68] detected V(IV) and V(V) in the coke pore water. However, no V(IV) was detected in OSPW. Redox chemistry could cause any V(IV) present in the OSPW to oxidize to V(V). If oxidation did occur, a method could be developed to prevent it and the oxidation rate could potentially be determined.

3.4. STABILITY OF VANADIUM IN OSPW

EDTA had been used to prepare stable vanadium standards (Section 2.2). The speciation of those standards was stable over a period of several months [144]. It was therefore tested to determine if EDTA could prevent the oxidation of V(IV) in OSPW and in the supernatant of a coke and OSPW mixture. The overall goal of the project was to determine what species of vanadium leached from coke into OSPW. Determining if the oxidation could be stopped in the supernatant of a coke and OSPW mixture would therefore be important later in the project.

A spiking solution was freshly prepared by dissolving vanadyl sulfate hydrate in DIW. The V(IV) standard was added to OSPW to give a nominal concentration of 100 µg/L V(IV). At different times after the addition of the spike, Na_4EDTA was added to give a concentration of 2.5 mM. The samples were filtered using a 0.45 µm Whatman filter, diluted 10-fold in 2.5 mM Na_4EDTA, and analyzed by HPLC-ICP-MS. In one sample, Na_4EDTA was added to the OSPW before the addition of the V(IV) standard to determine if the order of addition affected the results.

As the time between the V(IV) spiking and the addition of Na_4EDTA increased, the concentration of V(IV) decreased significantly (Figure 3.2). When the time was increased to 10 minutes, all the V(IV) added had oxidized. The order in which the chemicals were added was important. When the Na_4EDTA was added first, the speciation was better preserved than when the order was reversed.

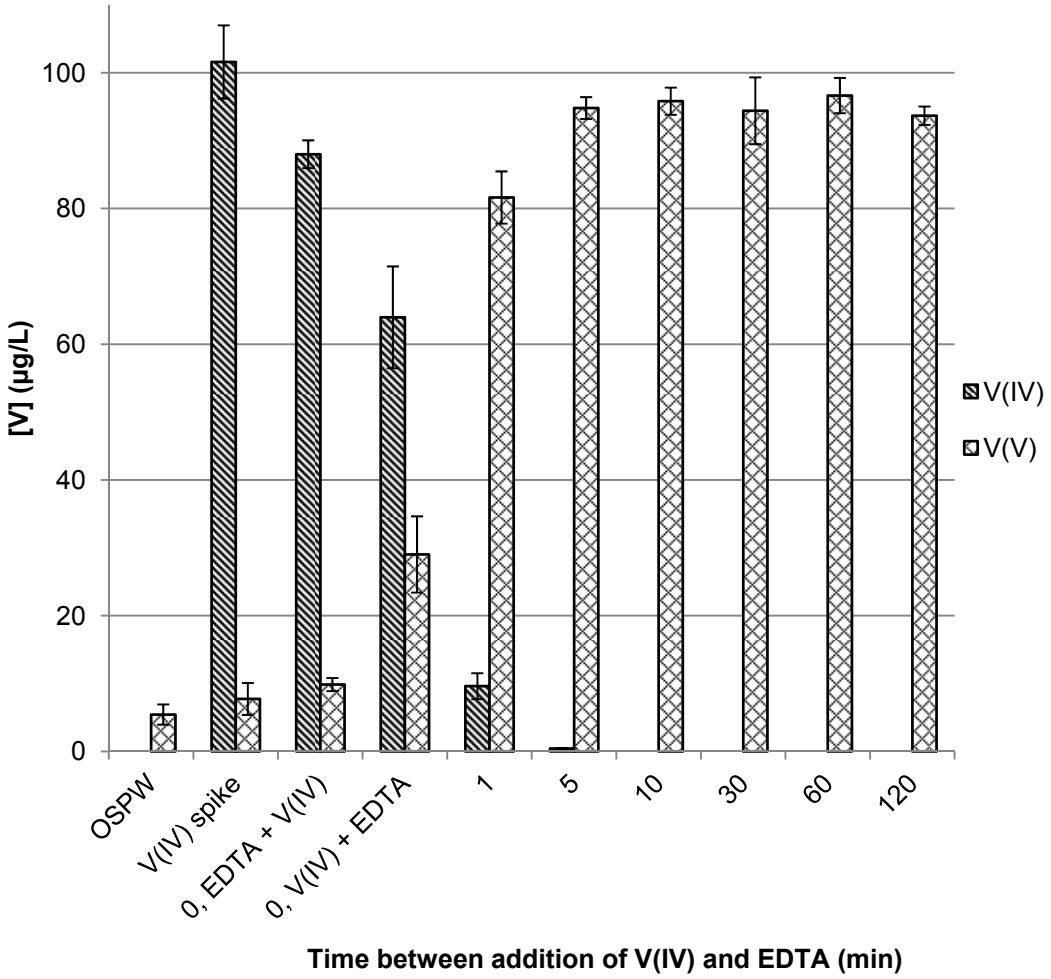

Figure 3.2. Change of vanadium speciation in OSPW after the addition of V(IV) and Na_4EDTA. For 0 minutes, the order in which the chemicals were added is listed. Error bars are 1 standard deviation.

The next experiment was to determine if the same phenomenon happened in the supernatant of a coke:OSPW mixture. A 10% coke:OSPW mixture was shaken for 4 hours. The supernatant was filtered and spiked with V(IV). EDTA was then added to the supernatant after a set time. As the time between the spiking of V(IV) and the addition of EDTA increased, the V(IV) peak decreased and the V(V) peak increased (Figure 3.3). Within 10 minutes, most of the V(IV) had

oxidized. The V(V) initially present in the supernatant had leached from the coke into the OSPW.

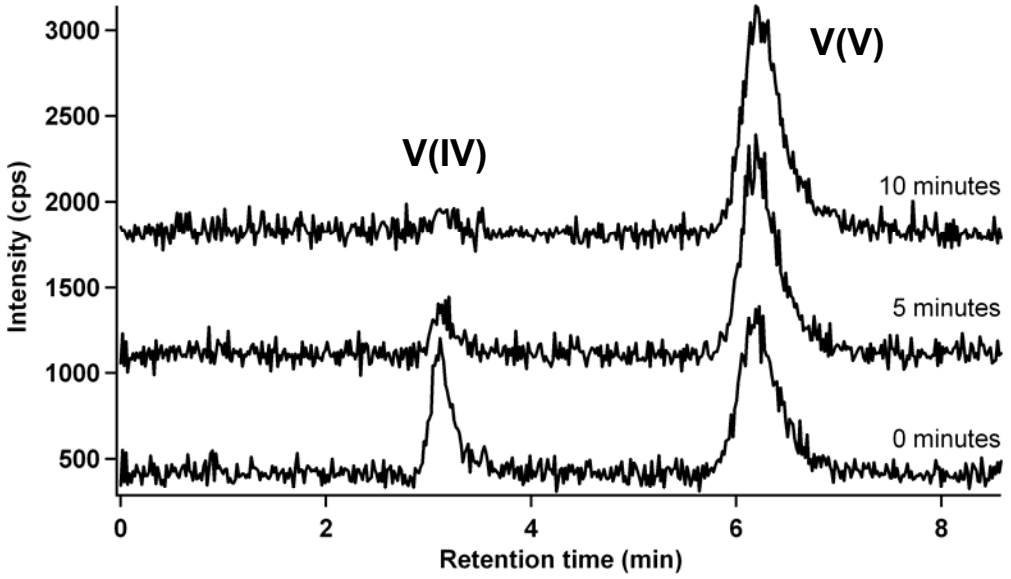

Figure 3.3. Chromatograms from the HPLC-ICP-MS analyses of the supernatant from a 10% coke:OSPW mixture supplemented with 1 mg/L of V(IV) and diluted 100 fold in 2.5 mM Na_4EDTA. EDTA was added to obtain a concentration of 2.5 mM at different time intervals after the addition of V(IV): immediately, 5 minutes, or 10 minutes.

3.4.1. Half-life of Vanadium in OSPW

As previously determined, V(IV) oxidizes rapidly to V(V) in OSPW. To determine how fast the oxidation occurred, a series of experiments were performed where OSPW was spiked with vanadyl sulfate (V(IV)) and the change in speciation was monitored over the course of an hour.

A concentrated V(IV) solution was prepared by diluting 0.125 g of vanadyl sulfate hydrate in 50.0 mL of DIW. The solution was diluted in DIW to prepare a spiking solution of the desired concentration. A 1.00 mL aliquot of the spiking solution

was added to 100.0 mL of unfiltered OSPW in a 250 mL Erlenmeyer flask. Each experiment was performed in triplicate. The solutions were mixed on an orbital shaker (180 rpm, room temperature, VWR DS-500 orbital shaker, VWR, Mississauga, Ontario, Canada). At predetermined time intervals, a 0.10 mL aliquot was removed from the flask and added to 0.90 mL of a 2.5 mM Na_2EDTA solution (Na_2EDTA, Sigma-Aldrich, Oakville, Ontario, Canada). The sample was then filtered through a 0.45 µm nylon membrane (PALL Life Sciences Acrodisc 13 mm Syringe Filter, Pall (Canada) Ltd., Mississauga, Ontario, Canada) and diluted in 2.5 mM Na_2EDTA to a concentration that fell within the calibration range. Nylon membranes should be used in the filtration of OSPW as OSPW can dissolve other types of membranes [152]. The change in speciation was monitored over a 1 hour period. To determine the initial V(IV) concentration in the solution, a serial dilution in 2.5 mM Na_2EDTA was performed until the spiking solution reached a nominal concentration of 20 µg/L. All samples were analyzed by HPLC-ICP-MS using the method described in Chapter 2.

The vanadium speciation change over the course of an hour after V(IV) was added to OSPW can be seen in Figure 3.4.

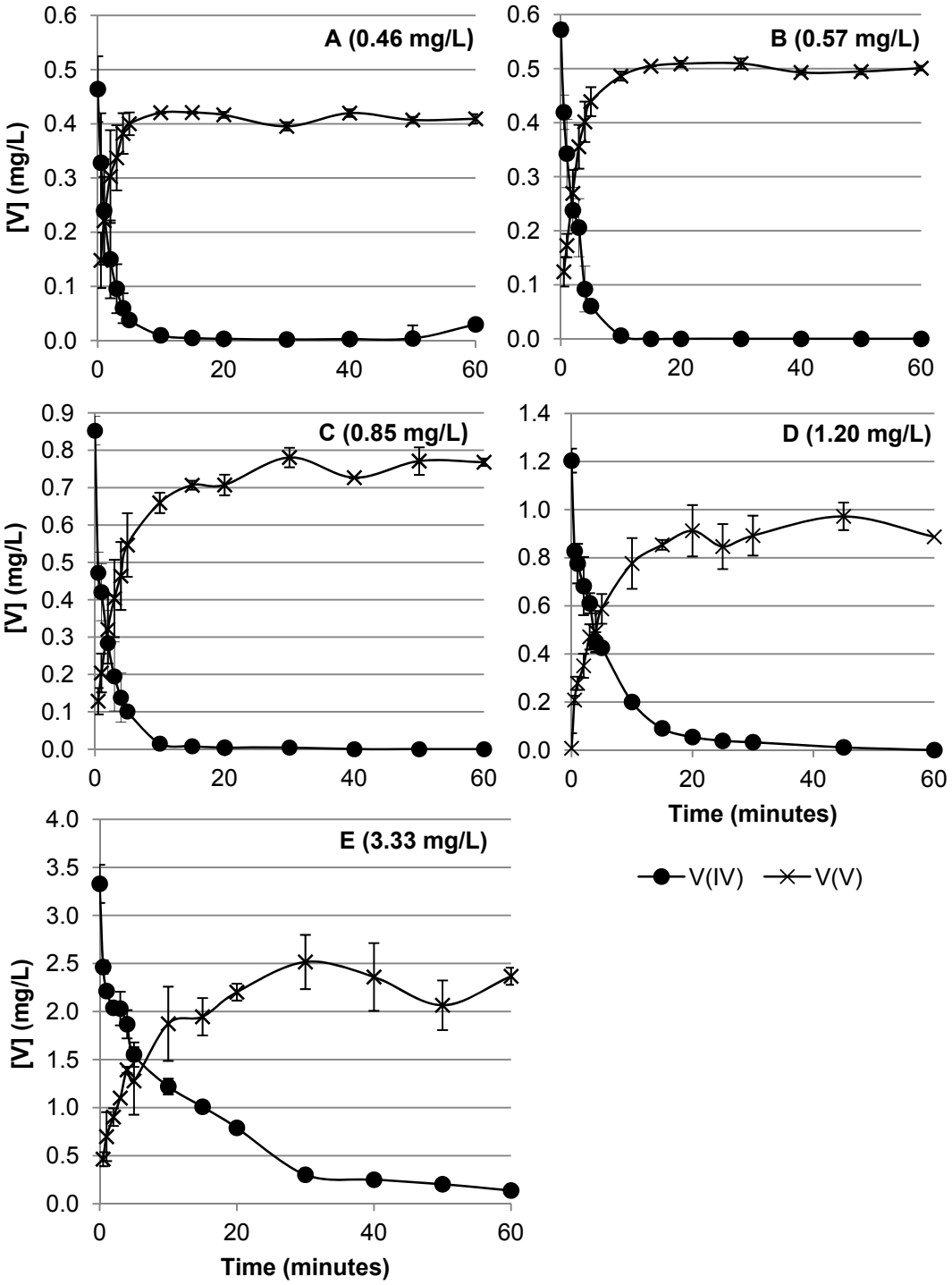

Figure 3.4. Monitoring the change in vanadium speciation in OSPW spiked with V(IV). The initial spiked V(IV) concentration: A) 0.46 mg/L B) 0.57 mg/L C) 0.85 mg/L D) 1.20 mg/L E) 3.33 mg/L. Error bars are 1 standard deviation. V(IV) is represented by closed-circles and V(V), by x.

The change in vanadium speciation appears to be an exponential decay; therefore, the reaction would be first order with respect to V(IV). Wehrli and Stumm [153] looked at the aquatic chemistry of vanadium in double-distilled water. They found that the rate of oxidation of V(IV) was a first order reaction that could be described by Equation 3.1. The observed results would agree with the work of Wehrli and Stumm [153].

$$-\frac{d[VO^{2+}]}{dt} = k_1[H^+]^{-1}[VO^{2+}][O_2] \quad (3.1)$$

Where k_1 is the rate constant (1.87 x 10^{-6} s^{-1}).

The reaction rate can be calculated from the first order rate equation (Equation 3.2) and the half-life calculated, from the first order half-life equation (Equation 3.3).

$$\ln[A] = -kt + \ln [A]_o \quad (3.2)$$

Where [A] is the concentration of the reactant, k is the rate constant, t is time, $[A]_o$ is the initial concentration of reactant A.

$$t_{1/2} = \frac{ln2}{k} \quad (3.3)$$

Where $t_{1/2}$ is the half-life

Figure 3.5 shows the results of applying Equation 3.2 to the different spiking experiments presented in Figure 3.4. For spikes below 1 mg/L only, the data for the first ten minutes were used because after ten minutes, the V(IV) concentration was below the calibration curve range.

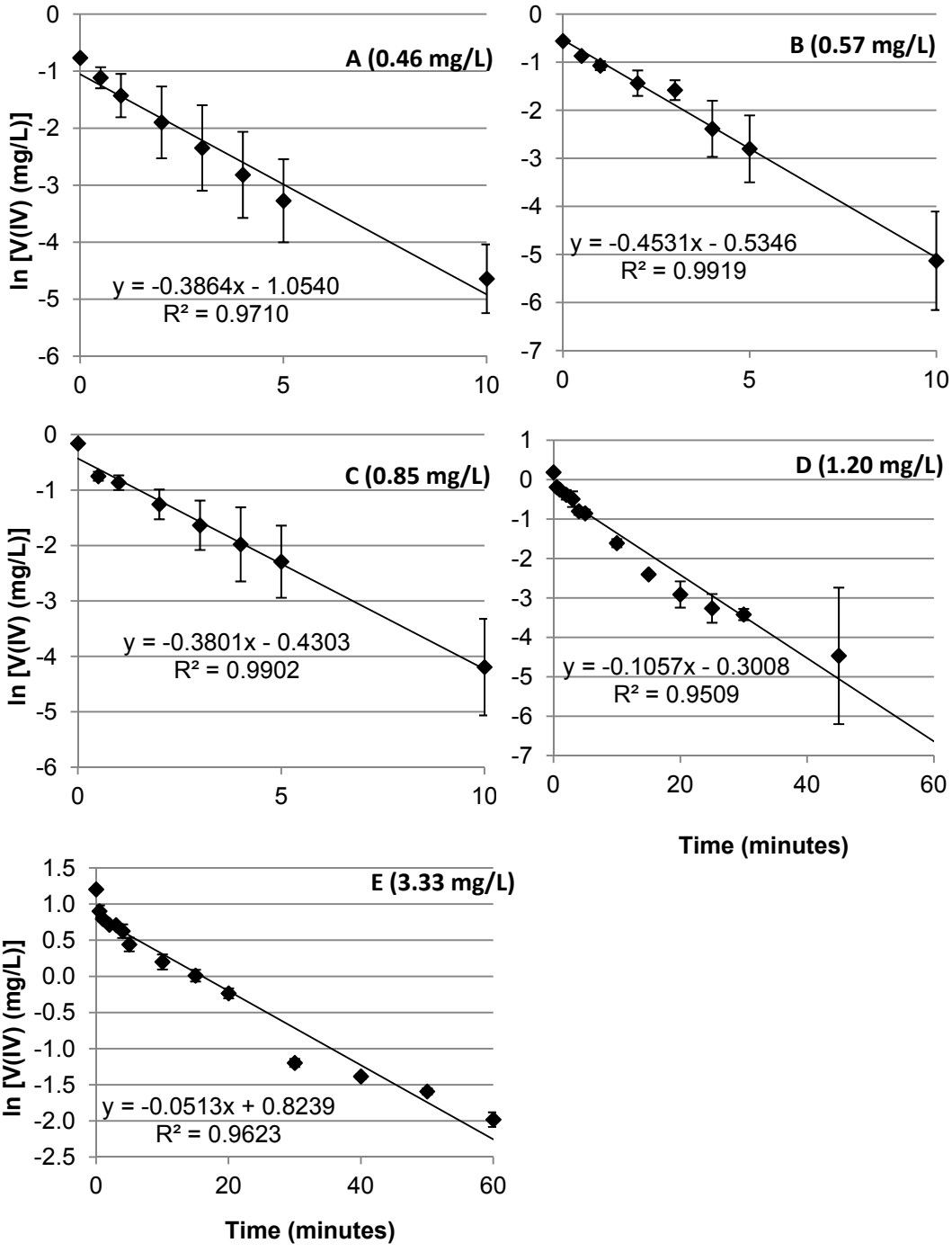

Figure 3.5. Changes in V(IV) concentration over time. The initial spiked V(IV) concentration: A) 0.46 mg/L B) 0.57 mg/L C) 0.85 mg/L D) 1.20 mg/L E) 3.33 mg/L. Error bars are 1 standard deviation.

The initial V(IV) concentration, the calculated rate constant, and the half-life are summarized in Table 3.1. The values were calculated using Equations 3.2 and 3.3.

Table 3.1. Initial V(IV) concentrations and the effect on the rate constant and the half-life

$[V(IV)]_o$ (mg/L)[a]	k ($minutes^{-1}$)[a]	$t_{1/2}$ (minutes)[a]
0.46 ± 0.01	0.39 ± 0.03[b]	1.8 ± 0.1[b]
0.57 ± 0.02	0.45± 0.02[b]	1.53 ± 0.06[b]
0.85 ± 0.01	0.38 ± 0.02[b]	1.82 ± 0.07[b]
1.20 ± 0.05	0.106 ± 0.007	6.6 ± 0.4
3.33 ± 0.11	0.051± 0.003	13.5 ± 0.8

a. The errors are 1 standard deviation.
b. The reaction rate and half-life were calculated using data from the first 10 minutes. After 10 minutes, the V(IV) concentration was below the range of the calibration curve.

For first-order reactions, the half-life is independent of the initial reactant concentration [154]. The oxidation reaction appears to be first order for the spike concentration below 1 mg/L. The rate constants, *k*, were compared using t-test (95% confidence interval) to determine if *k* was independent of the initial spiking concentration. The *k* for the 0.57 mg/L spike was statistically different from *k* for the 0.46 mg/L and 0.85 mg/L spikes which were statistically the same.

Based on Wehrli and Stumm's work [153], a one degree temperature increase will increase *k* by a factor of 1.2. The temperature was not controlled during the experiments. For the 0.85 mg/L spike, the temperature was 23.0 °C at the beginning of the experiment and reached 25.5 °C by the end of the one hour

mixing period. The initial temperature of the 0.57 mg/L spike experiment was 24.5 °C and for the 0.46 mg/L spike experiment, it was 24.0 °C. The rate constant for the 0.57 mg/L was divided by a factor of 1.2 and compared to the other rate constants. They were statistically the same. Such temperature differences could account for the difference in the observed k values. The half-life was independent of the initial V(IV) concentration for concentrations below 1 mg/L.

In order to use pseudo-first-order conditions, all the reactants except the one under investigation must have a constant concentration. The reactants must therefore be present in excess, a 10-fold excess being considered sufficient [155]. Okamura et al. [156] proposed that dissolved oxygen was the cause of the oxidation of V(IV) spiked into Milli-Q water. The concentration of oxygen in water saturated with air at 1 atm at 25 °C is 8.3 mg/L (258.22 μM) [157], and the concentration in seawater with a salinity of 40 is 6.6 mg/L (205.66 μM) [158]. The sample matrix is closer to that of seawater than fresh water so the dissolved oxygen concentration would be closer to 6.6 mg/L. In the experiments with the V(IV) spikes of concentrations below 1 mg/L, the oxygen was always in excess. For the 1.20 mg/L (23.6 μM) and 3.33 mg/L (65.3 μM) spikes, the vanadium was 11.5% and 31.8% of the concentration of the dissolved oxygen. The oxygen was therefore not present in excess. This could possibly explain why the rate constant and half-life for the higher test concentrations were different than those of the lower test concentrations. Pseudo-first-order conditions could not be applied.

The concentration of V(V) towards the end of the analysis is never as high as the initial V(IV) concentration. Some of the vanadium may be bound to the

suspended matter in the OSPW. Since the sample is filtered before analysis, this amount of bound vanadium would not be determined in the analysis. To confirm this hypothesis, V(IV) could be added to filtered and non-filtered OSPW. The change in vanadium speciation would then be observed over the course of an hour. If the total vanadium concentrations are different, then the suspended matter is the source of the difference.

The half-life values for the oxidation of V(IV) in OSPW ranged from 1.5 to 1.8 minutes for solutions spiked with concentrations below 1 mg/L. These values are much lower than those determined by Okamura et al. [156] for lake water and seawater spiked with V(IV), which were 15 minutes and 7 minutes, respectively. It should be noted that they tested only one spiking concentration for each water type.

Future work could include a more in-depth study of the oxidation of V(IV) to V(V) in OSPW. Modifications to the experimental design would be required. A more turbulent mixing (use of a Teflon coated magnetic stir bar) would promote better mixing of the initial V(IV) spike with OSPW and ensure that the solution is continually saturated with air. It has previously been shown that if the concentration of V(IV) exceeds the dissolved oxygen concentration, the initial oxidation is rapid and then slows down to a rate equal to the rate of oxygen diffusion into the solution [159].

To determine the different parameters affecting the oxidation of V(IV), the OSPW would require different treatments. Given the complexity of OSPW, there is potential for multiple oxidizers to be present. If dissolved oxygen is the only

oxidizing agent in OSPW, the rate of reaction would be different in the presence and absence of oxygen. Bubbling oxygen through the system would saturate OSPW with oxygen. Conversely, bubbling nitrogen would remove any dissolved oxygen. If the rate of reaction is higher for the OSPW saturated in oxygen, then oxygen is most likely the only oxidizing agent. If the determined half-lives are similar, then oxygen is not the main oxidizer. Pyrzynska and Wierzbicki [160] found that degassing a lake water sample (pH 7.1) for 20 minutes slowed the oxidation of added V(IV) over the course of 20 days.

Performing the test in a temperature controlled environment will allow for a better determination of the half-life of V(IV) in OSPW as reaction rates are temperature dependent. Determining the total organic carbon (TOC) and testing filtered versus non filtered OSPW would give information on the effect of dissolved organic substances on the oxidation of V(IV) and reduction of V(V) [74]. Different batches of OSPW should be tested as each batch has a different composition.

3.5. OPTIMIZATION OF THE EDTA CONCENTRATION

The concentration and species of chemicals that leach into water from petroleum coke affects the toxicological effects of the water. The most toxic species can leach or alternatively, a less toxic species can leach and then be converted to a more toxic form in the environment. It has been proposed that coke might be used as a medium to absorb naphthenic acids from OSPW, thereby removing naphthenic acids from OSPW. However, a concern is the potential leaching of other chemicals, for example vanadium, from the coke. It is not known what species of vanadium are leached into OSPW from the coke. Preserving the

speciation of vanadium as it leaches from coke would help determine which species leaches.

The effect of the EDTA concentration on the concentration and species of vanadium leached after 12 hours of mixing was investigated. The choice of the mixing period was based on work by collaborators [152]. They studied the adsorption of naphthenic acids from OSPW onto coke with different mixing times and ratios. From their preliminary work, they determined the optimal mixing period for the removal of naphthenic acids to be 12 hours. This time was used to test the fate of vanadium during mixing.

The different Na_2EDTA concentrations investigated were 1, 2.5, 5, 10 and 15 mM EDTA as well as a control (0 mM EDTA). Concentrated EDTA solutions were prepared and added to the OSPW to obtain a solution containing the desired concentration. The solution was added to coke in a 20% coke:water ratio. All mixtures were prepared in duplicate and mixed for 12 hours on an orbital shaker (270 rpm, 21.0 °C, New Brunswick Scientific, Enfield, Connecticut, USA). A coarse gravity filtration was performed using 185 mm Whatman no. 2 Qualitative filter paper, then a vacuum filtration was performed on the filtrate to remove the finer particulates using a SUPELCO Nylon 66 membrane (0.45 µm x 47 mm, SUPELCO, Bellefonte, Pennsylvania, USA). The supernatant was diluted with 2.5 mM Na_2EDTA to a concentration that fell within the range of the calibration curve. Each sample was analyzed in duplicate using the HPLC-ICP-MS method described in Chapter 2.

A sample chromatogram for this study can be seen in Figure 3.6. Both V(IV) and V(V) are present in the supernatant of the coke and OSPW mixture (20% coke:OSPW).

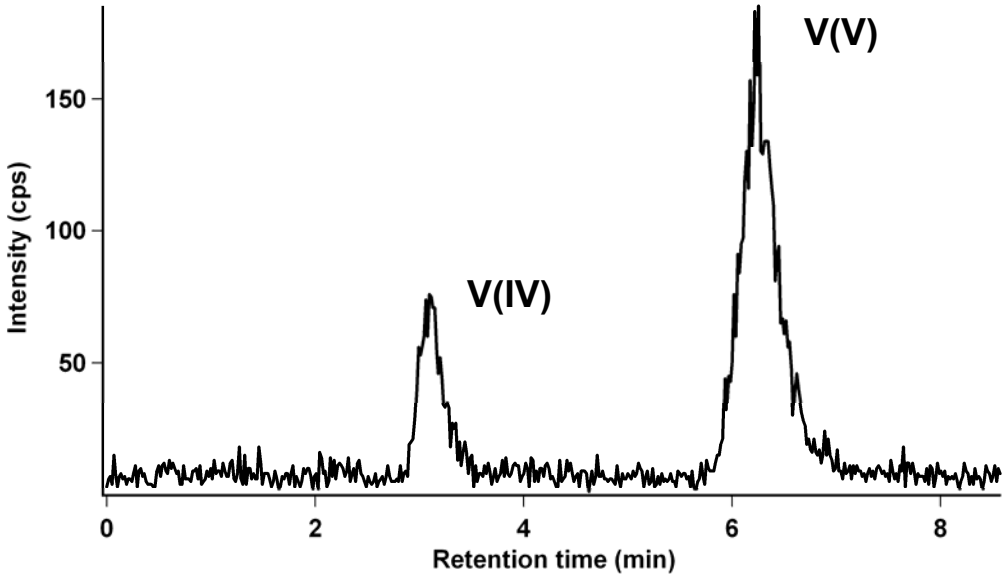

Figure 3.6. Chromatogram of supernatant from 10 mM EDTA solution, 20% coke:OSPW, 1:100 dilution. The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

The concentrations of V(IV), V(V) and total V present in the supernatant were determined (Figure 3.7). The V(V) concentrations were blank subtracted using the initial V(V) concentration in the OSPW as the blank concentration. The supernatant concentrations were converted into µg V/g coke for easier comparison (Figure 3.8). The results were compared statistically using a Tukey test.

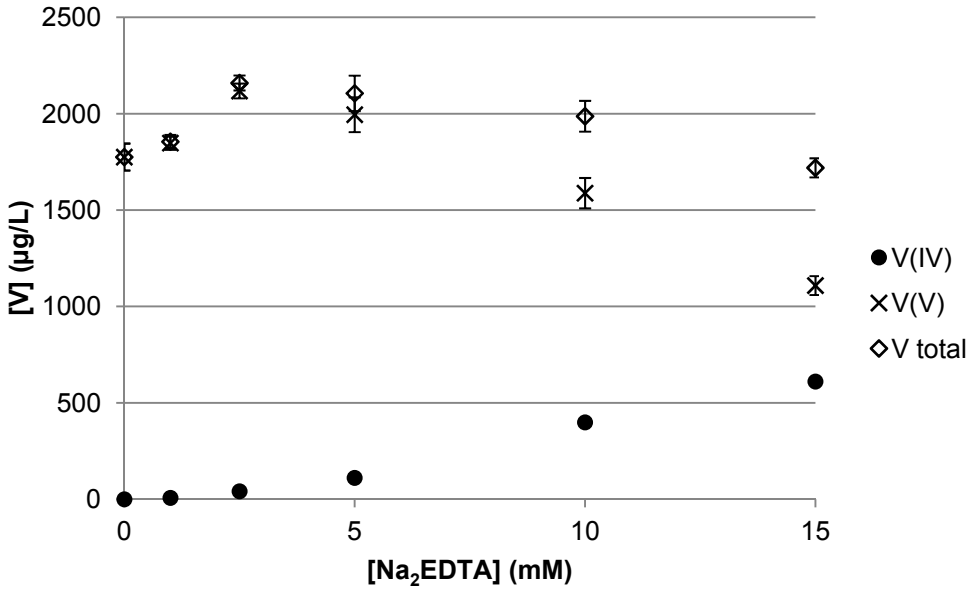

Figure 3.7. Concentration of leached vanadium in the supernatant of a 20% coke:OSPW mixture with different EDTA concentration. Error bars are 1 standard deviation.

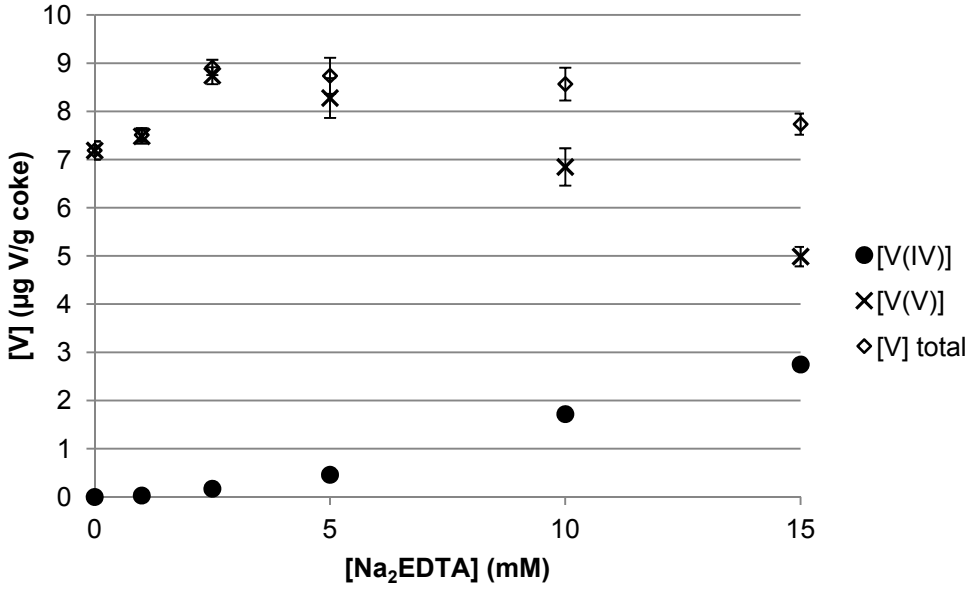

Figure 3.8. Concentration of leached vanadium from coke in a 20% coke:OSPW mixture with different EDTA concentration. Error bars are 1 standard deviation.

The amount of V(IV) leached from the coke increased as the concentration of EDTA increased. Only 0 and 1 mM EDTA gave a V(IV) concentration of 0 µg/g. A linear relationship can be established for EDTA concentrations of 2.5 mM and higher to the amount of vanadium leached ([V(IV)] = 0.1717[EDTA], R^2 = 0.9401).

The V(V) concentration increased with the EDTA concentrations to a maximum at 2.5 mM, beyond which there was no further increase. The total vanadium concentration varied between 7.1 ± 0.2 µg V/g coke and 8.8 ± 0.2 µg V/g coke. Statistical analysis indicated that there was no correlation between the total vanadium leached and EDTA concentration. The values for 2.5 mM, 5 mM and 10 mM were statistically the same while those for 1 mM and 15 mM were statistically the same.

To find the optimal EDTA concentration to use for the leaching studies, a few conditions must be met. The maximum concentration of total vanadium leached and the presence of V(IV) in the supernatant are the main ones. The maximum amount leached was for 2.5 mM, 5 mM and 10 mM EDTA. These concentrations were therefore options for optimal EDTA concentration. The 2.5 mM Na_2EDTA solution has a concentration that is more similar to the concentration in the mobile phase (2 mM EDTA) than the other solutions tested. Given these reasons, 2.5 mM EDTA was chosen as the optimal EDTA concentration for studying the leaching of vanadium from coke.

3.6. EFFECT OF THE COKE-TO-WATER RATIO

Once the optimal EDTA concentration was determined, it was applied to study the effect of the coke to water ratio on the amount and species of vanadium leached after 12 hours of mixing.

In the study of the effect of the coke-to-water ratio, there are two possibilities. In the first, the amount of vanadium leached per gram of coke is independent of the mixing ratio. For higher mixing ratios, there would be a higher concentration in the supernatant because of a larger source of vanadium but less water for it to leach into. In the second, the amount of vanadium leached is affected by the mixing ratio. If the maximum amount of vanadium is dissolved in the water, then there would be more vanadium per gram of coke leached for lower mixing ratios. Even with a smaller source of vanadium, in the presence of a larger volume of water, more vanadium could desorb thus increasing the mass of vanadium leached per gram of coke.

The coke to water ratios investigated were 0%, 5%, 10%, 20%, 30%, and 40% by weight. Both OSPW and DIW were used in this study. A concentrated Na_2EDTA solution was added to the water, either OSPW or DIW, to give a 2.5 mM Na_2EDTA solution. The solution was then added to coke to obtain the desired coke:water ratio. The samples were prepared, mixed, filtered, diluted, and analyzed using the same procedure as the samples for the optimization of the EDTA concentration.

A sample chromatogram obtained from this study can be seen in Figure 3.9.

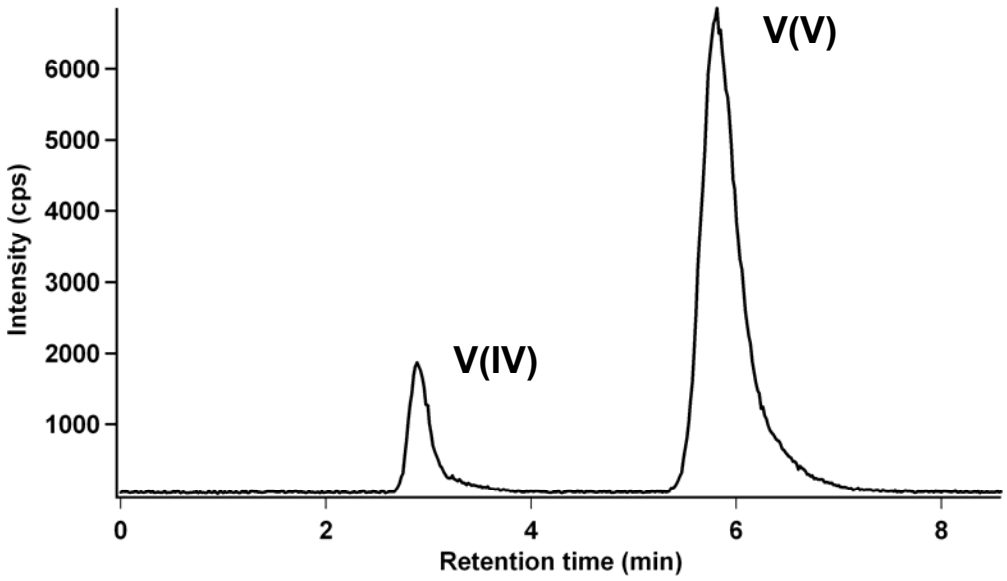

Figure 3.9. Chromatogram of supernatant from 30% coke:DIW, 2.5 mM Na_2EDTA solution, 1:10 dilution. The eluent was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate and pH 6.

All data were compared using the Tukey test. The results are found in Table 3.2.

The supernatant concentrations were converted to µg V/g coke (Figures 3.10 to 3.12). The V(IV) levels leached from the coke are presented in Figure 3.10.

Table 3.2. Concentration of vanadium in the supernatant obtained from mixing coke with different ratios and types of water

% coke	OSPW			DIW		
	[V(IV)] (µg/L)	[V(V)] (mg/L)	[V] total (mg/L)	[V(IV)] (µg/L)	[V(V)] (mg/L)	[V] total (mg/L)
0	0 ± 0	0.000 ± 0.007	0.000 ± 0.007	0 ± 0	0.000 ± 0.001	0.000 ± 0.001
5	0 ± 0	0.54 ± 0.02	0.54 ± 0.02	0 ± 0	0.56 ± 0.02	0.56 ± 0.02
10	0 ± 0	1.08 ± 0.07	1.08 ± 0.07	37 ± 9	0.99 ± 0.06	1.03 ± 0.06
20	18 ± 5	2.45 ± 0.04	2.47 ± 0.04	176 ± 9	2.05 ± 0.06	2.22 ± 0.06
30	36 ± 6	4.55 ± 0.14	4.59 ± 0.14	359 ± 51	2.92 ± 0.13	3.28 ± 0.14
40	60 ± 5	8.29 ± 0.99	8.35 ± 0.99	530 ± 45	5.11 ± 0.17	5.64 ± 0.17

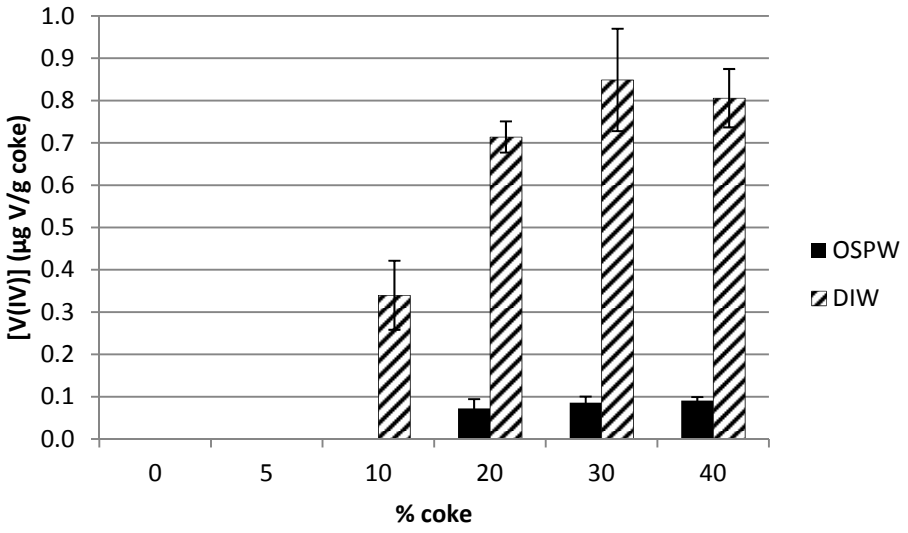

Figure 3.10. Concentration of leached V(IV) from coke for different coke:water ratios with 2.5 mM Na_2EDTA. Error bars are 1 standard deviation.

For the OSPW supernatant, no V(IV) was present in the 0%, 5% and 10% mixtures. There was V(IV) present for the higher coke:OSPW mixtures and the

concentrations were statistically the same (0.07 ± 0.02 to 0.09 ± 0.01 µg/g). For the DIW supernatant, there was a greater variation in the V(IV) concentrations. The 0% and 5% coke:DIW mixtures had no V(IV) present. The 10% coke:DIW mixture had a concentration of 0.3 ± 0.1 µg/g. The 20%, 30%, and 40% coke:DIW mixtures were statistically the same (0.71 ± 0.04 to 0.8 ± 0.1 µg/g). The type of water influenced the concentration of V(IV) leached from the coke. At 10%, 20%, 30% and 40% coke:water, the V(IV) was statistically different with higher concentrations in the DIW. Only for the 0% and 5% coke:water did the vanadium concentrations agree statistically as the V(IV) concentration was 0 µg/g.

The V(V) and total vanadium levels leached from the coke are presented in Figure 3.11 and 3.12 respectively.

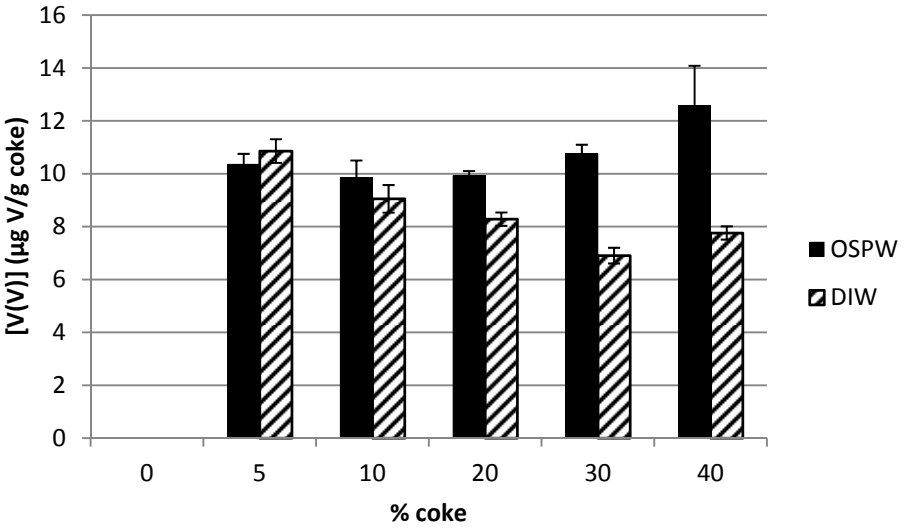

Figure 3.11. Concentration of leached V(V) from coke for different coke:water ratios with 2.5 mM Na_2EDTA. Error bars are 1 standard deviation.

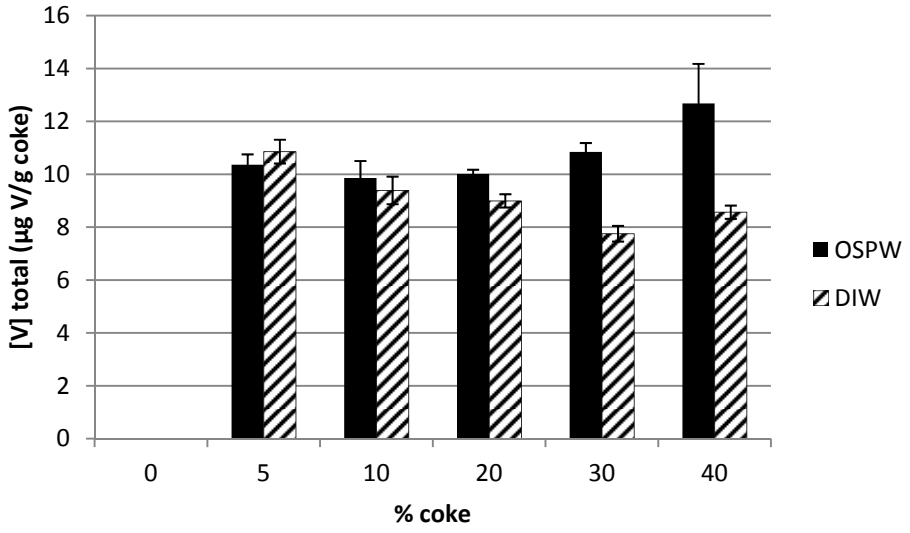

Figure 3.12. Concentration of leached total V from coke for different coke:water ratios with 2.5 mM Na_2EDTA. Error bars are 1 standard deviation.

For the 5 to 30% coke:OSPW mixtures, the amount of vanadium leached was statistically the same for both V(V) and total V concentrations. The highest concentration was with the 40% coke:OSPW ratio. This would indicate that the ratio of coke:OSPW does not affect the amount of vanadium leached.

For the DIW supernatant, the 20% and 40% coke:DIW mixtures were statistically the same for both V(V) and total V concentration. The other coke:DIW ratios were statistically different. The highest concentration was the 5% coke:DIW (10.9 ± 0.4 µg/g for both V(V) and total vanadium). The lowest concentration was 30% coke:DIW (6.9 ± 0.3 µg/g and 7.8 ± 0.3 µg/g for V(V) and total V respectively).

The effect of the water type was more pronounced when investigating the V(V) and total vanadium concentrations. The concentrations for 0%, 5% and 10% coke:water were the same statistically whether the leaching was performed in

OSPW or DIW. At higher coke:water ratios, more vanadium leached into the OSPW than into the DIW.

Kessler and Hendry [112] studied the leaching potential of elements from coke. They found that the water soluble fraction of V in the coke was 8.6 mg/kg. This is comparable to the values obtained in this leaching study (from 9.9 ± 0.6 to 12.7 ± 1.5 µg/g for OSPW and 7.8 ± 0.3 to 10.9 ± 0.4 µg/g for DIW).

In the supernatant for coke:water mixtures of 20% and higher, the concentrations of V(V) was higher than the LC50 for American flagfish (1.13 mg/L, 28 days) [49], juvenile rainbow trout (1.9 to 6.0 mg/L, 7 days) [50], and guppies (2.0 mg V/L, 7 days) [53]. The 30% and 40% coke:OSPW and the 40% coke:DIW supernatant had V(V) concentrations above the LC50 for zebrafish (2 to 3 mg V/L, 7 days), guppies (3.3 mg V/L, 7 days), and daphnids (3.4 to 4.8 mg V/L, 48h) [52]. The vanadium concentration in the supernatant could therefore have adverse effects on the aquatic animals that would be exposed to this water. This effect could be mitigated by using lower coke:water ratios as the amount of vanadium leached per gram of coke is independent of the mixing ratio.

More V(IV) leached into the DIW than into the OSPW and more leached for coke:water of 20% or higher. In the OSPW, more V(V) and total vanadium leached into the OSPW at higher coke:water ratios.

3.7. LEACHING PROFILE

The concentration and speciation of vanadium present in the supernatant after 12 hours was studied. The next question of interest was what occurred during the first few hours of mixing. The leaching could be gradual or could happen rapidly and reach a plateau after a few hours. Knowing the leaching profile could help minimize the amount of vanadium leached into OSPW. If the leaching was gradual, then as the mixing time increased, the amount of leached vanadium would increase and so would the toxicity of the water due to vanadium. If rapid leaching was followed by a slower leaching rate or even a plateau, then it would be possible to maximize the removal of naphthenic acids while minimizing the leaching of vanadium. A maximum amount of vanadium could also leach in a few hours. The increased mixing time required to reduce the concentration of naphthenic acids would not cause an increase in vanadium concentration and thus its toxicity.

Three flasks containing 250 mL of a 20% coke:OSPW mixture and 5 mM Na_2EDTA were prepared. The flasks were mixed on an orbital shaker (180 rpm, room temperature). At predetermined time intervals, a 1.00 mL aliquot was removed from the flask and filtered through a 0.45 µm nylon membrane. The aliquot was diluted in 5 mM Na_2EDTA to a concentration that fell within the range of the calibration curve. The change in speciation was monitored over a 7 hour period. The same experiment was performed on a different day using DIW instead of OSPW. The samples were analyzed by HPLC-ICP-MS using the method described in Chapter 2.

The results from the first 7 hours of leaching from coke into water can be seen in Figures 3.13 (OSPW) and 3.14 (DIW).

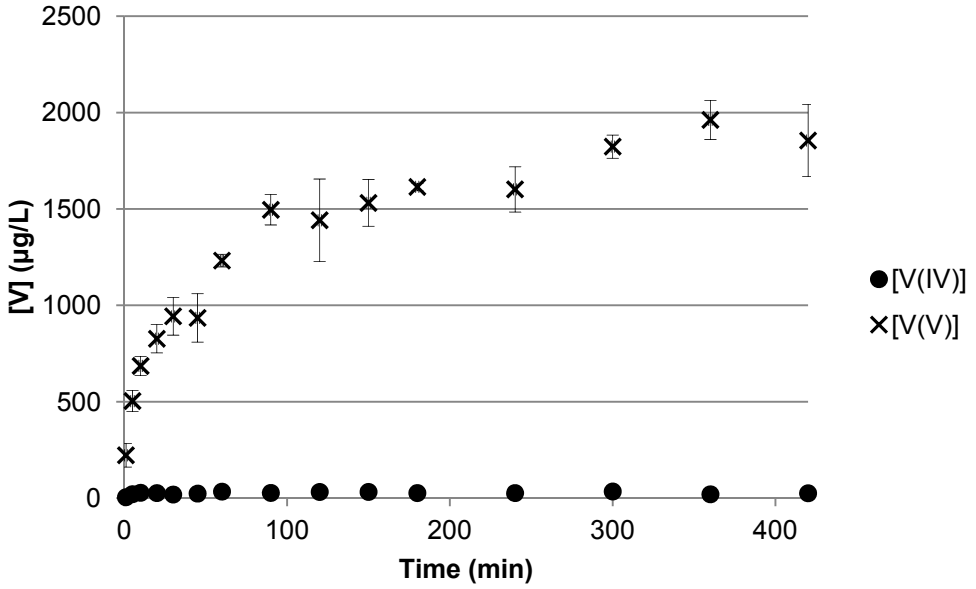

Figure 3.13. Concentration of leached vanadium from coke in a 20% coke:OSPW mixture with 5 mM Na_2EDTA. Error bars are 1 standard deviation. For clarity, total V was not shown but matches the V(V) concentration.

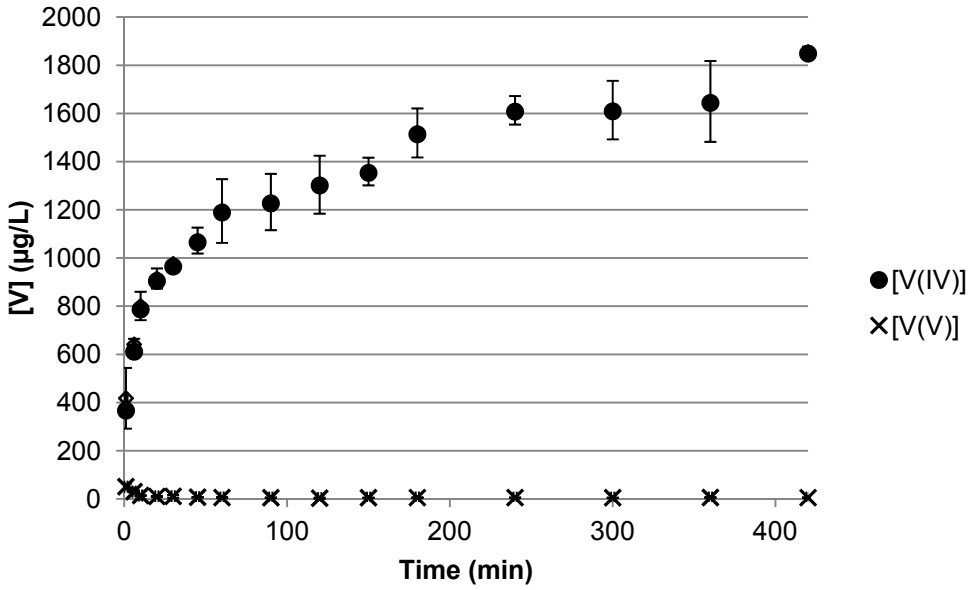

Figure 3.14. Concentration of leached vanadium from coke in a 20% coke:DIW mixture with 5 mM Na_2EDTA. Error bars are 1 standard deviation. For clarity, total V was not shown but matches the V(IV) concentration.

The speciation of the vanadium leached depended on the type of water used. The majority of the vanadium leached into OSPW was V(V), while in DIW, it was V(IV). The amount of vanadium leached seemed to be independent of the type of water used. In order to compare the leaching profiles, curve fitting was performed on the total vanadium leached.

A biphasic fitting[1] was used since a semi-log plot did not yield a good fitting curve. Equation 3.4 was used and the fitting can be seen for OSPW and DIW in Figures 3.15 and 3.16, respectively. The fitting parameters are found in Table 3.3.

$$V = V_f - \left\{ \frac{\left((V_i - V_f)k_1 + (V_o - V_f)k_2\right)e^{-k_1 t} + (V_f - V_i)k_1 e^{-k_2 t}}{k_1 - k_2} \right\} \qquad (3.4)$$

Where V_o is the vanadium concentration in supernatant at t = 0, V_i is the amount of V from the faster step, V_f is the final V concentration, k_1 is the rate constant for the faster step (minutes^{-1}), k_2 is the rate constant for the slower step (minutes^{-1}), t is time (minutes)

[1] Fitting performed by Dr. Robert B. Jordan, University of Alberta, Edmonton, Alberta, Canada

Table 3.3. Parameters from the biphasic fitting equation for the leaching of vanadium into OSPW or DIW

Parameter	OSPW	DIW
V_o (mg/L)	0.1413	0.3465
V_i (mg/L)	0.9231	0.9271
V_f (mg/L)	2.450	2.045
k_1(minutes^{-1})	0.1204	0.1338
k_2 (minutes^{-1})	0.003469	0.003717

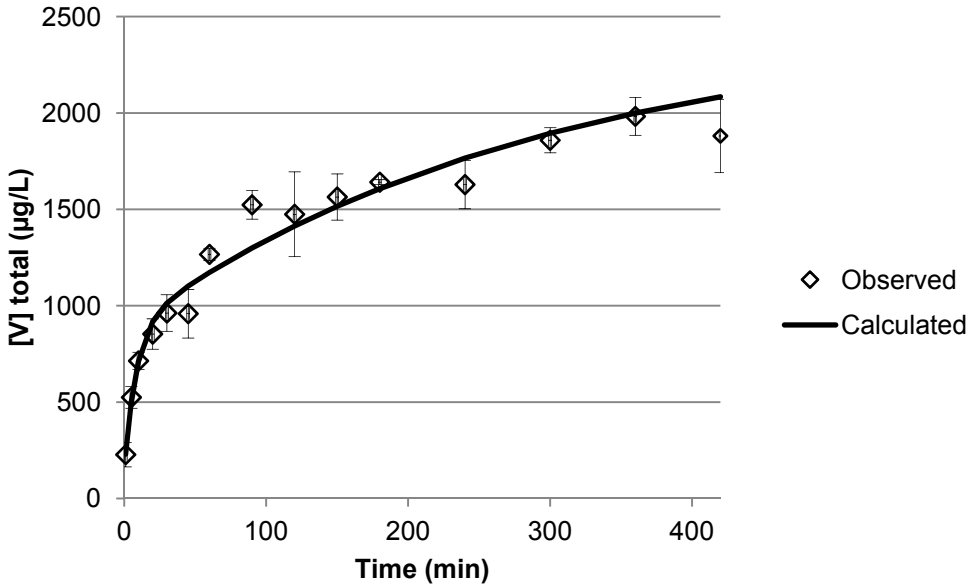

Figure 3.15. Observed concentration of total vanadium in the supernatant of a 20% coke:OSPW mixture with 5 mM Na_2EDTA and the calculated values using a biphasic fitting equation. Error bars are for the observed results and represent 1 standard deviation.

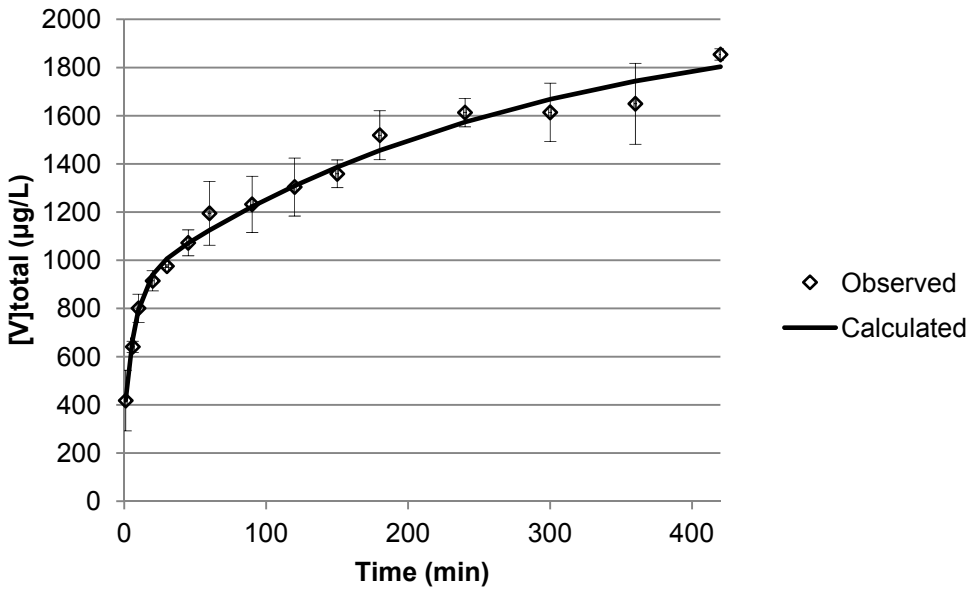

Figure 3.16. Observed concentration of total vanadium in the supernatant of a 20% coke:DIW mixture with 5 mM Na_2EDTA and the calculated values using a biphasic fitting equation. Error bars are for the observed results and represent 1 standard deviation.

The value used for V_f came from the 12 hour leaching test (Section 3.5). The calculated V_o is higher than is actually present in the water initially. This is mostly due to the experimental design. For example, samples were taken at 1 minute for each replicate. The sampling itself takes a few seconds so the time between the 1st and 3rd replicate could be 10 to 20 seconds. In the first few minutes of the experiment, those seconds are more significant as vanadium is rapidly leaching.

The biphasic shape of the leaching profile (Figures 3.15 and 3.16) suggests that multiple processes and mechanisms may be involved in the leaching of vanadium from coke. The first phase indicates a source of rapid leaching and is responsible for the rapid increase of vanadium in the supernatant over the course of the first two hours. The second is responsible for the slower increase of

vanadium in the supernatant after the first few hours of mixing. The vanadium concentrations after 7 hours of mixing were lower than those after 12 hours of mixing indicating that there is continual leaching occurring.

No further investigation was performed to determine what the processes or mechanisms were. More work could be performed by studying the leaching over the course of several days or weeks to determine if there is a maximum amount of vanadium that does leach from the coke.

3.8. CONCLUSIONS

The only vanadium species present in OSPW is V(V). The main reason is that V(IV) oxidizes when added to OSPW unless a preserving agent is present. EDTA can be used as a way to prevent the oxidation of V(IV) for a short period of time. In the absence of EDTA, the half-life of V(IV) in OSPW at concentrations below 1 mg/L is 1.5 to 1.8 minutes. At higher concentrations, the half-life is longer. Vanadium leaches as both V(IV) and V(V) from coke when it is mixed with water. However, when mixed with OSPW, any V(IV) that leached is oxidized to V(V). A less toxic species, V(IV), leached but was converted to the most toxic oxidation state of vanadium, V(V). The coke-to-water ratio does not seem to influence the amount of vanadium that leaches per gram of coke. For coke:water ratios of 20% and higher, the concentration of vanadium in the supernatant was higher than the LC50 for several aquatic species (American flagfish, juvenile rainbow trout, and guppies). The coke-to-water ratio can therefore be optimized to minimize leaching of vanadium from coke. Increasing the leaching time would increase the amount of leached vanadium. There appear to be at least two different types of

sites on coke from which vanadium can leach. The second type shows a slower rate of release as the concentration in the supernatant increases continually over the course of 7 hours without reaching the concentration obtained after 12 hours of mixing.

Chapter 4. Stabilizing Vanadium Species

4.1. INTRODUCTION

The common practice of sample acidification prevents metal adsorption onto container walls. However, acidification could affect the chemical species present in the sample. Unlike the determination of total element concentration, speciation analysis requires appropriate storage and preservation of samples to prevent changes in the chemical species.

Okamura et al. [156] studied the behaviour of vanadium in acidified lake water, seawater, and Milli-Q water (pH 2.0). They found that V(V) in the natural waters was reduced completely within a day while in the acidified Milli-Q water, V(V) was stable. The organic matter present in the lake water and seawater were thought to cause the reduction. Nukatsuka et al. [74] tested the speciation in artificial and natural seawater samples during storage. In an acidified natural seawater sample (pH 2.0), V(V) was reduced within 24 hours. They concluded that seawater samples should not be acidified if the goal is vanadium speciation. The general conclusion from these studies was that samples must be analyzed as quickly as possible after collection.

Other groups have tried preserving vanadium speciation in water samples. In the analysis of samples from the Athabasca Oils Sands, Li [144] added twice the molar ratio of EDTA acid to vanadium and allowed the samples to sit for 20 minutes. She found that any V(IV) present in the original samples had oxidized after 3 days of storage in at 4 °C. The three samples tested had concentrations

of V(IV) ranging from 48 µg/L to 1.1 mg/L and V(V) concentrations from 30 µg/L to 5.1 mg/L. The pH of the samples ranged from 8.1 to 8.4.

Aureli et al. [82] added Na_2EDTA to bottled water samples to reach a concentration of 25 mM. The samples were stable over a one week period when stored at room temperature. Speciation on 10 different bottled water samples was performed. Only 3 samples contained V(IV) and V(V), while the others contained only V(V). The vanadium concentrations were much lower than in Li's samples, V(IV) ranged from 0.57 to 3.19 µg/L and V(V) from 5.07 to 46.70 µg/L.

Sample analysis immediately after collection is not always possible due to the distance between the sample collection site and the laboratory, the remoteness of the sample collection location, and instrument availability. Therefore a method to stabilize vanadium species was developed.

4.2. METHODOLOGY

Different types and concentrations of EDTA were investigated as well as different storage temperatures. The types of EDTA of interest were Na_2EDTA and Na_4EDTA. The first was tested at 2.5 mM and 5 mM concentrations and the second at 2.5 mM. EDTA is used to preserve the speciation in the vanadium standards for several months. However, the solubility of EDTA is low compared to Na_2EDTA and Na_4EDTA [161-163] so these chemicals were chosen to be tested. The storage temperatures tested were room temperature (20 °C ± 5 °C) and 4 °C.

A 20% coke:OSPW (40 g coke, 160 mL OSPW) mixture was prepared in triplicate and mixed for 12 hours on an orbital shaker (270 rpm, 21.0 °C, New Brunswick Scientific, Enfield, Connecticut, USA). A coarse gravity filtration was performed using 185 mm Whatman No. 2 Qualitative filter paper, then a vacuum filtration was performed on the filtrate using a SUPELCO Nylon 66 membranes (0.45 µm x 47 mm, SUPELCO, Bellefonte, Pennsylvania, USA). The supernatant for each replicate was diluted 100 fold using the appropriate EDTA solution. The sample was either spiked with unchelated V(IV) (vanadyl sulfate hydrate, Aldrich, St. Louis, Missouri, USA) for a nominal spike concentration of 20 µg/L or not spiked. The samples were stored at room temperature or 4 °C in 15 mL polyethylene terephthalate (PET) conical vials (Corning Incorporated, Corning, New Jersey, USA), sealed with parafilm.

The samples were analyzed over a period of 56 days. Testing was performed on the first two days of the study, weekly for the next two weeks, then randomly until the end of the study. Before each analysis, the vial was shaken, vortexed, then shaken again. A 0.25 mL aliquot was removed and filtered through a 0.45 µm nylon filter (PALL Life Sciences Acrodisc 13 mm Syringe Filter, Pall (Canada) Ltd., Mississauga, Ontario, Canada). For samples stored in the fridge, the vial was only removed just before it was sampled. It was replaced in the fridge within 5 minutes of removal. Filtered samples were allowed to reach room temperature before injection. The samples were analyzed once per day by HPLC-ICP-MS using the method described in Chapter 2.

4.3. RESULTS AND DISCUSSION

The V(IV), V(V), and total V concentration from the supernatant of a 20% coke:OSPW mixture diluted 100-fold with an EDTA solution and either spiked with 20 µg/L V(IV) from vanadyl sulfate or not spiked are shown (Figures 4.1 through 4.6).

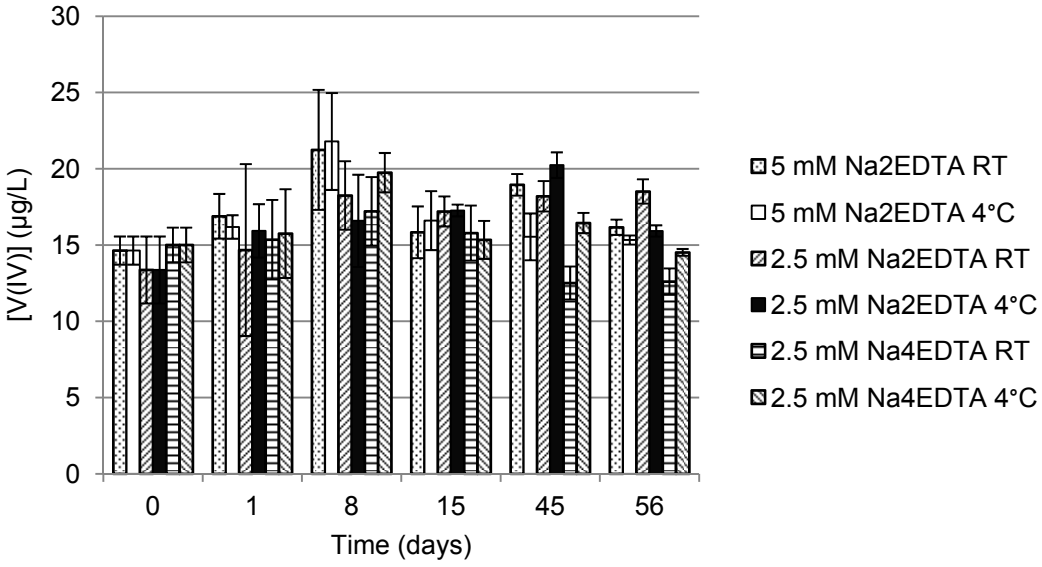

Figure 4.1. V(IV) concentration in the supernatant from an OSPW and coke mixture diluted 100-fold and spiked with 20 µg/L V(IV). The samples contained 5 mM Na_2EDTA, 2.5 mM Na_2EDTA, or 2.5 mM Na_4EDTA and were stored either at room temperature (RT) or at 4 °C for 56 days. Error bars are 1 standard deviation.

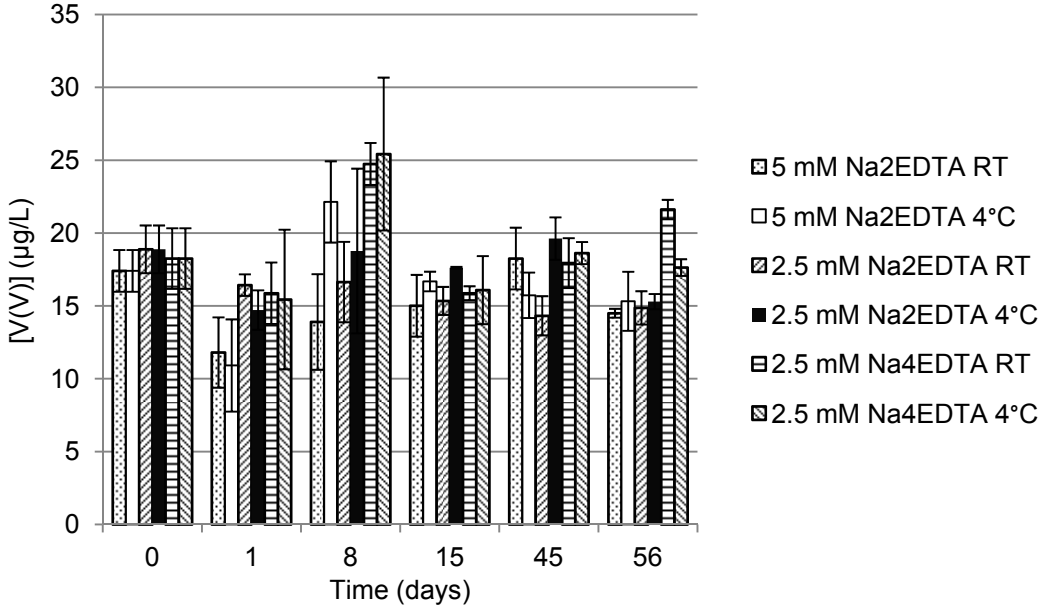

Figure 4.2. V(V) concentration in the supernatant from an OSPW and coke mixture diluted 100-fold and spiked with 20 µg/L V(IV). The samples contained 5 mM Na_2EDTA, 2.5 mM Na_2EDTA, or 2.5 mM Na_4EDTA and were stored either at room temperature (RT) or at 4 °C for 56 days. Error bars are 1 standard deviation.

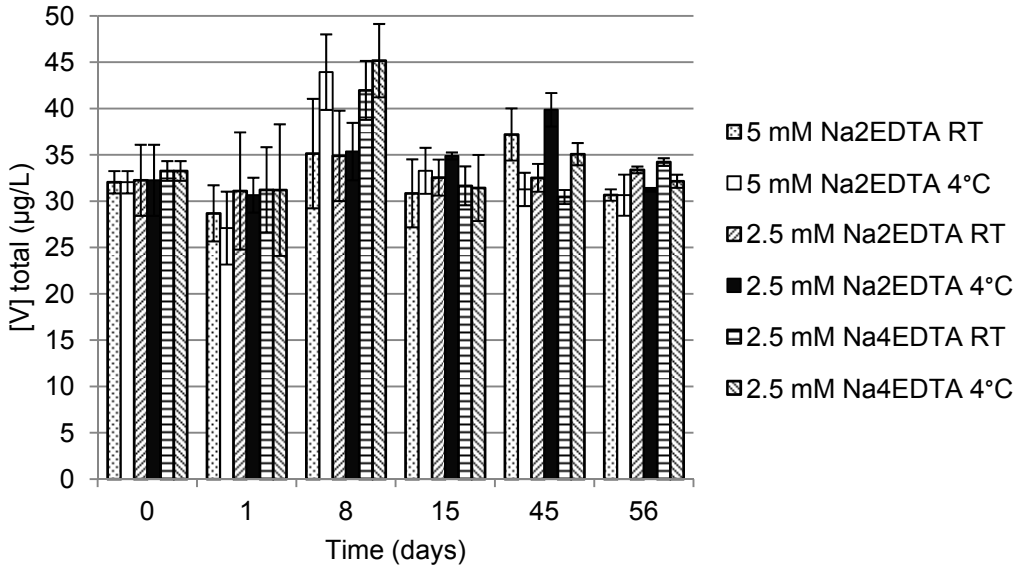

Figure 4.3. Total vanadium concentration in the supernatant from an OSPW and coke mixture diluted 100-fold and spiked with 20 µg/L V(IV). The samples contained 5 mM Na_2EDTA, 2.5 mM Na_2EDTA, or 2.5 mM Na_4EDTA and were stored either at room temperature (RT) or at 4 °C for 56 days. Error bars are 1 standard deviation. Total vanadium is the sum of V(IV) and V(V) from the speciation.

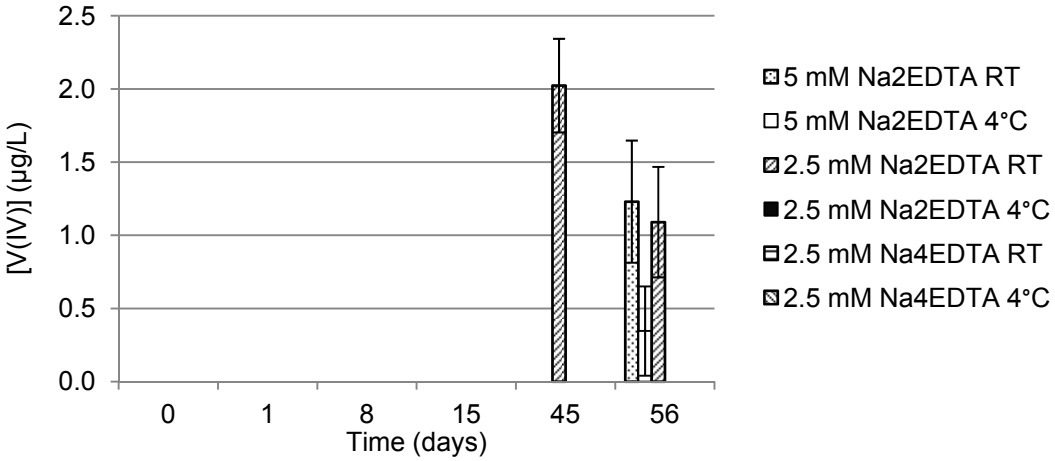

Figure 4.4. V(IV) concentration in the supernatant from an OSPW and coke mixture diluted 100-fold. The samples contained 5 mM Na_2EDTA, 2.5 mM Na_2EDTA, or 2.5 mM Na_4EDTA and were stored either at room temperature (RT) or at 4 °C for 56 days. Error bars are 1 standard deviation.

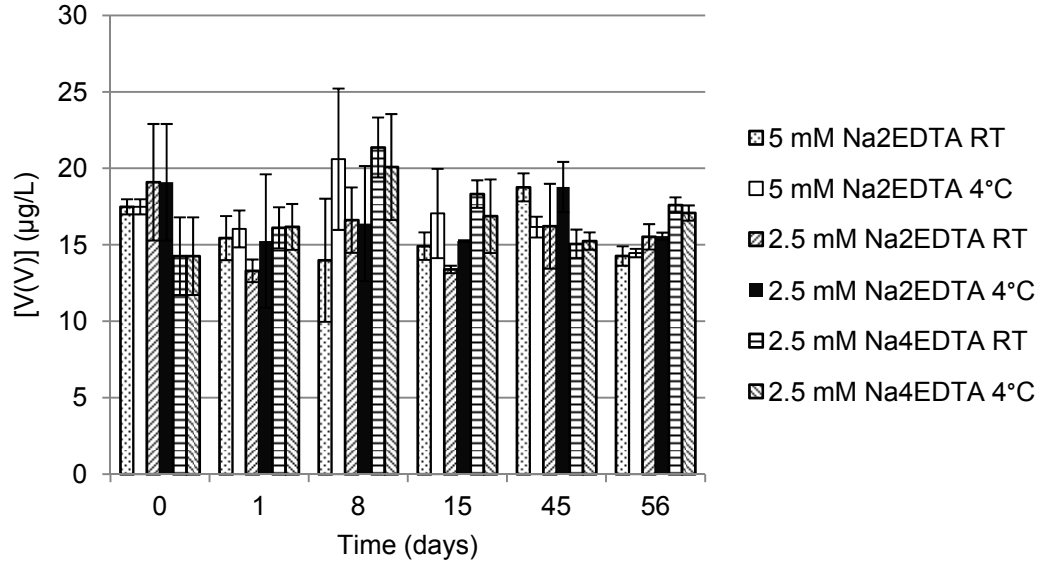

Figure 4.5. V(V) concentration in the supernatant from an OSPW and coke mixture diluted 100-fold. The samples contained 5 mM Na_2EDTA, 2.5 mM Na_2EDTA, or 2.5 mM Na_4EDTA and were stored either at room temperature (RT) or at 4 °C for 56 days. Error bars are 1 standard deviation.

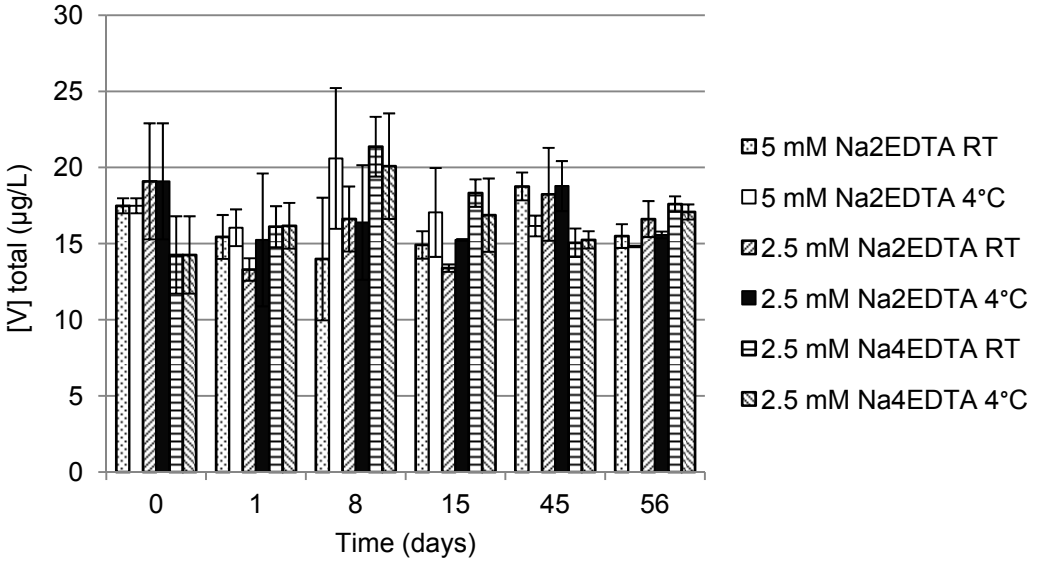

Figure 4.6. Total vanadium concentration in the supernatant from an OSPW and coke mixture diluted 100-fold. The samples contained 5 mM Na_2EDTA, 2.5 mM Na_2EDTA, or 2.5 mM Na_4EDTA and were stored either at room temperature (RT) or at 4 °C for 56 days. Error bars are 1 standard deviation. Total vanadium is the sum of V(IV) and V(V) from the speciation.

A linear regression was performed to determine the slope of the concentration vs. time for each preservation condition. The slope was then compared to a slope of 0 using GraphPad Prism 5 software. The closer the slope value was to 0, the smaller the variation in the concentration of each species present in the sample. In order to consider a storage method valid, all samples, both spiked and non-spiked, stored under a specific set of conditions had to be stable in both speciation and total concentration. The slopes and associated errors are found in Table 4.1.

Table 4.1. Slopes and errors for test preservation conditions

		Non-spiked			Spiked		
Concentration and EDTA type	Storage temperature	V(IV)	V(V)	V total	V(IV)	V(V)	V total
5 mM Na_2EDTA	Room temperature	0.015 ± 0.007	0.006 ± 0.040	0.02 ± 0.03	0.008 ± 0.050	0.03 ± 0.05	0.04 ± 0.06
	4 °C	0.004 ± 0.002	-0.05 ± 0.03	-0.04 ± 0.03	-0.03 ± 0.05	-0.01 ± 0.07	-0.05 ± 0.12
2.5 mM Na_2EDTA	Room temperature	0.030 ± 0.009	-0.005 ± 0.046	0.03 ± 0.05	0.07 ± 0.03	-0.05 ± 0.02	0.01 ± 0.03
	4 °C	0 ± 0	0.0007 ± 0.0371	0.0007 ± 0.0371	0.05 ± 0.04	-0.006 ± 0.042	0.04 ± 0.07
2.5 mM Na_4EDTA	Room temperature	0 ± 0	-0.005 ± 0.054	-0.005 ± 0.054	-0.06 ± 0.02	0.03 ± 0.07	-0.03 ± 0.09
	4 °C	0 ± 0	-0.001 ± 0.042	-0.001 ± 0.042	-0.02 ± 0.03	-0.01 ± 0.07	-0.03 ± 0.11

The V(IV) in the spiked samples stored at room temperature in 2.5 mM Na_4EDTA (Figure 4.1) had a slope (-0.06 ± 0.02) that differed significantly from 0. Therefore, that storage method was not considered suitable. V(IV) was first found in non-spiked samples on day 45 in all three samples stored at room temperature in 2.5 mM Na_2EDTA (Figure 4.4). On day 56, V(IV) was detected in 5 out of 6 non-spiked samples preserved with 5 mM Na_2EDTA. The sixth sample was stored in the fridge. Because of this, 5 mM Na_2EDTA was not considered as an appropriate storage solution. Since none of the samples stored at room temperature preserved the vanadium speciation, storage at 4 °C was determined as the preferred storage temperature.

Of the two remaining EDTA solutions, the 2.5 mM Na_2EDTA resulted in the lowest variation in concentration of V(V) for both the spiked and non-spiked samples (Figures 4.2 and 4.5) while 2.5 mM Na_4EDTA gave the lowest variation in V(IV) and total vanadium concentration for the spiked samples (Figures 4.1 and 4.3). The only species present in the non-spiked samples for these storage conditions was V(V), 2.5 mM Na_2EDTA kept the non-spiked samples more stable. For the spiked samples, the difference in concentration change between the 2.5 mM Na_2EDTA samples and the 2.5 mM Na_4EDTA samples was negligible. Given that both methods were equivalent, storing samples in the 4 °C fridge in a 2.5 mM Na_2EDTA solution was selected as the optimal storage method.

4.4. CONCLUSIONS

A method to preserve vanadium speciation in water samples was developed. Storing the samples in a 2.5 mM Na_2EDTA solution at 4 °C conserved the speciation and concentration for a period of 56 days.

The method was applied to real water samples from an acute toxicity study of vanadium (Chapter 5). More work could be performed to determine whether the storage method is valid for a longer period of time with weekly or monthly testing. Expanding the application of the method by determining if the vanadium speciation in higher concentration samples is preserved is another area that should be considered.

Chapter 5. Vanadium Speciation of Tissue and Water Samples from a Vanadium Toxicity Test on *Hyalella azteca*

5.1. INTRODUCTION

Hyalella azteca is a benthic freshwater shrimp commonly used in toxicity studies [130, 138, 164]. As part of the Integrated Oil Sands Environment Monitoring Program, *H. azteca* are used to determine the toxicity of water and sediment samples collected from various water sources in the Athabasca Oil Sands region [140].

There have been no studies on the exposure, uptake, or metabolism of different chemical species of vanadium to *H. azteca*. As part of a collaboration with Environment Canada and the University of Waterloo, the vanadium species were determined in the test waters and tissues of *H. azteca* following 7-day exposure to vanadium spiked water. Determining the uptake and acute toxicity of vanadium allows for a better understanding of the effect of vanadium on an aquatic system. To complement the standard practice of total analysis, speciation of the water and tissue samples was performed. The speciation analysis would determine to which vanadium species the animals were exposed and possible speciation changes that may have occurred after ingestion and metabolism. Vanadium speciation and bioaccumulation results can help decide the type of testing required, speciation or total analysis, in the assessment and future monitoring of the Athabasca Oil Sands region [140].

5.2. METHODOLOGY

5.2.1. Study Set-up Involving *H. azteca*

A series of test concentrations (25, 141, 451, and 1410 µg V/L for V(IV), and 19, 106, 339, and 1060 µg V/L for V(V)) was generated based on 23-day LC50 and the 23-day no observed lethal concentration (NOLC) for daphnids (*Daphnia magna*) for sodium metavanadate (2.0 mg V/L and 1.6 mg V/L, respectively) [52] and the one-week LC50 for *H. azteca* (1251 µg/L for V from an AAS standard and 368 µg/L for sodium orthovanadate) [137, 140]. The toxicity studies and total analysis were performed at Environment Canada, Burlington, Ontario, Canada. Vanadium speciation was performed at the University of Alberta, Edmonton, Alberta, Canada.

Vanadyl sulfate hydrate (Sigma-Aldrich, St. Louis, Missouri, USA) was used to prepare the test solutions for V(IV) and sodium metavanadate (Sigma-Aldrich, St. Louis, Missouri, USA), for the V(V) solutions. A blank group (without any *H. azteca*) and a control group (no addition of vanadium) were also prepared. For each of the nine experimental conditions, four replicates were prepared. Water samples were collected from all replicates for speciation analysis. For total analysis of the water, samples were taken only from the first replicate.

The dechlorinated (deChlor) water used for the experiments was Burlington city tap water (Ontario, Canada) which uses Lake Ontario water as a water source. The tap water is dechlorinated by passing it through activated charcoal filter cartridges and then aerated vigorously for two weeks [165].

For the set-up of the experiment and at the beginning of each renewal cycle, 2.5 mg of Tetra-Min fish food (Ulrich Baensch, Melle, Germany) was placed in each 400 mL plastic beaker. DeChlor water (200 mL) was added followed by the addition of the vanadium stock solution and then another 200 mL of deChlor water. A 5 x 5 cm piece of 100% cotton gauze was added to each container. The contents of the beakers were allowed to sit for 3–4 hours. Water samples were collected for speciation (all replicates) and total analysis (first replicate only) (Section 5.2.2) immediately before 20 *H. azteca* (6–10 weeks old) were added to the containers. The containers were placed in an incubator that was maintained at 25 °C and operated on a cycle of 18 hours of light and 6 hours of darkness. Water renewals were performed on days 0, 2, and 5. They are designated Day 0, Day 2.1 and Day 5.1, respectively, as shown in Figure 5.1.

At the end of each renewal period (Day 2, Day 5, and Day 7 on Figure 5.1), water samples were collected for analysis (Section 5.2.2). The contents of the containers were transferred into a sorting bowl and the surviving *H. azteca* were transferred to a petri dish, counted, and transferred into the appropriate fresh renewal solution.

For example, on Day 2, water samples were taken from the beaker for speciation and total analysis. The samples were designated Day 2. The surviving *H. azteca* were removed from the beaker, counted, and transferred to a freshly prepared solution containing vanadium. Prior to the addition of the animals, water samples, designated as Day 2.1, were collected from this fresh solution for speciation and total analysis. After three days of exposure, water samples were collected from the beaker and designated Day 5.

Tissue samples were collected on Day 7 (Section 5.2.3). This “static renewal” experimental design follows previously reported procedures [132, 164, 166].

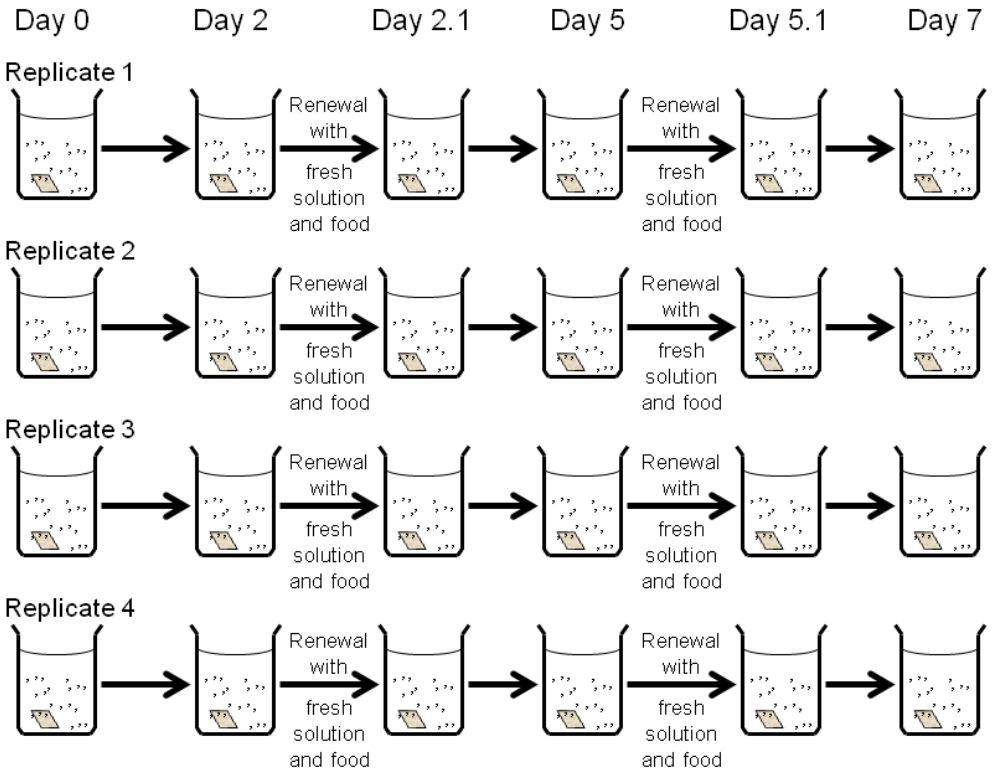

Figure 5.1. Experimental set-up for a 7-day acute toxicity study on *H. azteca* for a single test concentration. On Day 0, 20 *H. azteca* were added to freshly prepared vanadium solution. On Day 2, the surviving *H. azteca* were transferred to a new beaker containing freshly prepared V solution. This was repeated on Day 5. On Day 7, the surviving *H. azteca* were collected for speciation analysis. An aliquot of solution from each beaker was sampled for V speciation analysis. An aliquot from the first replicate was collected for total analysis.

5.2.2. Preparation and Analysis of Water Samples

5.2.2.1. Speciation Analysis of Water Samples

The water samples collected for speciation analysis were preserved using the method developed in Chapter 4. To a 10 mL water sample, 0.5 mL of 52.5 mM Na_2EDTA solution was added to produce a 2.5 mM solution. The samples were refrigerated at 4 °C until they were shipped on ice to the University of Alberta. The samples were then stored at 4 °C until analysis within a few days of arrival.

Samples were mixed on the vortex mixer. A 1.00 mL aliquot was filtered using a 1 mL syringe (BD, Franklin Lakes, New Jersey, USA) and 0.45 µm nylon filter membrane (PALL Life Sciences Acrodisc 13 mm Syringe Filter, Pall (Canada) Ltd., Mississauga, Ontario, Canada) and diluted in 2.5 mM Na_2EDTA solution to a concentration that fell within the range of the calibration curve. Each replicate was analyzed once by HPLC-ICP-MS using the method described in Chapter 2.

5.2.2.2. Total Analysis of Water Samples

A 1 mL aliquot was collected from replicate 1 of every treatment and preserved with 10 µL concentrated ultrapure HNO_3 (J.T. Baker, Canada). The samples were analyzed by AAS using the method described in Norwood et al. [164].

5.2.3. Tissue Collection and Analysis

At the end of the experiment, the surviving animals were transferred to a sample cup containing 60 mL of a 50 µM EDTA solution made with deChlor water, a piece of gauze, and 2.5 mg Tetra-Min food, for gut clearance. The *H. azteca*

were kept in the solution for 24 hours. After 24 hours, the animals were counted, transferred to a clean solution for rinsing, dried on a Kim-wipe and weighed. Two animals were removed to be analyzed for total vanadium by AAS at Environment Canada. The remaining *H. azteca* were reweighed and frozen at -80 °C until they were shipped on dry ice to Edmonton. The samples were kept frozen until analysis time.

5.2.3.1. Extraction and Speciation Analysis of the Tissue Samples

The sample extraction method was based on two previously published reports [70, 167]. Colina et al. [70] extracted the vanadium by shaking 0.2 g of lyophilized tissue in 15 mL of 2.5 mM EDTA for an hour. The work by Caruso et al. [167] and experience from our group [168] showed improved extraction efficiency by sonication. For the purpose of extracting vanadium from *H. azteca* tissue, the modified procedure involved sonication and the use of EDTA.

The tissue collected from all replicates for a given test condition was weighed in a tube (Cultube Sterile Culture Tubes, 12 mm x 75 mm height, Simport, Beloeil, Quebec, Canada). A 1.00 mL aliquot of 2.5 mM Na_2EDTA solution was added to the tube. The tissue was ground using a PowerGen 125 grinder (Fisher Scientific, Inc., Ottawa, Ontario, Canada). The grinder was rinsed with 1.00 mL of 2.5 mM Na_2EDTA solution into the vial. The contents of the tube were transferred to a 15 mL conical vial (Corning Incorporated, Corning, New Jersey, USA). The tube was rinsed with 1.00 mL of 2.5 mM Na_2EDTA that was added to the conical vial for a total of 3 mL of extract solution. The contents of the conical vial underwent sonication for 1 hour (Sonicor Instrument Corporation, Copiague, New

Jersey, USA), then were centrifuged for 15 minutes at 3500 rpm (Sorvall Biofuge primo, Mandel Scientific Co. Ltd., Guelph, Ontario, Canada). The supernatant was removed. Another 1.00 mL portion of 2.5 mM Na_2EDTA was added to the conical vial. The sample was vortexed, sonicated for an hour, centrifuged and the supernatant removed and combined with the previous supernatant. This process was repeated one more time for a total of 3 hours of sonication and 5 mL of supernatant. The supernatant was filtered through a 0.45 µm nylon filter (Pall (Canada) Ltd., Mississauga, Ontario, Canada) then analyzed in triplicate by HPLC-ICP-MS using the method described in Chapter 2.

5.2.3.2. Total Analysis of the Tissue Samples

The two animals removed for total analysis were dried at 60 °C for 72 hours. The samples were acid digested and analyzed by AAS using the method described in Norwood et al. [164].

5.3. RESULTS AND DISCUSSION

5.3.1. Water Analysis

The speciation results for the control group (Figure 5.2) and the water samples from exposure to V(IV) (Figures 5.3 to 5.6) and V(V) (Figures 5.7 to 5.10) are presented. Analysis of the blank samples was performed and no vanadium was detected in them.

The first thing to notice is that the samples from the V(IV) toxicity test were mostly V(V) (Figures 5.3 to 5.6). Some of the samples did contain V(IV) but it

was not the major species present. For the 25 µg/L V(IV) treatment (Figure 5.3), V(IV) was only observed in the fresh water samples prepared at the beginning of each renewal period (Days 0, 2.1, and 5.1), not in the samples collected at the end of the renewal period (Days 2, 5, and 7). These observations would suggest that the V(IV) oxidized completely between renewals. In the higher test concentrations (Figures 5.4 and 5.5), some V(IV) was detected in samples at the end of the renewal period (Days 2, 5, and 7) but the concentrations were significantly less than in the corresponding fresh solutions.

V(V) is the major species present in all the samples from the V(V) toxicity test (Figures 5.7 to 5.10). Only four of the 96 samples contained V(IV) and it represented less than 0.75% of the total vanadium in each of those samples.

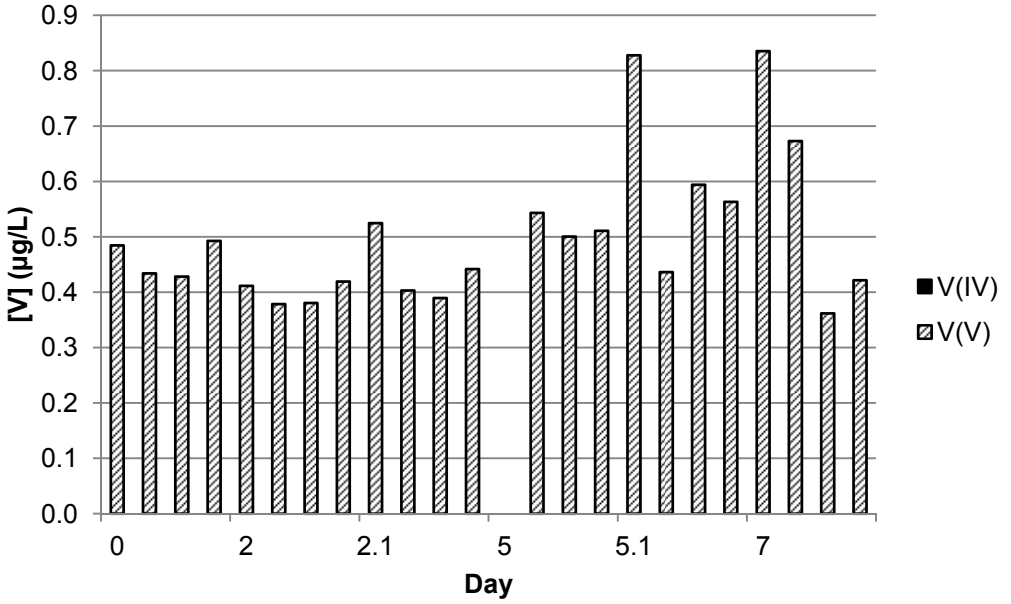

Figure 5.2. Speciation of water samples from a 7-day acute toxicity study on *H. azteca*. Samples are from the control group. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups. No vanadium was detected in the first replicate for Day 5.

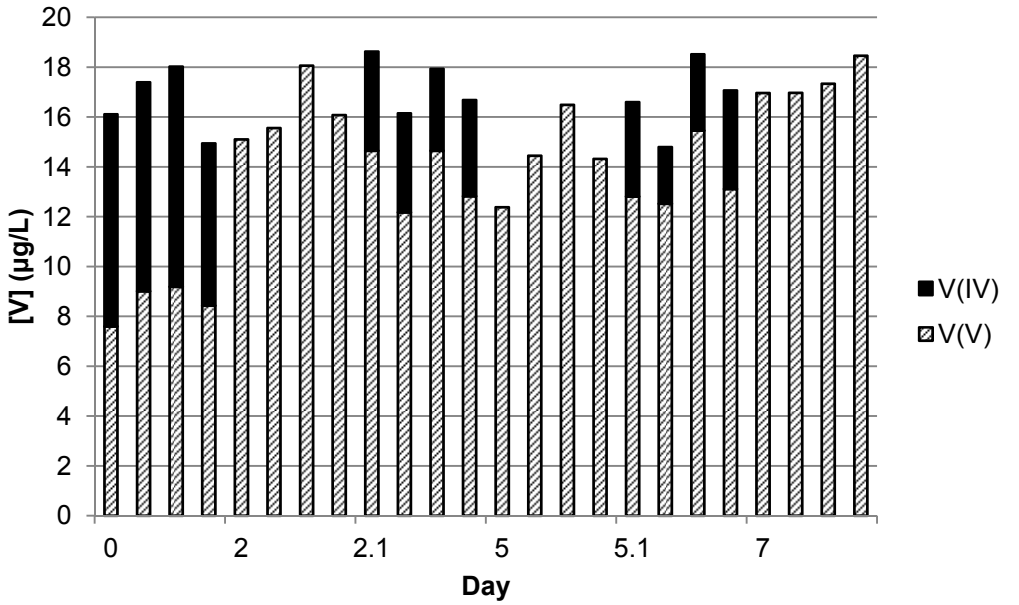

Figure 5.3. Speciation of water samples from a 7-day acute toxicity study on *H. azteca*. Samples for exposure to 25 µg/L V(IV) from vanadyl sulfate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

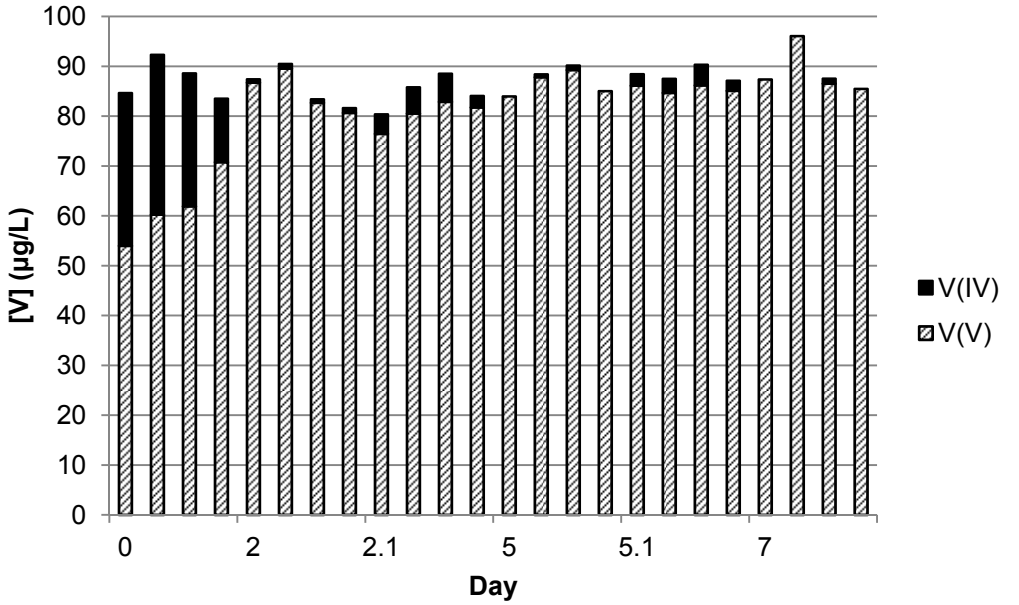

Figure 5.4. Speciation of water samples from a 7-day acute toxicity study on *H. azteca*. Samples for exposure to 141 µg/L V(IV) from vanadyl sulfate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

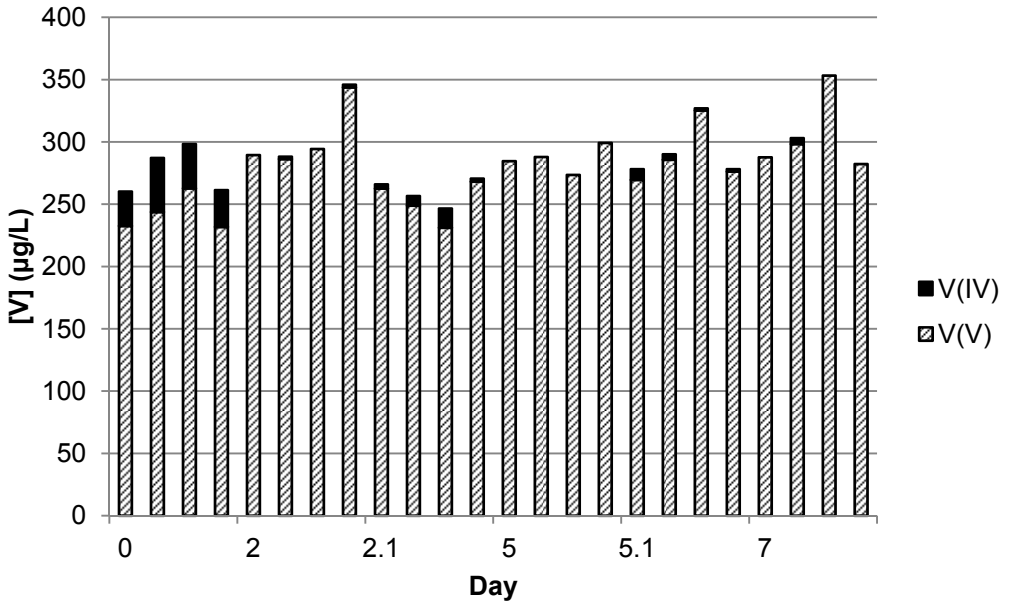

Figure 5.5. Speciation of water samples from a 7-day acute toxicity study on *H. azteca*. Samples for exposure to 451 µg/L V(IV) from vanadyl sulfate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

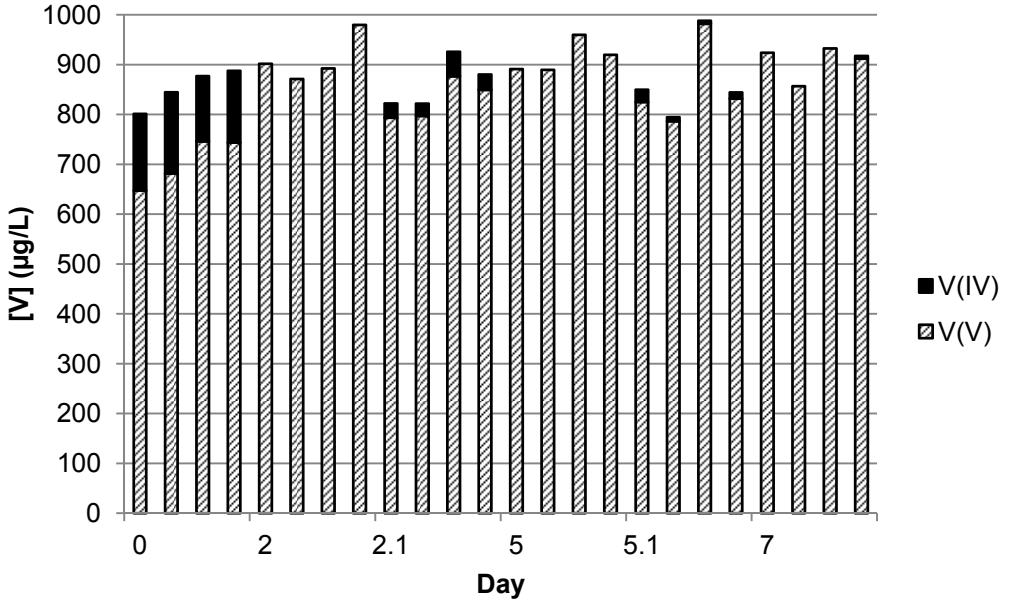

Figure 5.6. Speciation of water samples from a 7-day acute toxicity study on *H. azteca.* Samples for exposure to 1410 µg/L V(IV) from vanadyl sulfate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

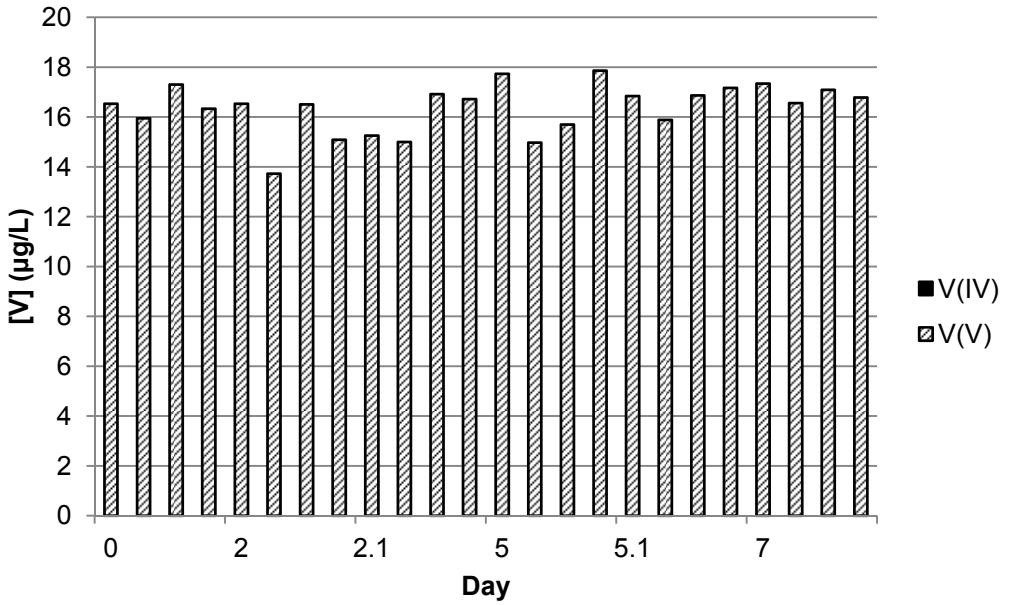

Figure 5.7. Speciation of water samples from a 7-day acute toxicity study on *H. azteca.* Samples for exposure to 19 µg/L V(V) from sodium metavanadate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

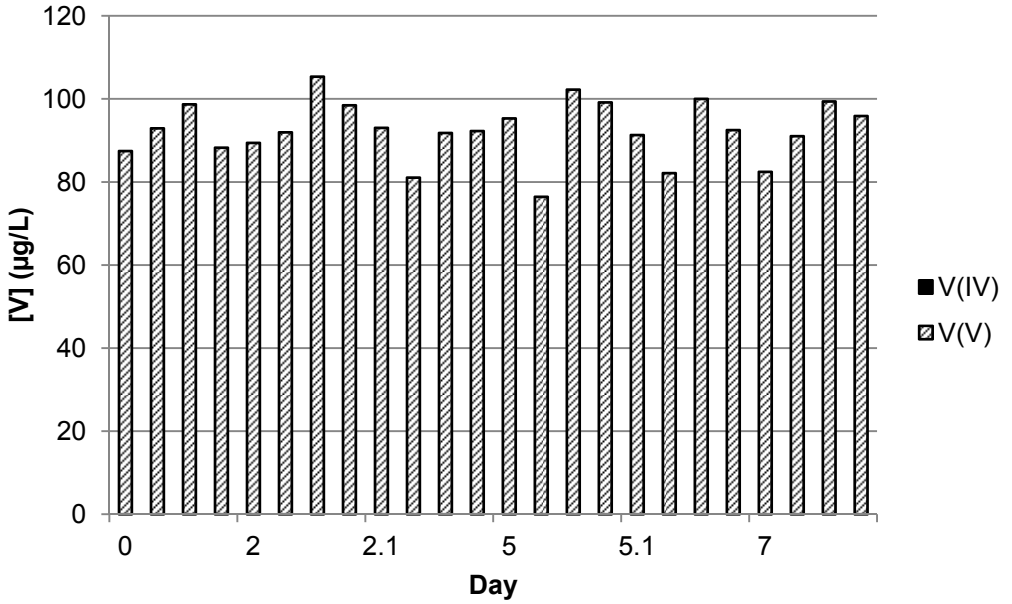

Figure 5.8. Speciation of water samples from a 7-day acute toxicity study on *H. azteca*. Samples for exposure to 106 µg/L V(V) from sodium metavanadate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

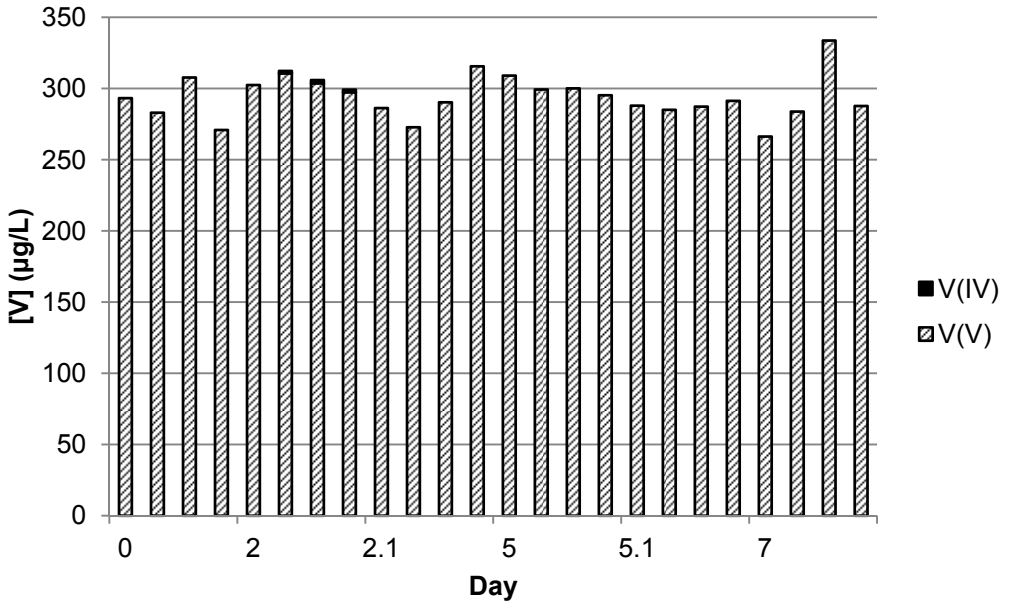

Figure 5.9. Speciation of water samples from a 7-day acute toxicity study on *H. azteca.* Samples for exposure to 339 µg/L V(V) from sodium metavanadate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

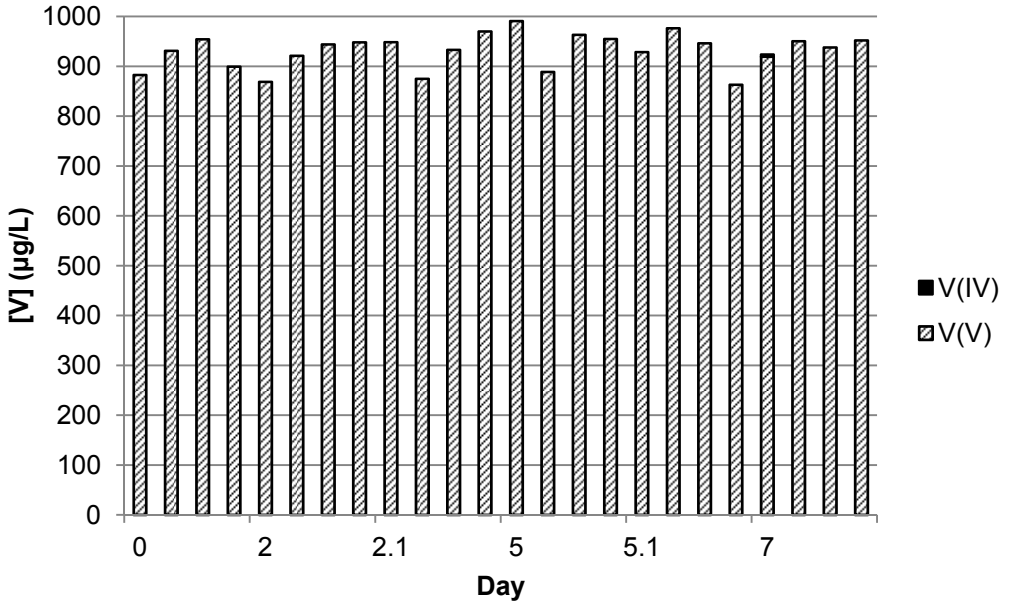

Figure 5.10. Speciation of water samples from a 7-day acute toxicity study on *H. azteca*. Samples for exposure to 1060 µg/L V(V) from sodium metavanadate. Day 0, 2.1, and 5.1 are fresh test solutions. Days 2, 5, and 7 are from the end of the renewal period. Each day has 4 replicate test set-ups.

To confirm that the oxidation was not due to the shipping and storage conditions, the samples that contained both V(IV) and V(V) were analyzed two weeks after the initial analysis (Figures 5.11 to 5.13). The first analysis was performed on either July 28th or 29th, 2011, and the second analysis was performed on August 9th, 2011. The concentration of V(IV) increased in all the samples except for two samples (Day 2, 1566 µg/L V(IV), replicate 1 and Day 5.1, 1566 µg/L V(IV), replicate 2) where there was a slight decrease (Figure 5.11). The increase could be due to analysis error or evaporation rather than a reduction process since the V(V) and total vanadium concentrations increased in all the samples (Figures 5.12 and 5.13).

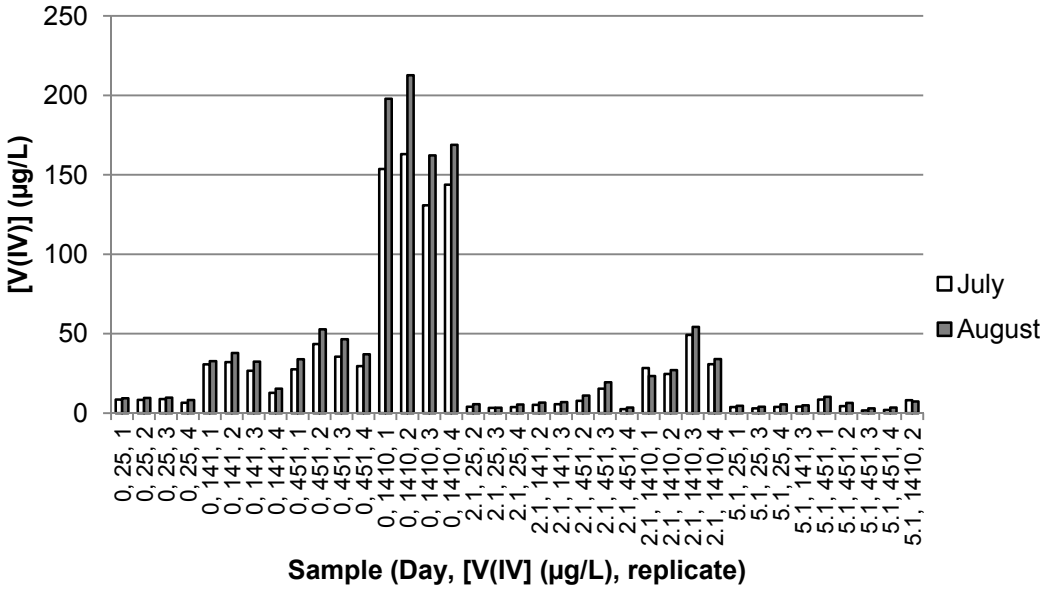

Figure 5.11. V(IV) concentration in water samples that contained both V(IV) and V(V). The samples were analyzed in July 2011, then analyzed again in August 2011.

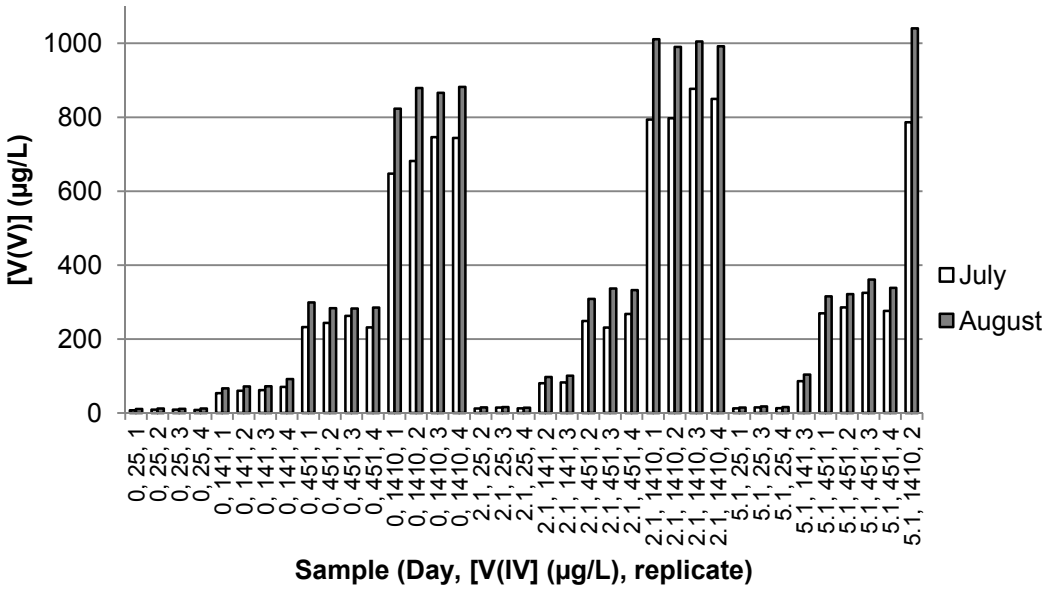

Figure 5.12. V(V) concentration in water samples that contained both V(IV) and V(V). The samples were analyzed in July 2011, then analyzed again in August 2011.

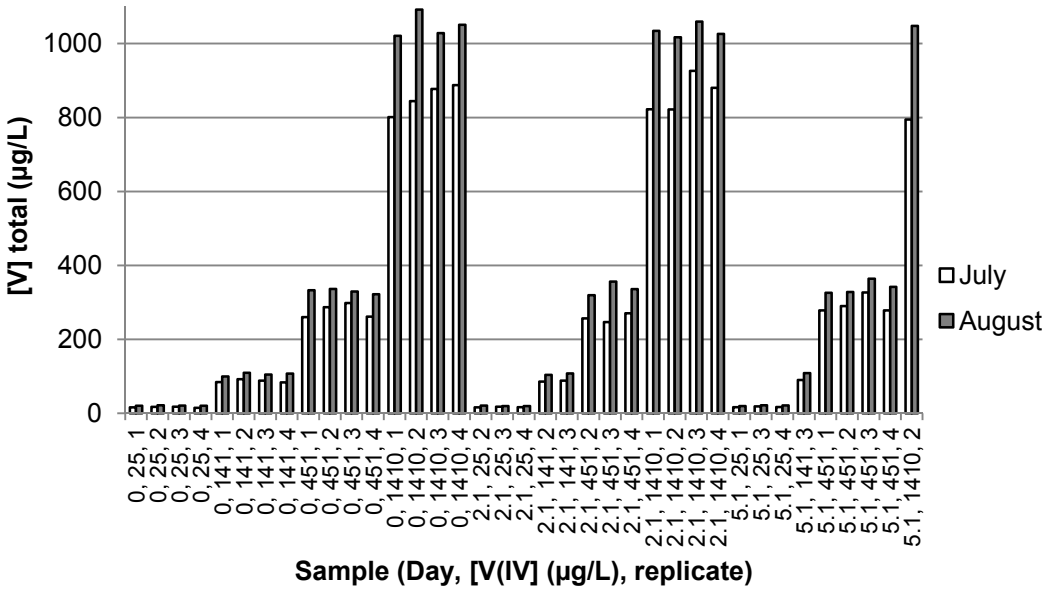

Figure 5.13. Total vanadium concentration in water samples that contained both V(IV) and V(V). Total vanadium is the sum of V(IV) and V(V). The samples were first analyzed in July 2011, then analyzed again in August 2011.

The % V(IV) present in the samples was calculated (Figure 5.14). Of the 37 samples analyzed, 20 of them had a higher % V(IV) when analyzed the second time, further confirming that there is no evident oxidation or reduction process occurring that would change the speciation in the sample.

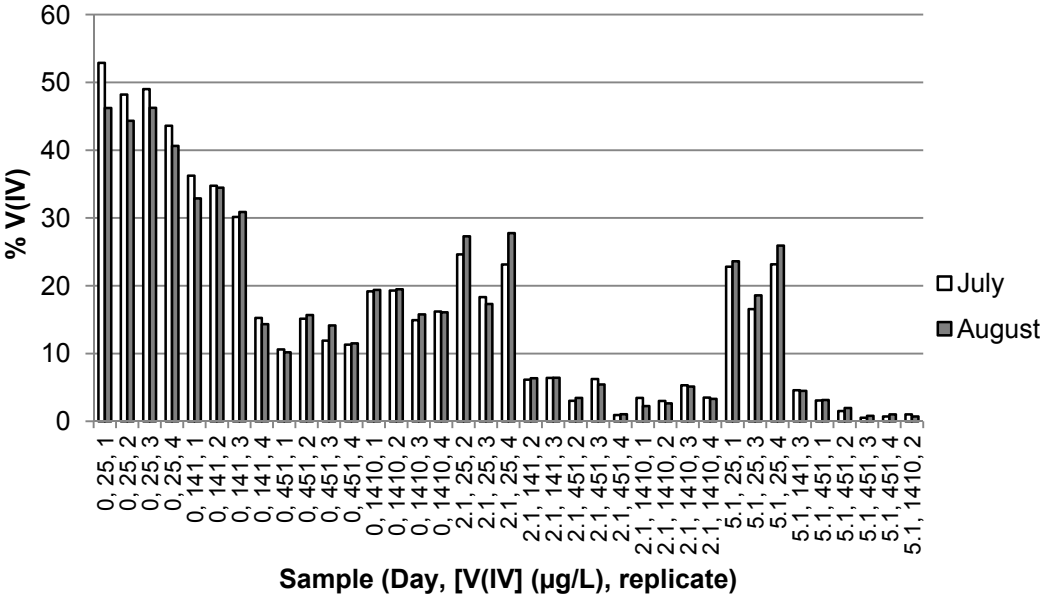

Figure 5.14. % V(IV) in water samples that contained both V(IV) and V(V). The samples were first analyzed in July 2011, then analyzed again in August 2011.

To support the hypothesis that the oxidation of V(IV) to V(V) occurred in the *H. azteca* test water where EDTA was absent, the stability of V(IV) in the test water was determined. The initial conditions of the *H. azteca* incubation study were reproduced. A water sample was spiked with V(IV) simulating the addition of the vanadyl sulfate solution to the test water. The vanadium speciation was monitored over the course of an hour by sampling this solution approximately every 10 minutes. DeChlor water was shipped from Burlington to Edmonton at ambient temperature and stored at 4 °C for 2 weeks before analysis. 200.0 mL of

room temperature deChlor water and 3.7 mg of Tetra-Min were mixed and allowed to sit for a few hours. A 5.0 mL aliquot of the deChlor water and Tetra-Min mixture was filtered to remove the Tetra-Min. A 0.90 mL portion of the solution was placed in a 2 mL vial (National Scientific, Rockwood, Tennessee, USA) and spiked to 160 µg/L V(IV) (0.10 mL spike volume).The vial was capped, shaken, and placed in the HPLC autosampler. The change in vanadium speciation was monitored by HPLC-ICP-MS for 85 minutes by successive injections from the vial directly into the LC system (50 µL injections, approximately 10 minutes between injections, analysis method described in Chapter 2). The resulting chromatograms can be seen in Figure 5.15.

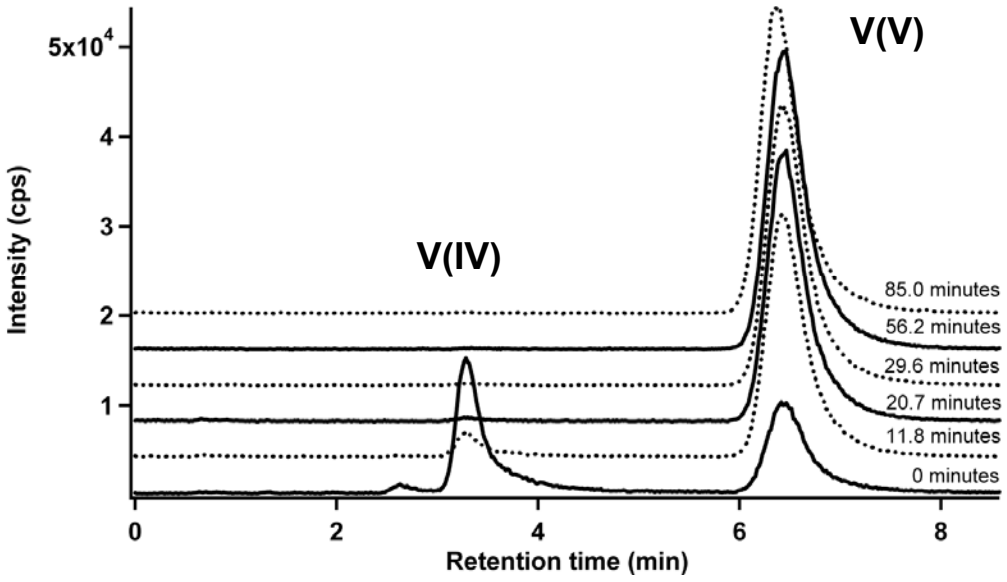

Figure 5.15. Change in vanadium speciation in deChlor water spiked with 160 µg/L V(IV). Times on the chromatograms represent the time between the beginning of the first injection and the beginning of the subsequent injection.

Most of the V(IV) oxidized to V(V) within 30 minutes (Figure 5.15). During the toxicity test, the water samples collected for speciation were taken 3 to 4 hours

after the initial mixing of the fresh vanadium stock solution with the deChlor water. During this time, oxidation of V(IV) could have occurred as the solution is exposed to air and no EDTA is present in the sample. This would explain why mostly V(V) was observed in the water samples from the toxicity study.

The sum of the speciation concentrations obtained from HPLC-ICP-MS were compared to the total vanadium as determined by AAS for each test concentration (Figure 5.16). On average, the sum of speciation represented 92% of the total analysis values (Figure 5.16) and there was a good correlation between the two ($R^2 > 0.99$). Further t-tests between the HPLC-ICP-MS and AAS analyses showed that the control, 25 µg V(IV)/L, 141 µg V(IV)/L, and 106 µg V(V)/L that were statistically the same. The HPLC-ICP-MS speciation analysis was performed as a blind study. The nominal concentrations of the water samples were known in order to dilute the sample appropriately. However, the results from the total analysis were not known when speciation was performed. Conversely, the speciation results were not available when the total analysis by AAS was performed.

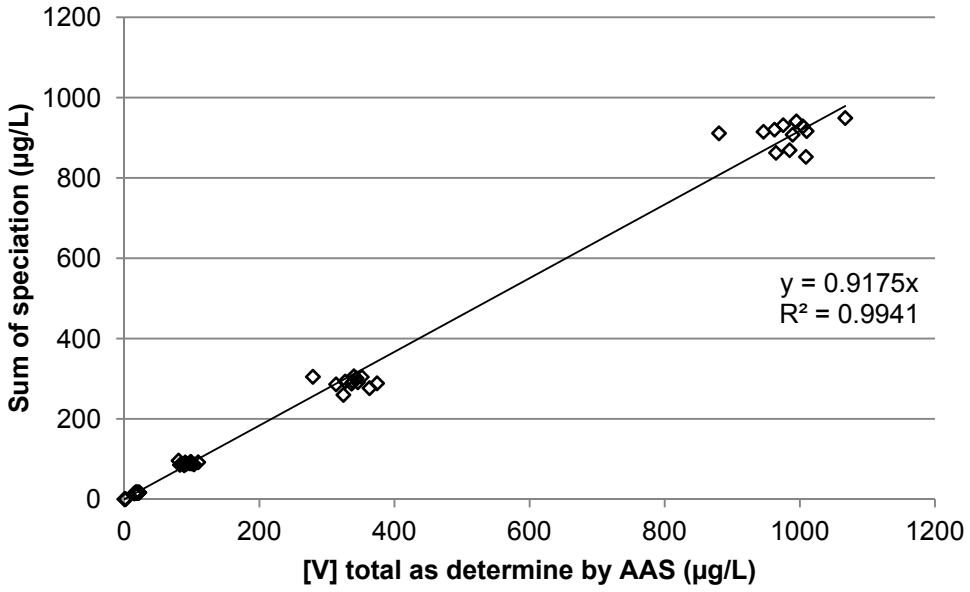

Figure 5.16. Comparison of total analysis as determined by AAS to the sum of the speciation analysis from HPLC-ICP-MS analysis of water samples from a 7-day acute toxicity study on *H. azteca*.

5.3.2. Tissue Analysis

Figure 5.17 shows the total concentrations of vanadium in the tissue of *H. azteca* exposed to varying concentrations of vanadium in water. In both sets of experiments, where either V(IV) or V(V) was initially added to the water, the concentrations of vanadium in *H. azteca* tissue increased with the exposed concentration of vanadium.

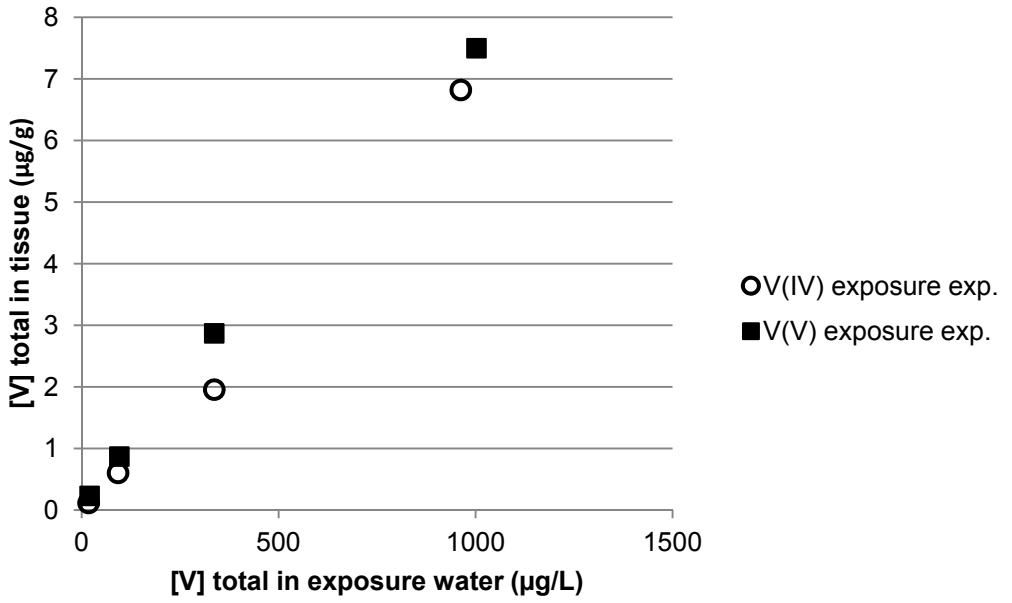

Figure 5.17. Relation between total vanadium concentration in the water and the total vanadium concentration in the tissue samples as determined by AAS.

HPLC-ICP-MS analysis of the *H. azteca* tissue extracts showed the presence of V(IV) and V(V) (Figures 5.18 and 5.19). There was V(IV) present in the animals exposed only to V(V). In several extracts, there is also an unidentified peak corresponding to the retention time of 1.5 minutes. This peak has a greater retention time than Cl^- (0.8 minutes) that would form ClO^+, a potential interferent. Considering that vanadium is the only element with an isotope at m/z 51, this is likely a vanadium-containing species. The unknown peak was not present in all tissue samples (Figure 5.18).

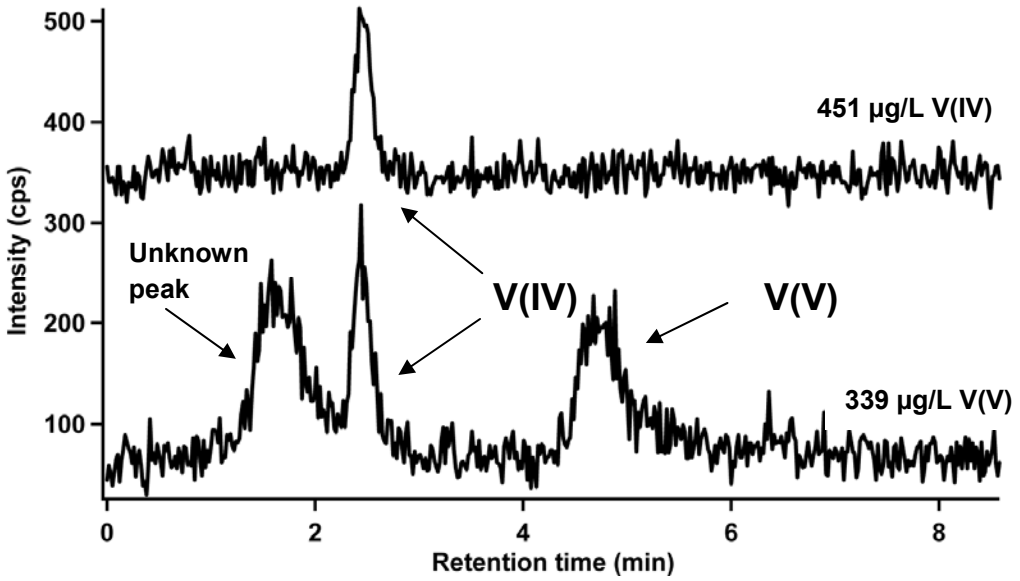

Figure 5.18. Chromatogram of vanadium speciation of tissue extract of *H. azteca* exposed to either 451 µg/L V(IV) or 339 µg/L V(V).

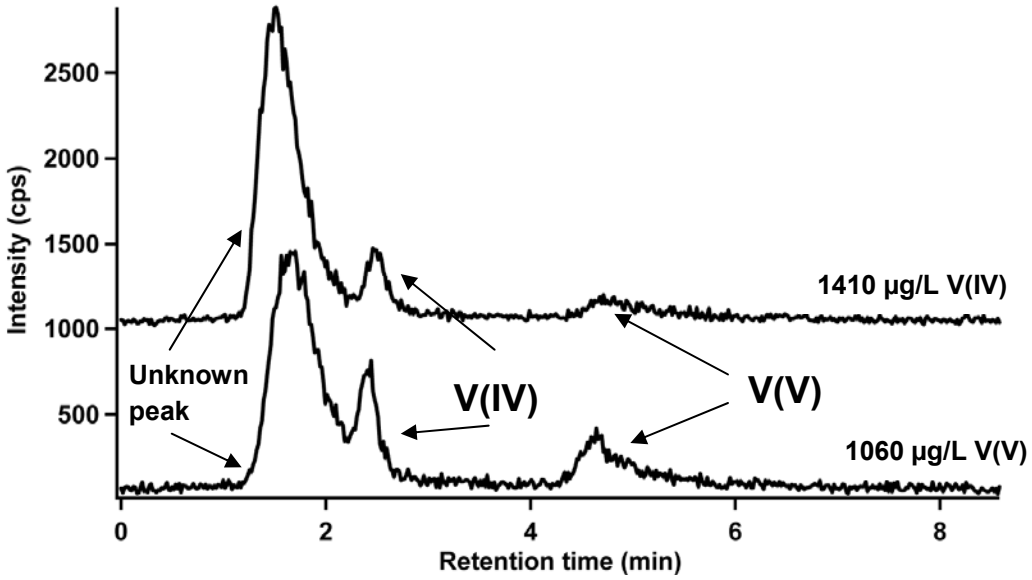

Figure 5.19. Chromatogram of vanadium speciation of tissue extract of *H. azteca* exposed to either 1410 µg/L V(IV) or 1060 µg/L V(V).

The speciation results of the tissue extract are found in Table 5.1. The concentration in the extraction liquid is close to the limit of detection of the method for most samples. However, the noise and background were low on the

day of analysis allowing for the quantitation of peaks. The limits of detection were also a little lower (0.2 µg/L for both V(IV) and V(V)) than the previously calculated LODs for the method (0.3 µg/L for V(IV)and 0.4 µg/L for V(V)). The values reported in the table are the average of the triplicate analysis of the extract. The peaks may have been quantifiable in one replicate injection but not in the next, therefore giving an average concentration below the LOD. The calibration curve for V(IV) was used to estimate the concentration of vanadium of the unknown peak.

Table 5.1. Concentration of vanadium in the extraction liquid from the extraction of *H. azteca*

Vanadium species	Nominal conc. (µg/L)	Measured exposure conc. (µg/L)	[V] in extraction liquid (µg/L)				
			V(IV)	V(V)	Unknown peak	V(IV) + V(V)	Sum of speciation
V(IV)	25	17	0.1	0.1	0.2	0.2	0.4
	141	92	0.1	0.2	1.3	0.3	1.7
	451	336	0.4	0.1	0.0	0.5	0.5
	1410	962	1.3	1.1	7.9	2.4	10.3
V(V)	19	20	0.2	0.1	0.0	0.3	0.3
	106	95	0.2	0.2	1.9	0.4	2.3
	339	336	0.5	0.8	0.8	1.2	2.1
	1060	1002	1.7	1.7	5.7	3.5	9.1

Most tissue samples contained both V(IV) and V(V), independent of the vanadium species that the animal was exposed to. The concentrations were on the same order of magnitude for both species, with increasing concentrations as the exposure concentration increased. The presence of V(V) in the animals exposed to V(IV) could be expected based on the analysis of the water samples. Most of the V(IV) oxidized to V(V) before the animals were exposed to the test solution. The presence of V(IV) in the animals only exposed to V(V) was not

predicted. The change in speciation could potentially be due to how the vanadium was metabolized. The relative distribution of vanadium species in the *H. azteca* tissue can be observed in Figure 5.20.

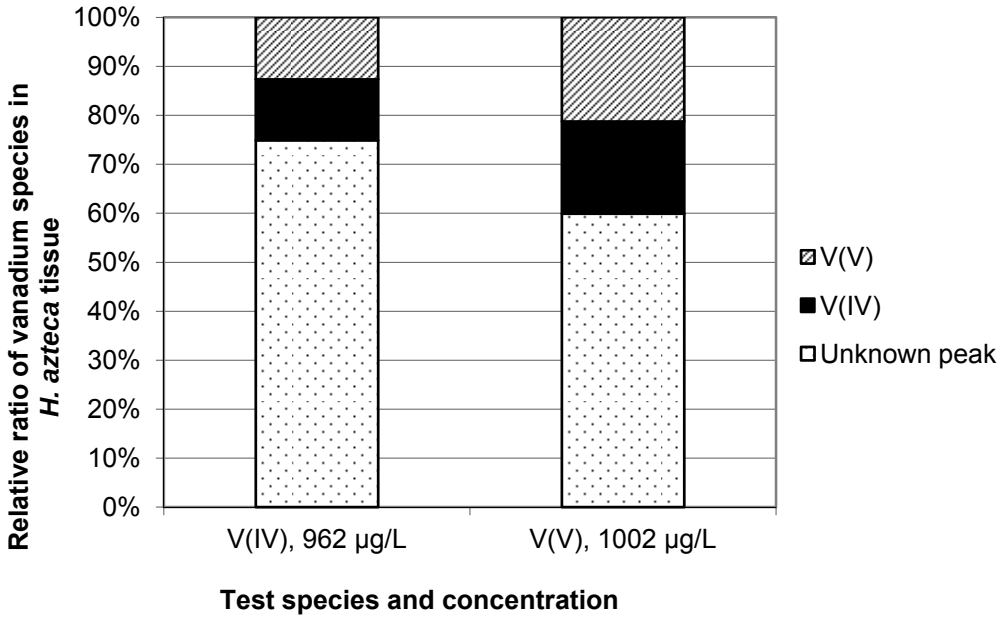

Figure 5.20. Relative distribution of vanadium species in *H. azteca* tissue after the 7-day exposure to vanadium in water.

To compare the sum of the speciation with the total analysis performed on the dried samples analyzed by AAS, the sum of the speciation was divided by the average of the dry weight to wet weight ratio as determined for each test condition (Table 5.2). The ratios ranged from 0.175 to 0.268.

Table 5.2. Concentration of vanadium in *H. azteca* tissue

Vanadium species	Measured exposure conc. (µg/L)	[V] in tissue (µg/g dw)					
		V(IV)	V(V)	Unknown peak	V(IV) + V(V)	Sum of speciation	Total by AAS
V(IV)	17	0.022	0.012	0.067	0.035	0.085	0.12
	92	0.016	0.040	0.222	0.056	0.278	0.61
	336	0.056	0.015	0.000	0.071	0.071	1.96
	962	0.362	0.369	2.178	0.731	2.365	6.82
V(V)	20	0.081	0.038	0.000	0.119	0.119	0.23
	95	0.032	0.031	0.289	0.063	0.352	0.87
	336	0.070	0.119	0.128	0.189	0.317	2.87
	1002	0.449	0.506	1.426	0.955	2.025	7.50

To determine the efficiency of the tissue extraction method, Equation 5.1 was used to calculate the extraction efficiency (Table 5.3).

$$Extraction\ efficiency = \frac{\sum[V]species}{Total\ [V]by\ AAS} \times 100\% \qquad (5.1)$$

Table 5.3. Extraction efficiency of vanadium from *H. azteca* tissue

Vanadium species	Nominal concentration (µg/L)	Measured exposure concentration (µg/L)	Extraction efficiency (%)	
			V(IV) +V(V)	Sum of speciation
V(IV)	25	17	29.6	72.7
	141	92	9.3	45.9
	451	336	3.6	3.6
	1410	962	10.7	34.7
V(V)	19	20	51.3	51.3
	106	95	7.2	40.5
	339	336	6.6	11.1
	1060	1002	12.7	27.0

The extraction efficiency was low with a minimum of 3.6% and a maximum of 51.3%. The average was 16.4 ± 16.2%. When the unknown peak was included, the maximum extraction efficiency increased to 72.7% and the average, to 36 ±

22%. The concentration of vanadium present in the tissue did not influence the extraction efficiency. The efficiency of the extraction method should be improved before it is applied to other tissue samples.

5.3.3. Unknown Peak

Based on previous work by Li [144], the peak was believed to be from a Ca-V(V)-EDTA complex. She mixed Ca^{2+} with V(V) overnight and found a peak that eluted at 1.5 minutes. She attributed the peak to the binding of Ca^{2+} with the $[VO_2Y]^{3-}$ complex.

To confirm this in the tissue extracts, a series of tests were performed replicating the extraction process. A Ca^{2+} solution was prepared using $CaCl_2 \cdot 2H_2O$ (BDH Inc., Toronto, Ontario, Canada). In the first set of experiments, 10.00 mL of a 1 mg/L Ca^{2+} solution was spiked with vanadium. The first vial contained 20 µg/L V(IV), the second 20 µg/L V(V) and the third 20 µg/L of both V(IV) and V(V). To serve as a control, a 20 µg/L V(IV) solution was prepared in DIW as was a 20 µg/L V(V) solution. The solutions were allowed to sit overnight at room temperature. The following day, they were analyzed by HPLC-ICP-MS using the method described in Chapter 2. All the peaks in the chromatograms corresponded to either V(IV) or V(V). No peak eluted at 1.5 minutes.

In the second set of experiments, the concentration of Ca^{2+} increased to 0.5 g/L and the vanadium concentration to 50 µg/L. The set of solutions, prepared in 2.5 mM Na_2EDTA, was otherwise similar to the one described above. An extra solution was prepared containing only Ca^{2+} and Na_2EDTA. The solutions were

allowed to stand overnight. An identical set of solutions was prepared the following morning and sonicated for 3 hours to represent the treatment of the *H. azteca* tissue extraction. The analysis by HPLC-ICP-MS showed the expected peaks for V(IV) and V(V). A peak did elute at 0.9 minutes in several samples. This peak was attributed to ClO^+ as it was the only peak present in the sample containing only Ca^{2+} and Na_2EDTA, and the concentration of Cl^- in all the solutions was very high (0.88 g/L Cl^-).

The third and final set of experiments was identical to the second set of experiments, except that the solutions were prepared in DIW. The results were the same as the second set of experiments with peaks corresponding to ClO^+, V(IV), and V(V).

Despite these experiments, the peak eluting at 1.5 minutes was not identified. When the extracts were re-analyzed 20 days later (501 and 1566 µg/L V(IV), and 339 and 1060 µg/L V(V)), the extra peak was no longer present. For the V(IV) exposure samples, the V(IV) to V(V) peak area ratio increased while the ratio remained constant for the V(V) exposure samples. The unknown vanadium containing compound did not convert to a V(IV)-EDTA or V(V)-EDTA complex. It may have precipitated during storage and been removed during the filtration of the sample before the second analysis.

It is possible that vanadium species could bind to proteins in *H. azteca* tissue. Others have shown vanadium binding to proteins [78, 79]. It would be useful to identify whether the unknown peak could be due to a vanadium-protein complex. This line of investigation could be explored further.

5.4. CONCLUSIONS

Vanadium speciation of water samples should be performed during toxicology studies to confirm that the animals or plants have been exposed to species under investigation. As can be seen from the V(IV) water samples, most of the vanadium oxidized before the *H. azteca* were exposed to the vanadium. Complete oxidation had occurred by the end of the renewal period. Speciation of the water samples used in the bioaccumulation studies performed on samples collected from the Athabasca Oil Sands region should be done. It would help determine what species are present in the water sources to which the biota of the region is exposed. Although the speciation method usually underestimated the total amount of vanadium present in the water samples, it did identify the species to which the animals were exposed.

The tissue extraction method should be improved as its efficiency was poor and inconsistent. There should be more animals for each test concentration under investigation during the improvement of the method. More identical tissue would be available to test using different extraction methods. Lyophilization of the tissue, as used by Colina et al. [70], could be one of the options explored. Total analysis should be performed on the residue after filtration to determine how much vanadium is left in the solid. Using a smaller volume of extraction liquid would increase its vanadium concentration, thus allowing for a better quantitation. At this point, tissue speciation should be used for qualitative analysis rather than quantitative analysis on low exposure concentrations. For higher exposure concentrations (above 900 µg/L), the method could still be used for quantitative analysis.

Chapter 6. Conclusions and Future Work

6.1. CONCLUSIONS

The toxicity of vanadium is linked to its oxidation states. As the oxidation state increases, so does the toxicity of the vanadium species. The oxidation states commonly found in nature are V(III), V(IV), and V(V). Knowing the species present in a sample provides valuable chemical information about the potential toxicity of the sample.

Vanadium is present in the Athabasca Oil Sands region in high concentrations. The concentration in the asphaltenes is 640 ppm [125]. Vanadium concentrates into the byproducts of the extraction and upgrading of bitumen. Petroleum coke has been investigated as a matrix to adsorb naphthenic acids from oil sands process water. Using this method could lead to the leaching of metals, such as vanadium, from the coke.

Determining the species and concentration of vanadium that leach into the water would contribute to a better understanding of what occurs during the process. HPLC-ICP-MS provides a good platform for the determination of vanadium species in the water samples.

An HPLC-ICP-MS method was optimized to separate the EDTA complexes of V(IV) and V(V) in water samples. The separation used a strong anion exchange column. The optimal mobile phase was 3% acetonitrile, 2 mM EDTA, 80 mM ammonium bicarbonate at a pH of 6. The combination of optimum column and

mobile phase conditions allowed for an 8 minute and 35 second separation, and the instrument was still functional after overnight use. The method was sensitive, had low limits of detection (0.3 µg/L and 0.4 µg/L for V(IV) and V(V) respectively), and the calibration was linear over 2 orders of magnitude. The method was successfully applied to the analysis of samples with complex matrices.

HPLC-ICP-MS analyses of the soluble fraction of OSPW showed that V(V) was the only detectable vanadium species. This speciation analysis only determined the dissolved vanadium since the samples were filtered before analysis. Other groups performed acid digestion to determine the total vanadium content in OSPW. The sum of vanadium species concentration from speciation analysis was generally lower than the vanadium concentrations previously reported. This difference is probably related to the filtration procedure used for speciation.

When V(IV) is added to OSPW, it rapidly oxidizes to V(V), thus explaining the observation of only V(V) in the OSPW tested. In order to study the leaching of vanadium from coke, a method to preserve the speciation was required. It was determined that the addition of EDTA to the solution prevented the oxidation of V(IV). EDTA was therefore used as a preservative for the rest of the experiments.

The half-life of V(IV) oxidation to V(V) was determined by adding V(IV) to OSPW, removing aliquots at predetermined times, adding EDTA to the aliquots to stop the oxidation, and measuring the remaining V(IV) and the converted V(V) species. For V(IV) concentrations below 1 mg/L, the half-life ranged from 1.5 to

1.8 minutes and followed first order kinetics. For concentrations above 1 mg/L, the half-life was longer.

To study the leaching of vanadium from coke, the first step was to optimize the EDTA concentration. The goal was to determine what species actually leached from coke and preserving the speciation is a key component. There was no statistical correlation between the concentration of EDTA used (0 to 15 mM) and the total amount of vanadium leached. However, for the 2.5 mM, 5 mM and 10 mM EDTA solutions, more V(IV) leached. The 2.5 mM EDTA solution was chosen because of the similarity of its EDTA concentration and the one in the mobile phase (2 mM).

Once the optimal EDTA concentration was determined, the effect of the coke to water ratio on vanadium leaching was studied, as well as the effect of the type of water (OSPW and DIW). The amount of vanadium that leached per gram of coke was not affected by the coke to water ratio. For the ratios tested (0 to 40% coke:water), the amount of leached vanadium was 9.9 ± 0.6 to 12.7 ± 1.5 µg/g into OSPW and 7.8 ± 0.3 to 10.9 ± 0.4 µg/g into DIW. The water soluble fraction of vanadium in coke, as determined by Kessler and Hendry [112], is 8.6 mg/kg. The results from their report agree with the results of this thesis. The water type used did affect the species of leached vanadium. More V(IV) leached into DIW than into OSPW. However, in total, more vanadium leached into OSPW.

While the mixing ratio did not affect the amount of vanadium leached per gram of coke, the concentration in the supernatant increased as the mixing ratio of coke to water increased. For mixing ratios of 20% and higher, the vanadium

concentration was higher than the LC50 for several aquatic organisms such as juvenile rainbow trout, a species native to the Athabasca Oil Sands region [50]. To reduce the potential increase in toxicity due to vanadium, a lower coke-to-water ratio could be used.

When coke and water are mixed, vanadium leaches with a profile that appears to be biphasic. Rapid leaching occurred within the first few hours of the mixing of coke with water, with slower leaching happening afterwards.

The use of EDTA to preserve vanadium speciation in water samples for more than a few hours was explored. Of the two storage temperatures and the three storage solutions tested, the optimal storage conditions were a 2.5 mM Na_2EDTA solution stored at 4 °C. The vanadium speciation and concentration was stable for a period of 56 days. The method was applied to real samples during the study of vanadium toxicity in *Hyalella azteca*.

Vanadium speciation of water samples from a toxicity study on *H. azteca* indicated that the speciation of vanadium can easily change. The chemical species to which the animals are actually exposed may not be the initial vanadium species added to the water. In this study, most of the V(IV) in solution oxidized before the animals were exposed to the test water. Speciation should therefore be performed on the water used in toxicity and bioaccumulation experiments to determine what chemical species are present.

Vanadium speciation on tissue extracts showed the presence of V(IV) and V(V) in the tissue of *H. azteca*. An unknown vanadium complex was also detected. It

was determined not to be a Ca-V(V)-EDTA as initially thought. It could potentially be a vanadium-protein complex. The developed vanadium extraction method could be applied in a qualitative manner in future studies involving vanadium concentrations below 0.9 mg/L. Once the extraction efficiency and reproducibility are improved, this method could be used for quantitative analysis. At this time, quantitative analysis should only be performed on tissue samples from animals exposed to water containing 0.9 mg/L or higher.

Several contributions to the field of vanadium speciation have been made with this work. A few examples include the determination of the vanadium species that leach from petroleum coke into water, the development of a method to preserve vanadium speciation in water samples, and the detection of a new, as yet unidentified vanadium species in the tissue extract of *H. azteca*. Speciation should be a part of sample analysis when different chemical species can have different effects on the system under investigation, be it vanadium leaching from coke or the study of animals exposed to vanadium.

6.2. FUTURE WORK

The presence of vanadium in a multitude of samples and its complex chemistry require the development of new applications and methods, and the optimization of current methods for vanadium speciation.

A method applicable to solid samples with little or no sample preparation, such as the use of synchrotron radiation, could be used to determine the vanadium species present in coke. Maintaining the species integrity of the sample would be

paramount in the experiment. The knowledge would provide a more complete picture as to the fate of vanadium from its origins in the bitumen ore to its concentration in petroleum coke.

More experiments could be performed on the oxidation of vanadium in OSPW. Performing temperature controlled experiments would be the first step, as the kinetics are governed by temperature. The range of concentrations tested should be similar to the concentrations found in the supernatant of the coke and water mixtures. These would provide a better representation of what is actually occurring during the oxidation. Preparing the OSPW in different ways would help determine the effects of the different components of OSPW on the oxidation process. Saturation with oxygen, degassing with nitrogen, and filtering to remove the suspended solids would be just a few of the different treatments that could be tested. Since the composition of OSPW changes as the source of the bitumen ore changes, different batches of OSPW should be tested. The information obtained from the experiments could therefore be applied on a wider scale.

The applicability of the speciation preservation method should be expanded. The expiry date of the method would be determined by testing the storage conditions for a longer period of time with weekly or monthly testing. A variety of sample concentrations should be included to test the suitability range of the preservation method. Different V(IV) to V(V) ratios should also be tested to determine how the species ratio affects the conservation of the vanadium speciation. Since the speciation distribution is not usually known before analysis, it is important to establish if the species ratio could change during transportation and storage.

The extraction method used on the *H. azteca* tissue should be improved. To expand the applicability of the vanadium speciation method to solid samples, an effective and efficient vanadium extraction method for solid samples should be developed. A method for tissue extraction would allow for a better understanding of what species accumulate in tissue and how this correlates to the vanadium species to which the animal is exposed. A method optimized for soil samples would permit the analysis of sediment. Toxicity studies sometimes involve determining the effect of sediment on the animal or plant of interest so the tissue extraction method could be used in conjunction with the sediment extraction method.

The unknown vanadium complex present in the *H. azteca* tissue samples should be identified. Elucidation of its composition would allow for a better understanding of the metabolization of vanadium in *H. azteca*. It would expand the number of species of vanadium that are identifiable by HPLC-ICP-MS. Expanding the understanding and identification of vanadium species in different samples presents an interesting challenge for researchers.

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