Influence of dissolved organic matter on dissolved vanadium speciation in the Churchill River estuary (Manitoba, Canada)

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Highlights
- Temporal and spatial changes in dissolved V speciation using DGT.
- Positive relationship found between protein-like DOM and DGT-labile V.
- Surface sediment is a significant source of DGT-labile V during spring freshet.

Abstract
Diffusive gradients in thin films (DGT) devices were used to investigate the temporal and spatial changes in vanadium (V) speciation in the Churchill estuary system (Manitoba). Thirty-six DGT sets and 95 discrete water samples were collected at 8 river and 3 estuary sites during spring freshet and summer base flow. Dissolved V concentration in the Churchill River at summer base flow was approximately 5 times higher than those during the spring high flow (27.3 ± 18.9 nM vs 4.8 ± 3.5 nM). DGT-labile V showed an opposite trend with greater values found during the spring high flow (2.6 ± 1.8 nM vs 1.4 ± 0.3 nM). Parallel factor analysis (PARAFAC) conducted on 95 excitation-emission matrix spectra validated four humic-like (C1–C4) and one protein-like (C5) fluorescent components. Significant positive relationship was found between protein-like DOM and DGT-labile V ($r = 0.53, p < 0.05$), indicating that protein-like DOM possibly affected the DGT-labile V concentration in Churchill River. Sediment leachates were enriched in DGT-labile V and protein-like DOM, which can be readily released when river sediment began to thaw during spring freshet.

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1. Introduction
Vanadium (V) is a transition metal widely distributed in natural environments (air, water, soil and biota) with potential mutagenic and carcinogenic properties (Stern et al., 1993). Natural sources of V in aquatic environments encompass rock weathering and sediment leaching (Hope, 1997; Rühling and Tyler, 2001; Shiller and Mao, 2000). Vanadium can also be introduced into aquatic environments through industrial activities especially from oil refineries and power plants that are burning V-rich fuel oil or coal (Evans and Landergran, 1975; Moskalyk and Alfanti, 2003; Kirk et al., 2014; Guéguen et al., 2006). Other sources of V to aquatic environments are fertilizers, sewage sludge, and discharge of domestic wastewater (Bhatnagar et al., 2008; McBride and Cherney, 2004). Vanadium is essential for normal cell growth at concentrations below 0.10 μM (Salice et al., 1999) but excessive amounts can cause adverse health effects on human and aquatic species. For example, the total V lethal concentration (LC50) was 37–118 μM for juvenile rainbow trout (Salmo gairdneri R.) (Frank et al., 1996), 39–59 μM for zebra fish (Brachydanio rerio) (Bishayee et al., 2010), and 65 μM for guppies (Poecilia reticulata) (Bishayee et al., 2010). In surface water, V with the oxidation state of +5 is the dominant and most toxic form to cells (Sabbioni et al., 1991) and organisms (Ma and Fu, 2009).

The toxicity of trace metals in aquatic environments is greatly influenced by its chemical speciation and particularly the concentration of its free form and small soluble complexes. On the other hand, metal bound to dissolved organic matter (DOM), iron and aluminum oxyhydroxides are not readily bioavailable (Koukal et al.,...
A change in DOM composition and concentration may affect bioavailability and mobility of metals in waters (Koukal et al., 2003). The diffusive gradients in thin-films (DGT) technique is a time-integrated, passive sampler technique for the in situ determination of free and weakly bound metals in natural waters (Davison and Zhang, 1994). The DGT device is composed of a diffusive layer, conventionally a polyacrylamide hydrogel, and a binding layer, typically ferrihydrite (Luo et al., 2010; Österlund et al., 2010; Price et al., 2013) or zirconia (Guan et al., 2015) impregnated within the polyacrylamide hydrogel. DGT with ferrihydrite binding gel has been deployed in synthetic and natural freshwater to measure V in his highest oxidation state (Huang et al., 2013; Panter et al., 2013), or zirconia (Guan et al., 2015) impregnated within the polyacrylamide hydrogel. DGT with ferrihydrite binding gel has been deployed in synthetic and natural freshwater to measure V in his highest oxidation state +5 (Luo et al., 2010; Österlund et al., 2010; Panter et al., 2013; Price et al., 2013).

Despite the fact that spring freshet is characterized by a dramatic increase in flux of dissolved and particulate species (Holemann et al., 2005; Kuzyk et al., 2008; Mann et al., 2012), it is also the least sampled period. The discharge of Churchill River 2010; Panther et al., 2013). This increased oxidation state +5 (Luo et al., 2010; Österlund et al., 2010; Panter et al., 2013; Price et al., 2013).

Prior studies on dissolved V speciation showed the DOM adsorption effect was significant in river and estuary (Shiller and Mao, 2000). Lu et al. (1998) found that aquatic humic substances strongly complexed vanadate and VO2+. Excitation-emission matrix (EEM) fluorescence spectroscopy and parallel factor analysis (PARAFAC) have been used to assess the composition (e.g. protein- and humic-like based on peak position in the EEM; Coble, 1996) and origin of DOM in natural waters (e.g. Dainard and Guéguen, 2013; Kothawala et al., 2013; Walker et al., 2013). Recent advances in ultra-high resolution mass spectroscopy allowed the identification of structural composition of DOM (e.g. D’Andrilli et al., 2013; Hertkorn et al., 2008; Mangal et al., 2016), a prerequisite for exploring the relationship between DOM and metal complexation. In this paper, the objectives were (1) to determine the concentration of dissolved and DGT-labile V in the Churchill estuary system (Fig. 1A) from August 23 to August 27, 2013 (summer base flow; Fig. 1B) and from May 8 to May 16, 2014 (spring pre-freshet; Fig. 1B) and from May 12 to May 21, 2015 (spring freshet). Mean water level in the Churchill River was 22.51 ± 0.02 m in late August 2013, 24.07 ± 0.31 m pre-freshet and 23.90 ± 0.19 m freshet (Government of Canada (2015)). Due to site accessibility in spring, all sites could not be monitored at all seasons. The estuarine DGT units (E1-E3) were deployed in winter 2013 for 4–5 h during high tide over a 6-day period for an overall accumulation period of 24–30 h in order to assess the influence of marine-derived DOM. The prefreshet (R1 and R5) and freshet DGT units (R2-R3 and R6) were deployed for 2–4 d in moving waters (300–350 m²/s) after drilling a hole through the river ice. The diffusive boundary layer was assumed to be negligible (Denney et al., 1999; Gimpel et al., 2001). R2 was the only site in the vicinity of a shallow wetland.

Water samples were daily collected at each site during the time of DGT deployment and immediately filtered through a pre-combusted, 0.7 µm glass fiber filter (Whatman) and stored at −20 °C. Dissolved V samples were obtained after filtration through 1 µm Nuclepore membrane filter (Whatman) and stored at −4 °C until analysis. Temperature of river was measured daily using a digital thermometer (Accumet APB5; ThermoScientific).

2.2. DGT preparation

Each DGT unit was composed of a 0.5 mm precipitated ferrihydrite binding gel layer (Luo et al., 2010), a 0.8 mm diffusive acrylamide-based gel layer and a 0.45 µm cellulose nitrate filter (Zhang and Davison, 1999). The binding and diffusive gels were casted using 0.25 and 0.5 mm thick, acid-bathed, polystyrene spacers, respectively. The binding gel was composed of a 0.5 mm diffusive gel immersed in 1 M iron nitrate solution for 12 h and in 2-N-morpholinoethanesulfonic acid (MES) buffer solution between 10 and 40 min (Luo et al., 2010). No significant difference in accumulated V was found in the 10 min soaked gel (p > 0.05). The binding gel was then rinsed with Milli-Q water (MQW) for 24 h and stored in MQW. The gel thicknesses (i.e. 0.5 and 0.8 mm for diffusive and binding gels, respectively) were verified using a digital caliper to an accuracy of 0.02 mm. The DGT preparation was conducted in metal-free, 10,000 class clean room to minimize metal contamination. Blank concentrations were assessed by measuring the mass of metal present in binding gels on unit brought to the field but not deployed. The field blank of binding gels (n = 3) was 0.37 nM, which was much lower than the lowest DGT-labile V concentrations measured in the Churchill River (i.e. 4.3 nM). Binding gels was eluted by 1 M nitric acid (HNO3; metal-free) for 24 h before ICP-MS analysis (XSeries II, Thermo). Indium and rhodium were used as internal standards. The accuracy of the ICP-MS measurements was assessed using SLEW-3 and SLRS-5 reference water (National Research Council, Canada). The measured V concentrations were within 5% of the certified values

The time-integrated concentration of DGT-labile V in the bulk solution, CDGT, was calculated based on Fick’s First Law of Diffusion (Zhang and Davison, 1995):

\[
CDGT = \left( M^* \Delta g \right)/(t*A*D)
\]

where M is mass of V accumulated in the binding gel, Δg is thickness of diffusive gel and filter membrane, t is deployment time, A is sampling area exposed to bulk solution and D is diffusion coefficient of vanadium in diffusive gel and filter membrane (D = 6.26 × 10⁻⁶ cm²/s at 25 °C; Luo et al., 2010). The D values were corrected to in situ average temperatures using the Stokes-Einstein relation (Li and Gregory, 1974).
2.3. Sediment leachate and DGT deployment

A bulk sample of surface sediment (0–20 cm) was collected from river sites R2 and R3 in May 20, 2015 and stored at −20 °C until analysis. Sediment samples were then oven-dried for 48 h and sieved to <2 mm for use in laboratory evaluations.

For DGT deployment experiments, 30 g of oven-dried sediment sample was weighed into acid-washed glass containers. Vanadium concentration in the acid-washed containers was 0.04 nM, which was well below concentrations measured in sediment. The samples were hydrating with 300 ml of Milli-Q water (MQW; Millipore) and gently mixed using a variable speed nutating mixer (Fisher Scientific) for 42 h at 20 ± 1 °C (Hooda et al., 1999). Leachate samples were filtered through 1 μm nitrocellulose filter (Millipore) for dissolved V and DOM analysis. Triplicate DGT units were deployed in filtered leachate and continuously stirred for 8 h. DGT binding gel was eluted and analyzed as previously for units deployed in the Churchill estuary system.

2.4. DOM analysis

Dissolved organic carbon (DOC) concentration was determined by a Shimadzu total organic carbon analyzer (TOC-VCPH). Three to five replicate injections (150 μL each) were performed for each sample, resulting in a typical variation coefficients < 2%. The absorbance spectrum was obtained using a Shimadzu UV-2550 spectrometer. MQW blanks were run after each sample to prevent cross-contamination. The EEM spectra were obtained by scanning excitation wavelength from 250 to 500 nm and emission wavelength from 300 to 600 nm, both in increments of 5 nm in ratio (S/R) mode using a Horiba Jobin-Yvon Fluoromax 4. EEMs were corrected using the manufacturer-generated emission and excitation correction factors and calibrated to Raman units using the Trent Water Quality Centre. Samples were injected at a rate of 50 μL/min and analyzed in negative ionization mode. Mass spectra were externally calibrated following Thermo protocol and internally calibrated using CH2 homologous series naturally present in the sample. The accuracy and precision in the relevant mass range (m/z 200–1000) was always <1 ppm. Molecular formula assignment, data filtering and processing were performed as per Mangal and Guéguen (2016).

2.5. High resolution mass spectrometry analysis

River water and sediment leachate DOM were diluted in 60:40 (v/v) trace metal grade methanol (Sigma Aldrich): MQW and continuously injected into a Thermo electrospray ion source of a Thermo Orbitrap (resolving power = 140 000 at m/z 400) housed in the Trent Water Quality Centre. Samples were injected at a rate of 1 μL/min and analyzed in negative ionization mode. Mass spectra were externally calibrated following Thermo protocol and internally calibrated using CH2 homologous series naturally present in the sample. The accuracy and precision in the relevant mass range (m/z 200–1000) was always <1 ppm. Molecular formula assignment, data filtering and processing were performed as per Mangal and Guéguen (2016).

2.6. Statistical analysis

Parallel factor analysis (PARAFAC) was conducted on 95 EEMs using Matlab R2010A, following the guidelines outlined in the DOMFluor toolbox (Stedmon and Bro, 2008). Emission wavelengths of 500–600 nm were cut as a means of eliminating fluorescent scattering that would otherwise be detrimental to modeling. This was acceptable because excitation and emission maxima of PARAFAC modeled fluorescent components were not expected to fall within these ranges based on previous findings (Coble, 1996, 2007). The model was constrained to non-negative values and run for three to seven components. The appropriate number of components was determined by split-half analyses (Stedmon and Bro, 2008). Composition of fluorescent components were determined by dividing each component by total fluorescence (e.g. C1% = C1/(C1 + C2 + C3 + C4 + C5)*100). Centered log ratio transformation (CLR) was run on the proportions of each fluorescent component for dimension reduction of compositional data (Cuss and Guéguen, 2015).

Principal component analysis (PCA) was conducted using the CLR-transformed proportions of PARAFAC components, DGT-labile V and dissolved V concentration, and DOC using XLSTAT 2014.

3. Results and discussion

3.1. Dissolved organic carbon concentrations

Concentrations of DOC in Churchill River (R1 - R7) and estuary (E1 - 3) ranged from 0.34 to 1.90 mM and from 0.25 to 0.75 mM, respectively (Fig. 2). The average river DOC concentration was...
Arctic rivers (Stedmon et al., 2011). Spring freshet DOC values were melting waters. No signiﬁcantly lower than at pre-freshet and summer (p < 0.05). DOC in R6 and R3 were signiﬁcantly greater in summer than during freshet (p < 0.05; Fig. 2) probably due to dilution with DOC-poor melting waters. No signiﬁcant differences were observed between pre-freshet and summer values (p > 0.05). The average estuary DOC concentration was 0.26 ± 0.10 mM in summer and 0.65 ± 0.25 mM in spring, which was consistent with other studies (Castelle et al., 2009). Estuary DOC was signiﬁcantly greater during pre-freshet (p < 0.05) than summer base ﬂow probably due to higher fresh-water pulses of DOC (higher discharge and water level) from Churchill River to the estuary.

3.2. Fluorescent DOM (FDOM)

A five-component model was split-half validated on 95 EEMs (Fig. S1). The components were compared with those in the OpenFluor database (Murphy et al., 2014). Component 1 (C1) had two excitation maximum (Ex) at <250 and 310 nm and one emission peak at 450 nm. This component was previously labeled as terrestrial humic-like DOM (Mostofa et al., 2010; Hong et al., 2012; Dong et al., 2014) and coastal environments (Dinant and Guéguen, 2013; Murphy et al., 2008; Søndergaard et al., 2003) and sediment (Yuan et al., 2014). Component C2 (Ex/Em = 275/475 nm) was attributed to UV humic-like DOM (Coble, 1996; Mostofa et al., 2010; Hong et al., 2012). Component C3 displayed two excitation maxima (<250 and 295 nm) and one emission maximum at 400 nm. This peak was previously attributed to UV and microbial humic-like DOM (Coble, 1996; Murphy et al., 2008; Hong et al., 2012; Dong et al., 2014; Kowalczik et al., 2009; Osburn et al., 2011; Yamashita et al., 2010b; Murphy et al., 2013; Kothawala et al., 2012, 2013; Cawley et al., 2012; Stedmon et al., 2003, 2007; Osburn and Stedmon, 2011; Walker et al., 2009; Shuto et al., 2014). Component C4 (Ex/Em = <250/440 nm) was attributed to UV humic-like DOM (Coble, 1996; Mostofa et al., 2010; Hong et al., 2012; Murphy et al., 2011; Yamashita et al., 2010b; Shuto et al., 2014). Component C5 (Ex/Em = 275/320 nm) was the only protein-like (tyrosine-like; Coble, 1996) component (Yamashita et al., 2010a, 2010b; Dong et al., 2014; Murphy et al., 2006; Kothawala et al., 2013; Yuan et al., 2014) found in this study.

Humic-like C1–C3 dominated the FDOM pool at all seasons (Fig. 3). The fluorescence of the humic-like C1–C3 increased from pre-freshet to summer base ﬂow at R1 and R5 (p < 0.05) but remained stable from freshet to summer at R3 and R6. All R sites except R1, showed an increase in protein-like intensity (i.e. C5) from summer to pre-freshet/freshet conditions. C5 has been associated with the autochthonous production of DOM (Determann et al., 1996) which was relatively strong during spring freshet when irradiance and nutrient inputs from the river increased (Parsons et al., 1988).

Some differences and similarities in the relative composition of PARAFAC components between ﬂow regimes were apparent (Fig. 4). Humic-like (C1%–C2%) was more abundant during summer base ﬂow than at pre-freshet (R1 and R5) and freshet (R6 and R3) (p < 0.05) (Fig. 4). On the other hand, protein-like C5% was signiﬁcantly greater during freshet than summer base ﬂow at R3 and R6 and signiﬁcantly greater during pre-freshet than summer base ﬂow at R5 (p < 0.05) (Fig. 4E). The temporal shifts in ﬂuorescence composition were consistent with a shift from poorly degraded plant litter during spring freshet to more degraded, humiﬁed DOM during base ﬂow conditions. Similar temporal patterns were reported in larger Arctic Rivers (Stedmon et al., 2011; Walker et al., 2013).

C5% in surface sediment averaged 28.6–34.1% of the total ﬂuorescence at R2–3, which was at least 2 times greater than in surface water (14.7–16.3%; Fig. 4E). The high protein contents in sediment leachates were also conﬁrmed using high resolution mass spectrometry (Fig. S3). While a variety of compounds such as lignin, lipid and tannins can be determined, compounds with protein characteristics can be found with and O/C ratio ranging from 0.15 to 0.5 and H/C ratios ranging from 1.5 to 2.25 (Ohno et al., 2013; D’Andrilli et al., 2013). Total dissolved compounds associated with protein structures in sediment leachate ranged from 359 at R2 to 380 at R3, contributing to 39.4% and 26.1% of total assigned peaks respectively. Total dissolved compounds associated with protein structures in surface water ranged from 541 at R2 to 750 at R3, contributing to 26.3% and 15.7% of total assigned peaks respectively. Percentage of dissolved compounds associated with protein structures of the total dissolved compound in sediment was about 50% higher than in surface water. Together these results conﬁrm that sediment leaching represented a signiﬁcant source of protein-like DOM in water.

3.3. V speciation in Churchill River sediment

Dissolved V concentration in sediment leachates ranged from 90.0 ± 4.4 nM at R3 to 580.2 ± 9.7 nM at R2, with an average of 335.1 nM which was up to 50 times more than the average (6.8 nM) in the Churchill River surface waters (Fig. 5). The high V concentrations in sediment leachates conﬁrm that surface sediment constitutes a signiﬁcant source of dissolved V in rivers (Emerson and Huested, 1991; Shiller and Mao, 1999, 2000). On the other hand, DGT-labile V in sediment leachates ranged from 50.4 ± 11.4 nM at R7 to 565.5 ± 28.3 nM at R3. Like surface waters (Fig. 5A and C), more DGT-labile V was found in sediment leachates at R2 (97 ± 2% vs 56 ± 13%). Together these results conﬁrm that sediment leaching can be a signiﬁcant source of dissolved V and DGT-labile V in rivers.

3.4. V speciation in surface waters

Concentrations of DGT-labile and dissolved V at R1–R7 ranged from 1 to 3 nM (Fig. 5A) and from 3 to 56 nM (Fig. 5B), respectively. The average dissolved V concentration (10.3 ± 9.4 nM) was consistent with previous studies (Shiller and Boyle, 1987; Ramani et al., 2014; Taylor et al., 2012).

Average DGT-labile and dissolved V concentrations at estuary...
sites (E1-3) ranged from 6 to 17 nM (Fig. 5A) and from 200 to 660 nM (Fig. 5B), respectively. The estuarine dissolved concentrations were higher than those reported in the open ocean (10-40 nM; Jeandel et al., 1987). However, the summer E sites were located in the surf zone in shallow waters where the interaction between oxic waters and V-rich sediments (up to 1400 nM; Emerson and Huested, 1991) increased the solubility of V; this may explain the high dissolved V concentration found in this study in aerobic water (Niencheski et al., 2014).

Dissolved riverine V concentrations were 5.6 fold greater in summer than during freshet whereas the abundance of DGT-labile V was significantly reduced from pre-freshet/freshet to summer base flow at all sites (Fig. 5C). For example, DGT-labile V at R1 accounted for 81% in pre-freshet conditions but only 15% in summer base flow. Sediment leaching is known to be a major source of dissolved V in aquatic systems (Emerson and Huested, 1991; Shiller and Mao, 2000). During pre-freshet period, bottom river sediment was still frozen, limiting the fresh leaching of dissolved V. As spring freshet progressed, river water temperatures increased above the freezing point and the bottom river sediments began to melt releasing large amount of dissolved V which were mostly DGT-labile as revealed by our sediment leachate experiments. This
process explains in part the increase in DGT-labile V associated with spring freshet. The DGT-labile V contribution was significantly higher at the R sites than that at the E sites (42.4% vs 3.1%), likely due to the interactions with V-rich river sediment.

Dissolved V concentrations were strongly positively correlated to DOC ($r = 0.48$), humic-like C1–C4 ($r = 0.39–0.66$) and C2% and C4% ($r = 0.36$ and 0.66), suggesting that dissolved V was strongly affected by DOC and humic-like intensity and abundance. Similar results were found by Shiller and Mao (2000). Lu et al. (1998) also reported that aquatic humic substances strongly complex V in aqueous solution. Dissolved and DGT-labile V concentrations were also weakly correlated with protein-like DOM. Stronger relationships were found at some sites. For example, DGT-labile V concentrations were positively correlated with C5% at R1 at all seasons ($r = 0.92$, $p < 0.05$), R6 during freshet ($r = 0.62$, $p < 0.05$) and R2 during freshet ($r = 0.89$, $p < 0.05$). The influence of protein-like DOM on DGT-labile V concentrations agreed well with the variation in DOM quality in sediment where protein-rich sediment leachates showed greater DGT-labile V concentrations.

3.5. Principal component analysis and influence of hydrography

To comprehensively assess spatial and temporal differences in dissolved V speciation, principal component analysis (PCA) was
performed using the DOC-normalized loadings, compositional loadings of the five PARAFAC components (Fig. 6). The first two principal components accounted for 73.4 and 20.6% of the total variance, respectively. The humic-like loadings (C1-4) showed dissimilar distributions along F1, suggesting that DOM quality (humic-like and protein-like) controls F1 (Fig. 6A). DGT-labile V was significantly positively correlated with F2 scores (r = 0.68, p < 0.05). Therefore, F2 can represent DGT-labile V. The loadings of protein-like C5 showed the most positive F2 loadings, suggesting that DGT-labile V was affected by the abundance of protein-like C5.

The scores plot (Fig. 6B) showed that pre-freshet/freshet and summer samples were displayed in opposite quadrants, confirming the seasonal impact on DOM and metal speciation. Freshet samples had higher F2 scores than summer samples suggesting that the abundance of C5 was influenced by spring melt conditions. This is congruent with higher concentration and composition of C5 during freshet than in summer (Figs. 3E and 4E).

The pre-freshet samples were site-dependent with R5 associated with the same quadrant as the freshet samples, indicating that the influence of C5 during pre-freshet had high abundance of C5. This is probably because of proximity of a shallow wetland, a V-rich and C5-rich viewer. Financial support for this study was awarded from NSERC, and we are also grateful for the helpful comments of two anonymous reviewers. Financial support for this study was awarded from NSERC, Churchill Northern Study Center (CNSC) and Canada Research Chair program.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.03.124.

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