Biogeochemical cycling of vanadium (+IV i +V) in aquatic systems and sediments

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DOCTORAL DISSERTATION

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Sveučilište u Zagrebu

PRIRODOSLOVNO-MATEMATIČKI FAKULTET

Lucija Knežević

BIOGEOKEMIJSKO KRUŽENJE VANADIJA (+IV I +V) U VODENIM SUSTAVIMA I SEDIMENTIMA

DOKTORSKI RAD

Mentor: Dr.sc. Elvira Bura-Nakić

Zagreb, 2023

This doctoral dissertation was carried out as a part of the postgraduate program at the University of Zagreb, Faculty of Science, Department of Geology, under the supervision of dr.sc. Elvira Bura-Nakić. The research was performed as a part of the Croatian Science Foundation scientific project „Geochemistry and redox proxies signature under the diverse environmental conditions: towards better understanding of the past redox (REDOX)“under the project number *HRZZ-2018-01* (principal investigator: dr.sc. Elvira Bura-Nakić), COST NECTAR (CA18202) Action and Ruđer Bošković Institute. The experimental part of the research was carried out at Ruđer Bošković Institute (Zagreb, Croatia) and Université Paris-Saclay, CNRS/IN2P3, IJCLab (Orsay, France).

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BASIC DOCUMENTATION CARD

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Doctoral thesis

BIOGEOCHEMICAL CYCLING OF VANADIUM (+IV AND +V) IN AQUATIC SYSTEMS AND SEDIMENTS

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The PhD thesis is based on the developed and adapted specific analytical methods for determination of vanadium redox species concentration in the water samples and bioavailable phase of surface sediment of Krka River esturay. The results show that V is predominantly present in the form of V(+V) species in the oxic water column of Krka River estuary, while determined presence of V(+IV) was linked with oxygen depletion in the water layer at the head of estuary, and stabilisation with organic ligands in the anthropogenic burdened sampling site. Determined slight V enrichment is mostly accompanied with retention of V to less mobile sediment phases, possibly due to the low mobility of reduced species and their binding to organic matter. Redox speciation in the bioavailable fraction of surface sediments of Krka River estuary showed that V is present in the form of V(+IV), thus it is not presenting toxic risk for sorrounding biota. In addition, stability of V(+IV)/V(+V) species was investigated in the presence of structurally simple organic ligand (succinic acid) and sulphurised organic ligands (3 mercaptopropionic acid, L-cysteine, thioacetic and ethanthiol) under modelled conditions. Stability constants for V(+IV) and V(+V) complexes with succinic acid in acidic aqueous solution are determined, where higher affinity of V(+IV) species, compared to V(+V), towards complexation was observed. In addition, stability of V(+V) species in the presence of sulpfurized organic ligands was monitored by novel combined approach based on the usage of spectrophotometric and chromatographic instrumentation. Results show that V(+V) reduction is a pH dependant and proton catalysed process which can play a significant role in decreasing V(+V) toxicity in natural systems.

(146 pages, 16 figures, 177 references, original in English)

Keywords: Vanadium redox speciation, ion chromatography, mobility, toxicity, succinic acid, sulphurized organic ligands

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BIOGEOKEMIJSKO KRUŽENJE VANADIJA(+IV i +V) U VODENIM SUSTAVIMA I SEDIMENTIMA

Lucija Knežević
Laboratorij za fizičku kemiju tragova
Zavod za istraživanje mora i okoliša
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Doktorski rad temelji se na razvijenim i prilagođenim postojećim specifičnim analitičkim metodama za određivanje koncentracije redoks vrsta vanadija u uzorcima vode i biodostupne faze površinskih sedimenata estuarija rijeke Krke. Rezultati pokazuju da je V pretežno prisutan u obliku V(+V) vrsta u oksičnom vodenom stupcu estuarija rijeke Krke, dok je utvrđena prisutnost V(+IV) povezana s niskom koncentracijom kisika u vodenom sloju na gornjem dijelu estuarija, te stabilizacijom s organskim ligandima na antropogeno opterećenom mjestu uzorkovanja. Utvrđeno blago obogaćenje V u sedimentu uglavnom je popraćeno zadržavanjem V u manje pokretnim fazama sedimenta, vjerojatno zbog niske pokretljivosti reduciranih vrsta i njihovog vezanja na organsku tvar. Redoks specijacija u biodostupnoj frakciji površinskih sedimenata estuarija rijeke Krke pokazala je da je V prisutan u obliku V(+IV), stoga ne predstavlja toksičan rizik za okolnu biotu. Nadalje, istražena je stabilnost V(+IV)/V(+V) vrsta u prisutnosti strukturno jednostavnog organskog liganda (sukcinske kiseline) i sumpornih organskih liganda (3 merkaptopropionske kiseline, L-cisteina, tioctene kiseline i etantiola) u modeliranim uvjetima. Određene su konstante stabilnosti za V(+IV) i V(+V) komplekse sa sukcinskom kiselinom u kiseloj vodenoj otopini, gdje je utvrđena viša stabilnost V(+IV) komplekasa, u usporedbi s V(+V). Dodatno, stabilnost V(+V) vrsta u prisutnosti sumpornih organskih liganda praćena je novim pristupom koji se temelji na usporednom korištenju spektrofotometrijske i kromatografske instrumentacije. Rezultati pokazuju da je redukcija V(+V) protonski kataliziran proces ovisan o pH, te može igrati važnu ulogu u smanjenju toksičnosti V(+V) u prirodnim sustavima.

(146 stranica, 16 slika, 177 literaturna navoda, originalno na engleskom jeziku)

Ključne riječi: Redoks specijacija vanadija, ionska kromatografija, mobilnost, toksičnost, sukcinska kiselina, sulfurizirani organski ligandi.

Mentor: Dr.sc. Elvira Bura Nakić, Senior Research Associate

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Dr.sc. Neven Cukrov,
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Dr.sc. Hana Fajković (zamjena)

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Acronyms:

ASV – Anodic stripping voltammetry

AdCSV – Adsorptive cathodic stripping voltammetry

CE – Capillary electrophoresis

DOM – Dissolved organic matter

ETAAS – Electrothermal atomic absorption spectrometry

FAAS - Flame atomic absorption spectrometry

FIA – Flow injection analysis

GFAAS- Graphite flame atomic absorption spectrometry

HMDE/ DME –Hanging mercury drop electrode

HPLC –High-performance liquid chromatography

IC- Ion chromatography

ICP- AES – Inductively coupled plasma atomic emission spectroscopy

ICP-MS- Inductively coupled plasma mass spectrometry

ICP-OES – Inductively coupled plasma optical emission spectrometry

LLE – Liquid-liquid extraction

NOM – Natural organic matter

RPLC – Reverse phase liquid chromatography

SC- Stripping chronopotentiometry

SPM – Suspended particulate matter

SPE – Solid phase extraction

SDME – Single-drop mikroextraction

SPME – Solid phase mikroextraction

SWAdCSV – Square wave adsorptive cathodic stripping voltammetry

TM – Trace metals

TOC – Total organic carbon

UV- Ultra violet

LIST OF RESEARCH PAPERS

Research papers on which the doctoral dissertation is based:

I. Knežević, Lucija; Omanović, Dario; Bačić, Niko; Mandić, Jelena; Bura-Nakić, Elvira. Redox Speciation of Vanadium in Estuarine Waters Using Improved Methodology Based on Anion Exchange Chromatography Coupled to HR ICP-MS System. Molecules, 26 (2021), 9; 2436. 15 doi:10.3390/molecules26092436

II. Knežević, Lucija; Cukrov, Nuša; Bura-Nakić, Elvira. Ion-exchange chromatography as a tool for investigating vanadium speciation in sediments: preliminary study. Journal of soils and sediments, 20 (2020), 2733-2740. doi:10.1007/s11368-019-02484-3

III. Bura-Nakić, Elvira; Knežević, Lucija; Mandić, Jelena. Chromatographic and spectrophometric studies of vanadate (+V) reduction by 3–mercaptopropionic acid. Journal of inorganic biochemistry, 230 (2022), 111747, 8. doi:10.1016/j.jinorgbio.2022.111747

PROŠIRENI SAŽETAK

Vanadij (V) je metal u tragovima s rastućim antropogenim opterećenjem u prirodnim vodenim sustavima. Ipak, složeno kemijsko ponašanje izraženo kroz brojna oksidacijska stanja (nevesti…), raznolikost redoks reakcija i reakcija kompleksiranja s prirodnim organskim ligandima, te laka promjena redoks vrsta utjecali su na trenutno ograničeno znanje o biogeokemijskom ciklusu V u prirodnom vodenom mediju. Osim toga, trenutačni eksperimentalni dokazi redoks raspodjele V u uzorcima vodenog okoliša su oskudni, pretežno zbog neprilagođenosti postojeće analitičke opreme složenom sastavu prirodnih vodenih i sedimentnih sustava (Chen and Owens, 2008a; Shaheen *et al.*, 2019). S obzirom na to, biodostupnost, toksičnost i interakcija V s organskim ligandima je trenutno nedovoljno razjašnjena (Gustafsson, 2019; White and Levy, 2021). Istraživanje redoks raspodjele V u prirodnim sustavima, kao i čimbenici koji utječu na promjenu redoks vrsta ili stabilnost u vodenom mediju, od velike su važnosti u razumijevanju biogeokemijskog kruženja V. Vanadij se u okolišu pretežno nalazi u obliku V(+IV) i V(+V) redoks vrsta, koje se znatno razlikuju u reaktivnosti, mobilnosti i toksičnosti. Vanadij(+V) koji je dominantno prisutan u oksičnim sustavima, ujedno je najmobilniji i najtoksičniji oblik vanadija, kada je prisutan u slobodnom ionskom obliku. Biogeokemijski fluks V snažno ovisi o redoks uvjetima i pH vrijednostima sustava, što ga čini važnim redoks indikatorom prošlih geokemijskih događaja. Posebno, interakcija V s organskim ligandima znatno utječe na raspodjelu V redoks vrste te posljedično na njegovu mobilnost i toksičnost u prirodnim sustavima (Huang *et al.*, 2015; Gustafsson, 2019). Dakle, za razumijevanje biogeokemijskog ciklusa nužno je doprinijeti s novim eksperimentalnim dokazima o redoks specijaciji V u prirodnim vodenim i sedimentnim sustavima. Također, procjena biodostupnosti potkrijepljena s utvrđenim dominantnim redoks vrstama V u sedimentu, značajno doprinosi razumijevanju toksičnog utjecaja V na okolnu biotu. Nadalje, reaktivnost V(+IV) i V(+V) redoks vrsta prema prirodnim organskim ligandima ključna je za razumijevanje moguće stabilizacije i/ili redukcije toksičnih V(+V) vrsta u prirodnim sustavima.

Predstavljeno istraživanje obuhvaća razvoj i prilagodbu postojećih analitičkih metoda za određivanje redoks stanja V u uzorcima okoliša, te modelnih otopina pri pretežno oksičnim fizikalno-kemijskim uvjetima. Hipoteza ove studije se temelji na pretpostavci da redoks uvjeti prirodnog sustava i prisutnost organskih liganada utječu na raspodjelu vanadijevih redoks vrsta, toksičnost i geokemijsko kruženje u prirodnim vodenim sustavima i sedimentima. Specifično,

pretpostavlja se da oksični uvjeti podupiru stabilnost V(+V) u vodenom stupcu, dok je dominantna zastupljenost reduciranih vrsta u ektrahibilnim frakcijama sedimenta pretpostavljena s obzirom na viši afinitet prema česticama i nižu mobilnost reduciranih kemijskih vrsta V, u usporedbi s V(+V). Nadalje, pretpostavlja se viša stabilnost V(+IV) vrsta s organskim ligandima te redukcija V(+V) u prisustvu biološki važnih sulfuriziranih organskih liganada.

Ovaj doktorski rad temelji se na tri znanstvena članka, pri čemu svaki od njih pridonosi dosadašnjim spoznajama o V redoks specijaciji i čimbenicima koji na nju utječu. Svi članci sadrže znanstveni doprinos s obzirom na razvijene i unaprijeđene analitičke metode te njihovu primjenu u prirodnim ili modelnim uzorcima.

U znanstvenom članku I. postojeća metodologija je prilagođena za V redoks specijaciju u uzorcima vodenog stupca estuarija korištenjem povezane tehnike koja se temelji na Ionskoj kromatografiji povezanoj s masenom spektrometrijom visoke rezolucije s induktivno spregnutom plazmom (engl. Ion Chromatography- High Resolution Inductively Coupled Plasma Mass Spectrometry, IC-HR ICP-MS) instrumentaciji. Prilagodbom analitičke metode omogućena je V redoks specijacija u vodenim uzorcima stratificiranog estuarija rijeke Krke. Redoks raspodjela pokazala je dominaciju V(+V), dok je visoki udio V(+IV) (do 26%) utvrđen u vodenom sloju u gornjem dijelu estuarija karakteriziran s niskom koncentracijom kisika. Osim toga, niska koncentracija kisika utjecala je na uklanjanje otopljenog V iz vodenog stupca, vjerojatno putem adsorpcije na prisutne suspendirane čestice u vodenoj fazi. Na lokacijama pod antropogenim utjecajem je također utvrđena prisutnost reduciranih vrsta V, unatoč oksičnim uvjetima. Stabilizacija reduciranih vrsta u oksičnom vodenom sloju u estuariju rijeke Krke protumačena je interakcijom V s prirodnim organskim ligandima. Stoga su rezultati u znanstvenom članku I. dodatno potkrijepljeni studijom o stabilnosti V(+IV) i V(+V) redoks vrsta s jednostavnim organskim ligandom (sukcinska kiselina), koji je odabran kao zamjena za strukturno složeniju prirodnu organsku tvar. Istraživanje je provedeno korištenjem afinitetne kapilarne elektroforeze u vodenim kiselim otopinama pri pH vrijednostima 1,5, 2,0 i 2,4 i različitim koncentracijama liganda. Ovom studijom su izvedene nove vrijednosti konstanti stabilnosti kompleksa V(+IV) i V(+V)-sukcinske kiseline. Logaritmi konstanti stabilnosti, izmjereni na ionskoj jakosti 0,1 mol L^{-1} ($NaClO_4/HClO_4$) i 25°C, su $\log\beta_{111}=7,4 \pm 0,2$ i $\log\beta_{122}=14,1 \pm 0,5$ za V(+IV), te $\log\beta_{111}=7,3 \pm 0,2$ 0,1 za V(+V). Vrijednosti, ekstrapolirane na nultu ionsku jakost korištenjem Daviesove jednadžbe, iznose $\log\beta°_{111}=8.3 \pm 0,2$ i $\log\beta°_{122}=15.6 \pm 0,5$ za V(+IV), te $\log\beta°_{111}=7,9 \pm 0,1$ za V(+V). Ova studija opisuje značajan doprinos poznavanju

prirode kompleksiranja V(+IV) i V(+V) s jednostavnim organskim ligandima. Naime, prvi put su dane termodinamičke konstante (pri nultoj ionskoj jakosti i 298 K) za V(+IV) i V(+V) protonirane komplekse sa sukcinskom kiselinom. Osim toga, numeričke vrijednosti opisuju višu stabilnost V(+IV) kompleksa sa sukcinskom kiselinom, što ukazuje na mogući viši afinitet V(+V) vrsta prema organskim ligandima u prirodnim sustavima.

U znanstvenom članku II., jednostavna kromatografska metoda je primjenjena za određivanje redoks vrsta V u ekstrahibilnoj frakciji površinskih sedimenata u estuariju rijeke Krke. Prethodno razvijena analitička metoda je optimizirana, čime je postignuto odvajanje V(+IV) i V(+V) redoks vrsta u uzorcima sedimenta složenog sastava, na temelju anion-izmjenjivačke ionske kromatografije. Opisana analitička metoda predstavlja novi pristup u procjeni biodostupnosti i toksičnosti V na temelju određenih V redoks stanja u uzorcima sedimenta. Znanstveni članak II., dodatno je potkrijepljen s rezultatima redoks specijacije V u ekstrahibilnoj frakciji površinskog sedimenta duž estuarija rijeke Krke, kako bi se utvrdio utjecaj prirodnih i antropogenih čimbenika na re-mobilizaciju V iz sedimenta. Navedenim istraživanjem dokazana je prikladnost kromatografske metode u analizi redoks vrsta V u uzorcima sedimenta složenog sastava. Rezultati ukazuju na nisku mobilnost V, dok izračunati faktor obogaćenja ne ukazuje na zabilježeno onečišćenje V u površinskom sedimentu estuarija rijeke Krke. Međutim, primijećeno blago obogaćenje V u površinskim sedimentima uglavnom je popraćeno zadržavanjem V u manje mobilnim frakcijama sedimenta. Najveći udio ekstrahibilnog V uočen je u površinskim sedimentima u kojima dominiraju karbonati. Redoks specijacija je pokazala dominantnu zastupljenost reduciranih V(+IV) vrsta u svim obrađenim uzorcima. Stoga, čak i u slučaju utvrđene visoke ektrahibilnosti V, dobiveni rezultati ukazuju na nizak toksični rizik za okolnu biotu.

U znanstvenom članku III., proučavana je kinetika redukcije vanadata s 3-merkaptopropionskom kiselinom primjenom novog pristupa koji se temelji na kombinaciji spektrofotometrijske i kromatografske metode. Ova studija je također nadopunjena dodatnim eksperimentima u kojima se prati interakcija V(+V) i bioloških te ekološki važnih tiola (L-cistein, tioctena kiselinu i etantiol). Također, po prvi put je proučavana sposobnost redukcije V(+V) s tioctenom kiseline i etantiolom. Dobiveni rezultati ukazuju na pH-ovisnu, protonski kataliziranu, redukciju V(+V) u vodenoj otopini. Uočeno je stvaranje stabilnih kompleksa između V(+IV) i tiola. Monodentatni tioli (tioctena kiselina, etantiol) bili su znatno manje uspješni u redukciji V(+V) u usporedbi s polidentatnim tiolima (3-merkaptopropionska kiselina, L-cistein). Formiranje stabilnih V(+IV)-

tiolnih kompleksa također je uočeno u slučaju polidentatnih tiola. Provedeni eksperimenti ukazuju na mogući mehanizam smanjenja V(+IV) toksičnosti i stabilizacije V(+IV) u prirodnim sustavima u prisutnosti tiola koji u svojoj strukturi sadrže dodatne proton-donorske skupine. Kada se uspoređuju monodentatni tioli, tiokarboksilne kiseline posjeduju veći redukcijski afinitet prema V(+V) u usporedbi s tioalkoholima. Etantiol, koji je također poznat kao dominantni organski zagađivač u sedimentu i vodenom okolišu, pokazao se najmanje uspješnim u redukciji V(+V) u vodenim otopinama pri ispitivanom širokom rasponu pH vrijednosti.

Rezultati prikazani u ovom doktorskom radu upućuju na to da redoks uvjeti, pH i interakcija s organskim ligandima (karboksilna kiselina i tioli) utječu na redoks raspodjelu V u vodenoj fazi i sedimentu. Pri oksičnim uvjetima, V(+V) su dominantno zastupljene redoks vrste V u vodenom stupcu estuarija. Stabilizirane reducirane vrste u oksičnim uvjetima ukazuju na moguću redukciju V s organskim ligandima i posljedičnu stabilizaciju V(+IV) redoks vrsta. Osobito, interakcija V s organskim ligandima može uzrokovati redukciju V(+V) i stabilizaciju reduciranih vrsta u oksičnom vodenom stupcu, unatoč termodinamičkim predviđanjima. Redukcija V(+V) također utječe na nisku biodostupnost i toksičnost u sedimentima estuarija. Vanadij ima tendenciju zadržavanja u manje mobilnim frakcijama sedimenta, što je vjerojatno posljedica prevladavanja reduciranih vrsta u ukupnoj koncentraciji V. Dakle, čak i kada je utvrđena visoka ektrahibilnost V, toksičnost vanadija ostaje niska. Također, proučavanje interakcije V(+IV) i V(+V) redoks vrsta s važnim organskim ligandima doprinose razumijevanju stabilnosti formiranih komplekasa i redukciji V(+V) u vodenoj otopini.

Znanstveni doprinos doktorskog rada temelji se na (i) razvoju i prilagodbi analitičkih metoda za jednostavnu, izravnu, robusnu i ekonomičnu analizu prirodnih uzoraka složenog sastava i modelnih uzoraka, s ciljem određivanja redoks raspodjele V, ii) razumijevanja utjecaja fizikalno-kemijskih čimbenika i antropogenog opterećenja na V redoks raspodjelu u oksičnim prirodnim vodenim i sedimentnim sustavima, iii) opisivanje stabilnosti V(+IV) i V(+V) redoks vrsta u prisutnosti sukcinske kiseline te biološki i ekološki važnih tiola pridonosi razumijevanju mehanizma interakcije V vrsta s organskim ligandima, i posljedični utjecaj na toksičnost V i njegovu biogeokemiju u prirodnim sustavima, iv) prvo istraživanje redoks specijacije V u estuariju rijeke Krke doprinosi razumijevanju biogeokemijskog ponašanja V u navedenom sustavu.

THESIS SUMMARY

Vanadium(V) is a trace metal and an emerging pollutant in the natural aquatic systems. Still, complex chemical behaviour expressed through number of oxidation states, variety of redox and complexation reactions with natural organic ligands, and facile redox interconversion caused limited current knowledge on biogeochemical cycle of V in natural aqueous medium. In addition, current experimental data set for V redox distribution in the environmental samples are scarce, mainly due to the maladjustment of existing analytical instrumentation to complex matrix of natural aquatic and sediment systems (Chen and Owens, 2008b; Shaheen *et al.*, 2019). Especially, studies on redox distribution of V in natural systems, as well as factors influencing redox species conversion or stability in aqueous medium, are of high importance in deciphering V biogeochemistry. Current knowledge gaps in V biogeochemistry are related to limited studies on V bioavailability, toxicity, and understanding the mechanism of V interaction of with organic and sulphurised organic ligands and their impact on V biogeochemical cycling (Gustafsson, 2019; White and Levy, 2021).

The redox speciation of V in the environment is mainly dominated by the V(+IV) and V(+V) species, which possess different characteristics of reactivity, mobility, and toxicity. Vanadium(+V), which is dominantly present in oxic systems, is also most mobile and toxic form of vanadium, when present in free ionic state. The V biogeochemical flux is strongly governed by redox conditions of the system and chemical reactivity towards natural ligands, which makes it important redox indicator of past geochemical events. Especially, rich chemistry with organic ligands enables formation of organic complexes and/or reduction of V species which highly affects V mobility and toxicity in natural systems (Huang *et al.*, 2015; Gustafsson, 2019). To gain complete insight in the biogeochemical cycle of V, knowledge on how certain environmental conditions affect V species distribution is necessary. In addition, reactivity of V(+IV) and V(+V) redox species towards naturally occurring organic ligands, is crucial in understanding possible stabilisation and/or reduction of toxic V(+V) species.

The goals of this research were the development and adaptation of existing analytical methods for determining V oxidation states in environmental samples, and model solutions under mainly oxic physico-chemical conditions. Established hypothesis of this study presumed that redox conditions of the natural system and the presence of organic ligands affect the distribution of vanadium redox

species, toxicity and geochemical cycling in natural water systems and sediments. More specifically, it is hypothesized that oxic conditions support the stability of V(+V) in the water column, while the dominant presence of reduced species in acid-extractable sediment fractions is proposed due to the higher particle affinity and lower mobility of reduced chemical species V, compared to V(+V). Furthermore, higher stability of V(+IV) species with organic ligands and reduction of V(+V) in the presence of biologically important sulfurized organic ligands is assumed. This doctoral thesis is based on three research papers, where each of them contributes to the current knowledge on V redox speciation, and factors affecting it. Each paper contributes to the current knowledge with developed and adapted analytical methods for V redox speciation and their application in natural and model samples.

In the research paper I., an improved methodology was developed for the V redox speciation in the estuarine waters using a hyphenated technique based on the Ion Chromatography- High Resolution Inductively Coupled Mass Spectrometry (IC - HR ICP-MS) instrumentation. Adaptation of the analytical method enabled V redox speciation in samples taken in the vertical salinity gradient of the highly stratified Krka River estuary. Redox distribution showed predominance of V(+V), while high share of V(+IV) (up to 26%) was determined in the oxygen-depleted water layer at the head of estuary. Additionally, oxygen depletion affected removal of dissolved V from the water column, possibly due to the particle scavenging. Higher concentrations of reduced species were also determined at the anthropogenically burdened site, despite oxic conditions. Preservation of reduced species in oxygen saturated water layers of Krka River estuary is possibly affected by interaction of vanadium species with natural organic ligands present in the water column of Krka River estuary. Thus, results obtained in the research paper I., are further supported with study on stability of V(+IV) and V(+V) redox species with simple organic ligand (succinic acid), which was chosen as a proxy for structurally complex natural organic matter. Study was done using affinity capillary electrophoresis in aqueous acid solutions at pH values 1.5, 2.0 and 2.4 and different ligand concentrations. Results of the study yielded novel stability constants values on V(+IV) and V(+V)-succinic acid complexes. The logarithms of the stability the constants, measured at 0.1 mol L^{-1} ($NaClO_4/HClO_4$) ionic strength and 25°C, are $\log\beta_{111}=7.4 \pm 0.2$ and $\log\beta_{122}=14.1 \pm 0.5$ for V(+IV), and $\log\beta_{111}=7.3 \pm 0.1$ for V(+V). The stability constant values, extrapolated to zero ionic strength with the Davies equation, are $\log\beta°_{111}=8.3 \pm 0.2$ and $\log\beta°_{122}=15.6 \pm 0.5$ for V(+IV), and $\log\beta°_{111}=7.9 \pm 0.1$ for V(+V). This study describes a significant contribution to the knowledge of

V(+IV) and V(+V) chemistry with simple organic ligands. Namely, the thermodynamic constants (at zero ionic strength and 298 K) for V(+IV) and V(+V) protonated complexes with succinic acid are given for the first time. In addition, numerical values describe higher stability of V(+IV) complexes with succinic acid, which points to the possible significant stabilisation of V(+IV) species with organic ligands in natural systems.

In the research paper II., application of simple chromatographic method for the determination of redox species in the acid-extractable sediment fraction of surface sediments of Krka River estuary was conducted. A previously published procedure was further optimized, and separation of V(+IV) and V(+V) redox species on anion-exchange based Ion Chromatography (IC) in a complex matrix of sediment samples was accomplished. The described analytical method represents a new approach in assessing potential bioavailability of V based on chromatographic techniques. Research paper II., is supported with additional processed samples along the Krka River estuary to depict how natural and anthropogenic factors affect bioavailability of V. With this study, suitability of the chromatographic method for the analysis of V redox speciation in complex environmental matrices is presented. Overall, acid-extractability of V along the Krka River estuary sediment remained low, and calculated enrichment factor didn't show any recorded V pollution in the surface sediments of Krka River estuary. However, recorded slight enrichment of V in the surface sediments is usually accompanied by retention of V in less soluble sediment fractions. Highest share of bioavailable V was noticed in the surface marine sediments dominated by the carbonates. Redox speciation showed predominance of reduced V(+IV) species in all processed samples. Thus, even in the case of determined high V extractibility, obtained results imply low risk of V toxicity to surrounding biota.

In the research paper III., the kinetics of vanadate reduction by 3-mercaptopropionic acid was studied using novel approach based on the combination of spectrophotometric and chromatographic method. This study is also supported with additional experiments using biologically and environmentally relevant thiols (L-cysteine, thioacetic acid and ethanethiol). In addition, thioacetic acid and ethanethiol capability of reducing V(+V) was studied for the first time. Obtained results imply proton catalysed pH-dependant reduction of V(+V). Formation of stable V(+IV)-thiol complexes were observed. Monodentate thiols (thioacetic acid, ethanethiol) were far less successful in V(+V) reduction compared to the polydentate thiols (3-mercaptopropionic acid, L-cysteine). Formation of stable V(+IV)-thiol complexes were observed in case of polydentate

thiols, as well. Conducted experiments point to the possible mechanism of lowering the V toxicity and V(+IV) stabilisation in natural systems by reduction mechanism of V(+V) to less toxic V(+IV) species, with thiols containing additional proton donor groups. When comparing monodentate thiols, thiocarboxylic acids possibly own higher reduction affinity towards V(+V) compared to thioalcohols. Ethanethiol, which is also known as a dominant organic pollutant in sediment and water environment, proved as least successful in reducing V(+V) over a wide pH range.

Results presented in this doctoral thesis suggest that redox conditions, pH and interaction with organic ligands (carboxylic acid and sulphur donor ligands) govern V redox distribution in aqueous and sediment medium. In the oxygen rich waters, predominance of V(+V) is established. Stabilised V(+IV) species in oxic conditions depict possible reduction of V with organic ligands, and consequent stabilisation of V(+IV). Especially, interaction of V with organic ligands can cause V(+V) reduction and stabilisation of reduced species in oxic water column, despite thermodynamic predictions. Reduction of V(+V) also affects low extractibility and toxicity in estuarine sediments. Vanadium tends to retain in less mobile sediment fraction, which is presumably a consequence of predominance of reduced species in the total V. Thus, even when bioavailability of V is established as high, toxicity of vanadium remains low. This study also provided results on the V(+IV) and V(+V) species interaction with important organic ligands to understand stability of formed complexes and reduction kinetics.

Scientific contribution of doctoral thesis is based on the (i) development and adaption of analytical methods for simple, direct, robust and cost-effective analysis of complex environmental matrices and model samples, with the goal of V redox distribution ii) understanding the effect of physico-chemical factors and anthropogenic burdening on V redox distribution in oxic natural aqueous and sediment systems iii) describing the stability of V(+IV) and V(+V) redox species in the presence of succinic acid and biologically and environmentally relevant thiols in order to understand the interaction mechanisms of V species with organic ligands, and consequent impact on V toxicity and biogeochemistry in natural systems iv) pioneer study of V redox speciation in Krka River estuary contributes to the understanding V biogeochemical behaviour in the stated natural system.

1. INTRODUCTION

The chemical speciation of trace metals (TM) is of great importance in various fields of research (analytical chemistry, geochemistry, toxicology, and environmental chemistry). Importantly, chemical speciation is essential for cohesive outlook on biogeochemical cycling of trace metal constituents in natural aqueous systems. Although information on total TM concentrations can give helpful insight on TM flux and enrichment in natural aqueous systems, chemical speciation studies enable assessment of their chemical reactivity, bioavailability and toxicity (Hirose, 2006). The official IUPAC definition refers to a chemical species as a specific forms of a particular element defined in terms of isotopic composition, electronic or oxidation state, and molecular structure (Jrc *et al.*, 2000).

Elemental vanadium (V) is a soft white metal which was first discovered in 1801., and rediscovered later in 1831. (Gustafsson, 2019). Although V is one of the most abundant trace metals in natural systems, little attention was given to its biogeochemistry until 1980s. More specifically, V redox speciation in environmental samples has only recently been receiving appropriate scientific attention, mainly due to the complex V redox chemistry and analytical constraints (Huang *et al.*, 2015; Shaheen *et al.*, 2019). Additionally, V readily enters variety of complexation and reduction reactions with naturally occurring ligands, which affects facile interconversion of V redox species. Consequently, V is one of the most understudied trace metals, compared to other potentially toxic elements (e.g., As and Hg). Current knowledge gaps on V biogeochemical behaviour are especially concerning in the light of growing anthropogenic V burdening of natural aqueous systems. Leading world health and environmental agencies recommended strengthening research efforts on the environmental behaviour and biological toxicological characteristics of V (Yang *et al.*, 2022).

Naturally occurring V is a mixture of two isotopes (^{51}V - 99.76% and ^{50}V - 0.24%) and can be present in several oxidation states (+II, +III, +IV, +V), from which +IV and +V are most commonly found in natural systems (Gustafsson, 2019). The V redox distribution and solubility is to a great extent controlled by redox and pH conditions, making it prominent redox indicator of past geochemical events. Other factors, such as V concentration and complexation reactions with natural organic and/or inorganic ligands influence V redox distribution, as well (Huang *et al.*, 2015). In turn, biogeochemical cycling of V is controlled by its distribution between redox states and characteristic chemical behaviour of each redox specie. Different redox species of V own

different aqueous chemistry, affinity towards complexation with natural ligands, and adsorption affinity towards natural particulate matter. The difference in toxicity between different V redox species is particularly important, since V(+V) represents the most toxic and mobile redox form of V (Costa Pessoa, 2015; Huang *et al.*, 2015; Gustafsson, 2019).

Experimental studies of V redox speciation in environmental samples are evolving together with the analytical instrumentation used for environmental studies. Since natural systems have usually complex matrix for standard analytical instrumentation, modifications and adjustments are often needed in the field of environmental studies. Generally, the process of speciation of trace metals is defined as analytical process during which one or more chemical species of a particular TM are quantitatively and qualitatively determined in a specific sample, as defined by IUPAC (Jrc *et al.*, 2000). The mentioned process must contain information about the distribution of a certain element among defined chemical species in the system under investigation (Hirose, 2006). The analytical process for the determination of TM redox species in natural samples always consists of the following steps: defining the problem to be investigated and selecting appropriate analytical instrumentation, selecting sampling locations of scientific interest, collecting samples which are representative of the specific sampling area, storing samples, appropriate sample preparation, analysis of samples using selected analytical instrumentation, analysis and processing of results, and finally drawing conclusions of scientific and social interest based on the obtained results (Hill, 1997).

Analytical methods developed for the purpose of V redox species distribution in marine samples must ensure high accuracy of the analytical process (preserving the original distribution of chemical species in the sample), low risks of contamination, and detection limits suitable for analyte quantification (Hirose, 2006). Reliable management of the entire analytical process is often a demanding process, especially considering general unsuitability of standard analytical instrumentation to the complex matrix of natural aqueous samples and low concentration of V in these systems. Therefore, the analytical processes of V redox speciation determination still represent a challenge in the study of natural aquatic systems (Hirose, 2006; Chen and Owens, 2008a).

Presented thesis is based on the three published research papers dealing with V redox speciation in the water column and acid-extractable fraction of surface sediments of highly stratified Krka River estuary. In addition, stability of V(+IV) and V(+V) species in the presence of organic ligands

(carboxylic acid and thiols) was studied in model solutions. Obtained results contribute to the current knowledge on V redox cycling in natural aqueous systems. Additionally, studies are of analytical importance for the current usage of the chromatographic techniques in the field of redox speciation of V, due to the performed adjustments to complex matrix of natural samples and novel approach in studying of V redox species and thiol interactions. Results presented in research papers are accompanied by additional results on stability of V(+V)/V(+IV) complexes with simple organic ligand (succinic acid) which serves as a support to the observed redox behaviour of V in the natural oxic water column. In addition, expanded study on V acid-extractibility from surface sediments of Krka River estuary has been also performed, now covering critical points in understanding how detrital material and anthropogenic burdening affects V potential remobilisation to surrounding biota. Lastly, published results on V(V) - reduction by 3-mercaptopropionic acid is supported with study of pH dependant V(V) reduction with other biologically and environmentally relevant thiols. Overall, presented results contribute to current ambiguities about V(+IV)/V(+V) biogeochemistry, with the emphasis on understanding V mobility, toxicity, and its interaction with other organic and sulphur donor ligands in aqueous solutions.

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2. LITERATURE OVERVIEW

2.1. Natural sources of vanadium

Vanadium(V) is present in various natural minerals with an average concentration of 97 mg kg^{-1} in the upper continental crust while approximately ~$20x10^9$ g V/year enters biogeochemical cycle of surface systems (Schlesinger, Klein and Vengosh, 2017). Shiller *et al.* (2000) determined constant correlation of V to Si, which suggests that V is entering aquatic natural systems dominantly via chemical weathering of silicate minerals (Shiller and Mao, 2000). Other pathways of V in the natural aqueous systems include volcanic activity, atmospheric deposition and aeolian processes. About 8.4 t of V is emitted from the natural sources (volcanic emissions, continental dust, forest fires etc.) to the atmosphere, and can eventually be deposited in the natural aqueous system (Imtiaz *et al.*, 2015). Atmospheric soil dust, followed by marine aerosols, are presumably main sources of natural atmospheric V (Orecchio *et al.*, 2016; Gustafsson, 2019). However, other stated sources contribute to much lower extent in the transport of V in the aquatic environment when compared to weathering processes (Gustafsson, 2019; Bian *et al.*, 2022).

Consequent to the high lithogenic concentrations and dominant weathering pathway to environment, V is second most abundant trace metal (TM) in seawater (~ 1.8 µg/L) (Wang and Sañudo Wilhelmy, 2009; Gustafsson, 2019). The long average residence time of V (91 ka), compared to ocean water mixing time of 1-2 ka, influenced homogenous isotopic composition of V (δ^{51} V) in modern seawater (Fan *et al.*, 2021). The dissolved concentration of V in the groundwater and river systems varies dependant on the geological background and atmospheric deposition processes. The highest concentrations of V in the groundwater systems were observed in alkaline and/or oxic conditions, while deep groundwater systems may contain up to 147 µg L^{-1} of V (Gustafsson, 2019). As previously mentioned, concentration of dissolved V in rivers correlates to Si concentrations with an average V/Si ratio of 66×10^{-6}, as chemical weathering process is a predominant pathway of V in natural aqueous system (Shiller and Mao, 2000). However, in sedimentary regions V/Si ratio seems to be influenced by strong surface complexation with iron-oxide and organic colloids (Wällstedt, Björkvald and Gustafsson, 2010). Significant variability of total V concentrations in the open sea waters compared to riverine waters suggests additional input of V into the seawater column (Gustafsson, 2019). Generally, V seems to be enriched in sediments under prevailing anoxic and euxinic conditions (>500 µg g^{-1}) whereas low V concentrations in the sediment suggest oxic conditions at the burial time (Bian *et al.*, 2022). This is additionally

supported with the suggested upward V flux from the sediment into the overlaying water column where remobilisation of V is known to be supported by the appropriate oxic phsyico-chemical parameters. On the other hand, anoxic sediments support downward V flux, where low Eh values and oxygen depletion promote V removal from the water column and stabilisation in the sediment (Schlesinger, Klein and Vengosh, 2017; Gustafsson, 2019). Stated behaviour define V as a redox sensitive element, and it's the reason why it is increasingly used as a proxy to reflect physico-chemical conditions that governed in the natural aquatic systems during significant past geochemical events (Moore *et al.*, 2020).

2.2. Anthropogenic sources of vanadium

Modern biogeochemical V cycle shows increasingly strong anthropogenic burdening of the aquatic natural systems (Schlesinger, Klein and Vengosh, 2017). Observed global anthropogenic flux of V is mainly connected with the exploitation of crude oils and its derivatives, burning of fossil fuels, and the direct extraction and processing of V minerals. Further on, V has been used as an important component in the chemical synthesis, oxidation processes and production of redox batteries as well (Schlesinger, Klein and Vengosh, 2017; Gustafsson, 2019). Global production of V has significantly increased over past 15 years, parallel to the increased production of high-quality steel, where V is used as an additive in alloys. Survey on V industry showed significant rise in the V production from 76 166 mt in 2011 to 102 365 mt in 2019, which has led to the point that V entered critical status as an emerging pollutant (White and Levy, 2021). Concerning studies have even compared V cancerogenic and toxic effect to those of most severe trace metal pollutants (Pb, As, and Hg). So far, it has been found that serious V pollution is concentrated in industrial areas (thermal power plants, V-Ti magnetite areas, smelters, etc.) that use heavy oils and coal as fuel (Yang *et al.*, 2022). Most of the V from metallurgy processing is discharged into the environment mainly in the form of gas, dust, wastewater and sludge, where V concentrations can amount up to few hundreds mg L^{-1}(Schlesinger, Klein and Vengosh, 2017). Out of all natural mediums, atmosphere receives most of the anthropogenic V ($37x10^9$ g/year), and as a result 10% of the V is being deposited to the oceans through natural deposition processes (Schlesinger, Klein and Vengosh, 2017; Awan *et al.*, 2021). De Foy *et al.* (2021), identified potential pollution of V in the atmosphere concentrated around industrial points. Additionally, elevated concentrations were

found to occur mainly during wind stagnation events suggesting that local industrial sources dominate over regional transport (de Foy *et al.*, 2012). Local atmospheric V deposition near polluted areas can amount to concerningly high percentage, proved by the study in the Russian Arctic where it was found that 50 % of V pollution is caused by V deposition from the atmosphere (Shevchenko *et al.*, 2003). Atmospheric motions can also increase the risk of the expansion of V pollution further from the polluted areas (Fei *et al.*, 2022). To evaluate whether V represents a hazard for an investigated location it is important to obtain information on the total enrichment of V, as well as the speciation distribution. Latter is especially important since V species own different toxic characteristics (Gustafsson, 2019). Higher concentration of V in modern sediments clearly depicts growing anthropogenic component of Vin its modern biogeochemical cycle (Awan *et al.*, 2021). However, even in this case it is necessary to evaluate its mobility, given that V species can also readily form complexes with natural organic and inorganic ligands and can be potentially stabilised in the sediment phase (Gustafsson, 2019; White and Levy, 2021). Since recent studies predict increased V anthropogenic burdening in the future, due to the increased V demand in the field of energy store and production, comprehensive investigation of V redox species pathways in natural aquatic systems is necessary (White and Levy, 2021).

2.3. Chemical behaviour of vanadium in aquatic marine environment

2.3.1. Vanadium aqueous redox chemistry

Vanadium has various oxidation states (+II, +III, +IV and +V), but in the natural aquatic environment it is mainly present in the form of V(+IV) and V(+V) species (Gustafsson, 2019). The redox conditions and different redox pairs of a certain system (NO_3^-/NH_4^+, Fe^{3+}/Fe^{2+}, MnO_2/Mn^{2+} and SO_4^{2-}/H_2S) highly influence V distribution between different oxidation states (Yang *et al.*, 2022).

Highest oxidation state (V(+V)) owns richest hydrolytic chemistry among all the V species, where its aqueous speciation is dependent on the redoks potential and pH of the system (Figure 1). At lower pH values, it is present as an oxocation, while hydrolysed forms prevail ($H_nVO_4^{(3-n)-}$) with increasing pH (Huang *et al.*, 2015; Gustafsson, 2019). On higher concentrations, V(+V) forms oligomerised species of different degree (i.e., pentamers, decavanadates etc.) which own more

complex chemical behaviour compared to monomeric species (Aureliano M., 2009). Vanadium(+V) forms both inorganic and organic complexes, although presumably of lower stability and affinity compared to V(+IV). Vanadium(+IV) occurs in the solution in the form of oxovanadium ion which is stable in the reducing conditions as observed on Figure 1. In addition, vanadyl is the most stable oxocation found in the natural environment (Gustafsson, 2019). Vanadium(+IV) species are highly reactive and can easily enter complexation reactions with various organic and inorganic ligands. Along with affinity for complexation and variety of reactions that V species can enter in natural system, facile conversion between V(+IV) and V(+V) redox species contributes to the complexity of V chemical behaviour in natural aqueous systems (Gustafsson, 2019). Vanadium(+IV) can be easily oxidised with increasing pH if present in free ionic form in natural system. Conversely, V(+V) can also be reduced to V(+IV) in the presence of natural organic ligands (humic substances, sulphurised organic ligands and other soil organic components) (Yang *et al.*, 2022). Reduction of V(+V) was observed even on the oxic conditions in the natural aqueous systems despite thermodynamic predictions, and was mainly connected with organic ligands interaction (Wang and Sañudo-Wilhelmy, 2008; Wang and Sañudo Wilhelmy, 2009; Shi, Mangal and Guéguen, 2016; Gustafsson, 2019). Under suboxic-anoxic conditions facile reduction of V(+V) species to V(+IV) is expected. Vanadyl ions, once formed in the water column, can interact with various ligands and particles (organic particles, Fe/Mn oxyhydroxides) or even form insoluble oxyhydroxides, that can subsequently be removed to the sediment. Thus, mobility of V decreases as the V oxidation state is reduced (Huang *et al.*, 2015).

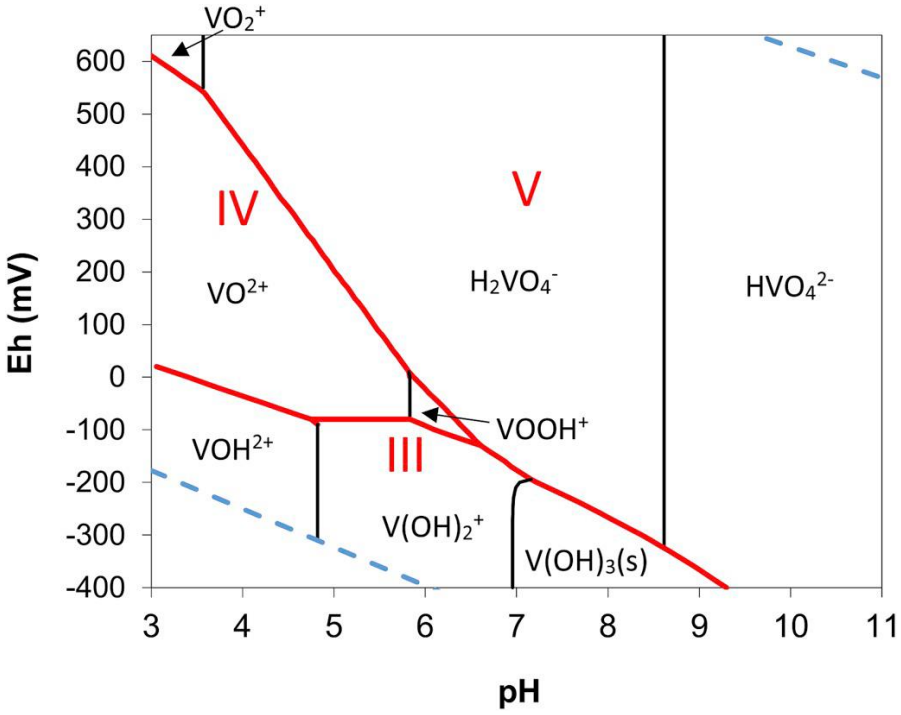

Figure 1. Predominance diagram showing the V speciation as a function of pH and Eh at total dissolved [V] = 1 μmol L^{-1}. T = 25 °C, I = 0.01 mol L^{-1} (as NaCl). Taken from Gustafsson et al. 2019 (Gustafsson, 2019).

2.3.2. Vanadium redox speciation in the sediments

Sediment is considered as a sink of V in the global biogeochemical cycle. Approximately, 80 different minerals have V incorporated in its structure (Gustafsson, 2019; Yang *et al.*, 2022). Most V deposits are associated with V-Ti magnetite, V uranium and petroleum-associated minerals. Vanadium oxides, $VO(OH)_{2(s)}$ and $V(OH)_{3(s)}$, can be formed in sediments at higher V concentrations (Yang *et al.*, 2022). In the minerals, V can be present in several oxidation states (+III, +IV and +V), dependant on the physico-chemical conditions of the medium (redox conditions, concentration of organic and inorganic particles etc.) (Shaheen *et al.*, 2019). Higher oxidation state mainly imposes greater mobility and solubility of V, which facilitates re-mobilization of V from the sediment to the water column (Schlesinger, Klein and Vengosh, 2017; William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018). In the marine sediment, physico-chemical conditions generally support stability of reduced V species, which own higher affinity towards solid phase (clay minerals, Fe-oxidohydroxides) and

organic matter present in the sediment (Gustafsson, 2019). Vanadium(+IV) and (+V) can also be easily adsorbed on metal oxides, where V(+IV) tends to form bidentate complexes, while the formation of monodentate complexes is characteristic of V(+V). Under oxic conditions, V presumably forms V(+V)-O species which are removed from the water column mainly by adsorption to Fe/Mn-oxyhydroxides (Breit and Wanty, 1991). In the clay minerals, which are among most favourable mineral phases for V enrichment, V(+III) and V(+IV) can be incorporated in the crystal structure by replacement of Al(+III) and Fe(+III) due to their structural similarity (Gustafsson, 2019; Shaheen *et al.*, 2019; Bian *et al.*, 2022). Enrichment of V in organic rich sediments is mainly due to the adsorption, complexation, and reduction reactions with organic matter. Specifically, significant enrichment of V is found to occur in black shales where V remains stabilised even during diagenetic processes (Breit and Wanty, 1991; Awan *et al.*, 2021; Bian *et al.*, 2022). However, exact redox speciation of V in the organic rich environment has been poorly studied and there is a significant lack of experimental data (Bian *et al.*, 2022). Widely accepted model for V species burial mechanism in reducing conditions is based on the research of Breit and Wanty *(*1991) (Breit and Wanty, 1991). In nitrogen/manganese rich environment, V(+V) can be reduced to V(+IV), which easily form V(+IV)-O complexes, presumably with organic ligands. In ferric rich conditions, Fe(+II) can reduce V(+V) to V(+IV) which can be easily incorporated into organic phases or Fe minerals (siderite, magnetite). Increase of reducing conditions would cause reduction of V(+V) with Fe(+II)-sulphur minerals. In strong reducing conditions, Breit and Wanty (1991) suggest that soluble V(+V) species are being reduced to V(+IV) by organic acids and then removed from the water column by adsorption on organic particles. Authors also presume reduction of V(+IV) species to V(+III) by dissolved sulphides and fixed in the sediment by clay minerals or geoprophyrins (Breit and Wanty, 1991). However, in recent study by Bian *et al.* (2022), dominant occurrence of V(+IV)-S structure (80-100% of the total V) in the organic rich Cambrian-Ordivicial Alum Shale in euxinic conditions was experimentally proven for the first time (Bian *et al.*, 2022). The stated study strongly indicates that high sulphide concentrations can cause different burial patterns of V species. Herein authors suggested an updated model describing the processes of V burial, but unlike previous studies, with a clear distinction between anoxic and euxinic conditions governing burial time. Thus, it is suggested that in anoxic conditions V(+IV)-O and V(+III)-O species are dominant in the sediment phase. In this case, the host phase of V(+IV) is organic matter, while V(III) species can be incorporated into clay minerals, organic matter or even form V

oxyhydroxides. In euxinic conditions, V(+IV)-S structure would be predominantly formed. Results obtained in this study suggest that V(+V) is reduced directly to V(+IV)-S by sulphide, and it is likely that this compound is later associated with organic matter. In strong euxinic conditions, reduction to V(+III) is not as favoured as is the formation of V(+IV)-S complexes. Scheme of the proposed mechanism is shown on the Figure 2 (Bian *et al.*, 2022).

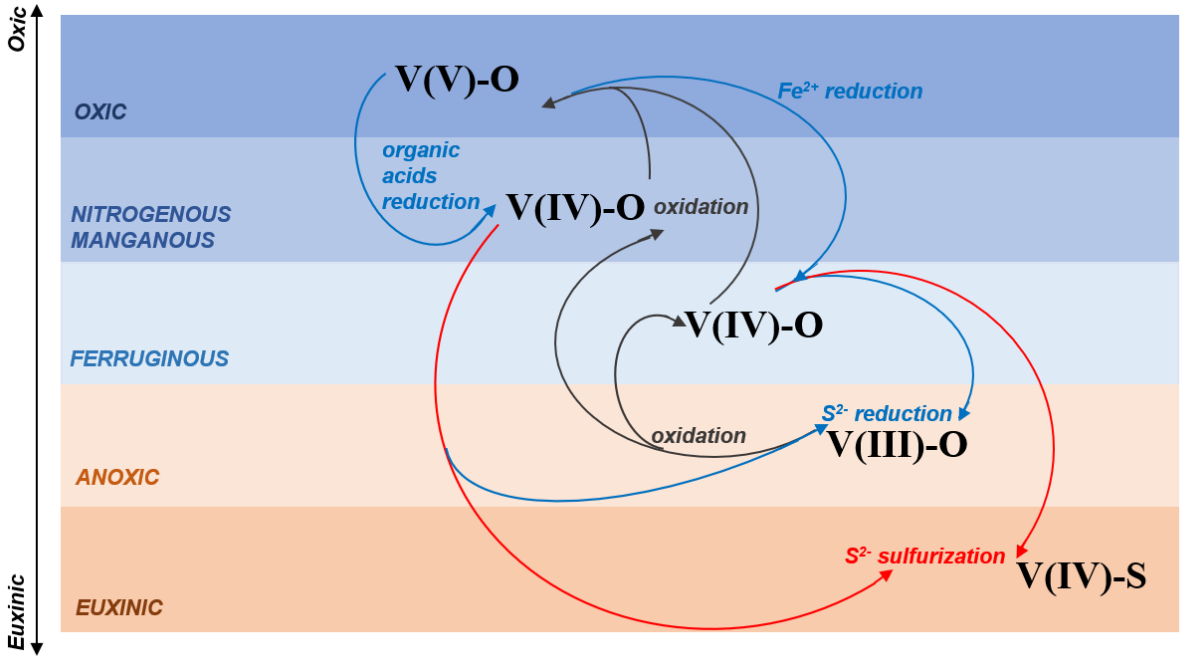

Figure 2. Scheme for V burial pathways in carbonaceous rocks. Taken from Bian *et al*. 2022. and adapted (Bian *et al.*, 2022).

2.3.3. Influence of organic matter on vanadium redox distribution in aqueous environment

Positive correlation of total V concentration and total organic carbon (TOC), which is often observed in marine shales, supports assumption of either strong complexation of V and organic matter or favourable conditions for V enrichment in the organic matter rich environment (Awan *et al.*, 2021). Significant binding to colloidal organic matter as well as V removal in deep anoxic zones of Pavin lake was determined by Albèric *et al.* (2000) further implying the influence of organic ligands on mobility of V species in the aquatic system (Albéric *et al.*, 2000). Positive

correlation between total dissolved V, DOC and humic compounds is found in the water column of the natural systems, as well (Shi, Mangal and Guéguen, 2016). Abbase *et al.* (2003) found that in the total concentration of dissolved V, a large share was occupied by the colloidal fraction of V. Compounds with a molecular mass lower than 10 kDa had a 70% share in dissolved V, with 45% of that being completely dissolved, low- molecular compounds (< 1kDa), presumably in the form of $H_2VO_4^-$ (Abbasse, Ouddane and Fischer, 2002). Further on, enrichment of V(+IV) cations in planktonic biomass and organic rich sediments were noticed, which implies rich V redox chemistry in organic rich environment (Breit and Wanty, 1991; Wang and Sañudo Wilhelmy, 2009; Bian *et al.*, 2022). Reduced species were found to exist even in oxic aquatic environment, which is usually explained with V(+IV) stabilisation towards oxidation through formation of stable complexes with natural organic ligands (Wang and Sañudo-Wilhelmy, 2008; Wang and Sañudo Wilhelmy, 2009; Shi, Mangal and Guéguen, 2016). However, current knowledge on V complexation mechanism with organic ligands in the marine environment and its effect on V redox speciation suffers from significant lack of experimental data on this subject, namely due to the complex V aqueous and redox chemistry (Gustafsson, 2019). Considering that in oxic seawater, V(+V) is dominantly present in anionic form (HVO_4^{2-}), thermodynamic presumptions discard its strong affinity for natural organic ligands due to the electrostatic repulsion with organic ligands, which are mainly negatively charged as well. However, this doesn't necessarily mean that V(+V) species are completely inert in the interaction with natural organic ligands (Linnik and Linnik, 2018). In the study by Mercê *et al.* (1999) binding constants of V(+V)-humic acid complexes were determined. In the models with one ligand (ML) the numerical values varied from 10^7 to 10^{13} M^{-1} (Mercê *et al.*, 1999). Studies on various analogues of naturally occurring organic ligands showed that V (+IV) species, compared to V(+V), form complexes more readily with organic compounds causing its increased stability towards oxidation (Shelke and Jahagirdar, 1977; A. Lorenzotti, D. Leonesi, A. Cingolani, 1981; Bartušek and Šustáček, 1983; Gonçalves and Mota, 1987; Tracey, Li and Gresser, 1990; Bruyère *et al.*, 2001). Additionally, V species seem to bond to humic acids through oxygen atoms with the affinity towards binding sites of salicylate type (Mangrich and Vugman, 1988). Given that organic compounds (humic and fulvic acid) have reducing abilities, it is assumed that V(+V) is first reduced to V(+IV), which then forms complexes with organic ligands (Gustafsson, 2019). Templeton *et al.* (1980) monitored the formation of VO^{2+} complexes with FA fractions (Mr = 750-300 Da). The binding of VO^{2+} took place through the carboxyl and phenolic hydroxyl groups

of FA, and the formation of V(+IV)-fulvic acid complexes were proposed (Templeton and Chasteen, 1980). Recently, Hu *et al.* (2019) followed release of V species with naturally occurring low molecular weight DOM substances containing carboxyl, hydroxyl, and amidogen functional groups from stone coal oxide ore. Thus, a ligand-promoted release mechanism of V is based on the rapid dissociation of organic ligands followed by the adsorption of organic ligands on the surface of minerals through complexation with oxovanadium. Surface complex is protonated and detached from the surface, inducing release of V(+V) species in the solution. The dissolved V(+V)-organic ligand complex can be reduced to V(+IV) through electron transfer self-exchange. Re-dissolution of V(+V) complexes was promoted by carboxyl functional group in acidic conditions, and catechol under basic conditions (Hu, Yue and Peng, 2019). Graphic scheme presenting possible mechanism of interaction of V(+V) and V(+IV) species with organic ligands, and consequent effect on V mobility is shown on Figure 3.

Clearly, mechanism of V species interaction with organic matter is complicated due to the rich chemistry of both V and organic ligands. However, interaction with organic ligands is a significant part of biogeochemical V cycle and as such requires additional studies to complete current knowledge gaps.

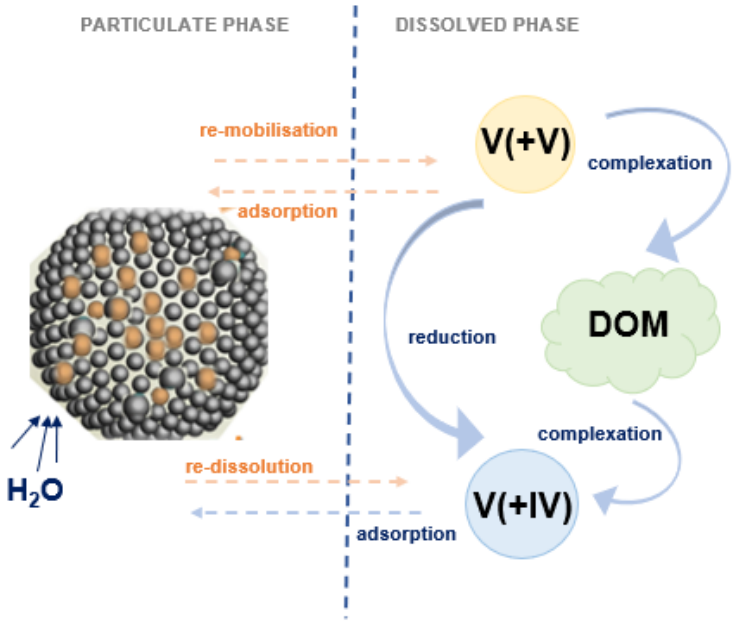

Figure 3. Scheme of proposed interaction mechanism of V(+IV) and V(+V) species with organic ligands, based on hypothesis presented in Hu *et al.* (2019) and Szalay *et al.* (1967) (Szalay and Szilágyi, 1967; Hu, Yue and Peng, 2019).

2.4. Biological importance of vanadium

Vanadium is known to participate in biological reactions and has been shown to own essential properties (Crans *et al.*, 2013; Gustafsson, 2019). Due to its specific biological functions and rich redox chemistry, V has also proven as an important element in biological evolution. Essential properties of V in biological media evolved with the chemical differentiation of Earth's surface environments where its redox chemistry potentially made this element an important cofactor in electron transfer reactions of primitive life forms (Moore *et al.*, 2020). Nowadays, research on the modern biochemical behaviour of V proved its several essential functions: as an alternative cofactor in the nitrogen-fixing enzyme nitrogenase, structural incorporation in haloperoxidases, as a primary electron acceptor in the form of vanadate (VO_4^{3-}) and as a phosphate mimicking enzyme inhibitor (Crans, Mahroof-Tahir and Keramidas, 1995; McLauchlan *et al.*, 2015; Moore *et al.*, 2020). Studies so far also clearly point to the importance of redox speciation for distinguishing effects of these metal compounds in biological systems. Two oxidation states of V are most frequently encountered in the biological medium: V(+IV) and V(+V) (Crans *et al.*, 2013). Both redox species show complex behaviour in aqueous solution and readily enter complexation and redox reactions with important biological ligands, with the end products often varying greatly in their ecotoxicity (Crans, Mahroof-Tahir and Keramidas, 1995). Vanadium(+V) is a structural analogue of phosphorus (Crans *et al.*, 2013; Rehder, 2015). As such, it is known to readily replace phosphate in enzymes such as phosphatases and kinases. However, since pentacoordination is a stable form in the case of V, but only a transitional state in the case of phosphate, the replacement of phosphate by V(+V) leads to the inhibition of the enzymatic activity of proteins, such as ATPase, protein tyrosine phosphatases (PTPases) and ribonucleases (Rehder, 2015). Consequently, toxic effect of V is greater in the case of V(+V), compared to V(+IV) species.

Biological uptake of V impacts redox cycling of V in natural aquatic systems as well, since implications of bio-reduction of toxic V(+V) to V(+IV) species is often observed in natural aqueous mediums (Gustafsson, 2019). Direct microbial V(+V) respiration by electron transfer and binding of V to reductases of other electron acceptors were found to be the two main pathways for V(+V) bio-reduction. In this case, V(+IV) was found as main product of these electron transfer processes (Zhang *et al.*, 2018). Authors in the study Wang *et al.* (2009) found positive correlation between phytoplankton biomass and V(+IV) concentrations in the coastal waters which suggest that

reduction of V(+V) can be also considered as an V uptake mechanism for marine phytoplankton (Wang and Sañudo Wilhelmy, 2009). Zhang *et al.* (2018) observed autotrophic bio-reduction of V(+V) using S(0) or Fe(0) in groundwater systems as well (Zhang *et al.*, 2018). Vanadium is known to inhibit microbiota at higher concentrations (on average 250 mg V/kg), where it particularly affects the processes of nitrogen mineralization and nitrification (Gustafsson, 2019). In some studies, it was found that a very low concentration of V can inhibit important metabolic processes in the sediment (the lowest value found was 8.4 mg V/kg), which shows that some bacteria in the sediment are extremely sensitive to V (Maja A. Larsson, Stijn Baken, Erik Smolders, Francesco Cubadda, 2015). Fei *et al*. (2022) showed that vertical V(+V) migration in soils is slowed by microbial bio -reduction to V(+IV) (Fei *et al.*, 2022). Larsson *et al.* (2013, 2015) conclude that the toxicity of V depends on the amount of V introduced into certain biomass in the sediments, which in turn depends on its solubility in the sediment, i.e. on the process of deposition of V on the surface of particles (e.g. Fe(III) and Al(III) (hydro)oxide) (Larsson M., Baken S., Gustafsson J.P., Hadialhejazi G., 2013; Maja A. Larsson, Stijn Baken, Erik Smolders, Francesco Cubadda, 2015). Clearly, bio-reduction of V(+V) is an important mechanism for decreasing V ecotoxicity and can often be traced down to V interaction with biological sulphur donor ligands on a cellular level. Vanadium is often found bound to sulphur compounds in the proteins which govern the most essential biochemical functions of V in living organisms (protein tyrosine phosphatases, V nitrogenase and V-dependent haloperoxidase) thus inhibiting functional thiolate groups (Nekola H., Wang D., Gruning C., Gatjens J., Behrens A., 2002; Monga V., Thompson. K.H., Yuen V.G., Sharma V., Patrick B.O., McNeill J.H., 2005; McLauchlan *et al.*, 2015). In the study by Macara *et al.* (1980), it was observed that a thiol-rich environment, such as tissue, containing millimolar glutathione concentrations can reduce total cytoplasmic vanadate to V(+IV). This observation may possibly explain the resistance of (Na+ +K+) - ATPase to inhibition by V(+V) (Macara, Kustin and Cantley, 1980). Similarly, the proteins VanabinX acts as a reductase in the accumulation of V(+V) by ascidians. The activity of this protein reductase has been attributed to cysteine residues and it was found that the binding of V(+IV) occurs via amino groups in the protein (Rehder, 2015; Adi *et al.*, 2022). Thus, non-enzymatic cell reduction of V(+V) with sulphur-containing bio-ligands is a powerful mechanism in preventing the inhibition of important proteins by vanadate (Bruech M., Quintanilla M.E. , Legrum W., Koch J., Netter K.J., 1984; Wang D., Behrens A., Farahbakhsh M., Gatjens J., 2003; Crans D.C., Zhang B., Gaidamauskas E., Keramidas A.D., Willsky G.R.,

2010). However, there are still inconsistencies regarding which thiol structural properties, V concentrations and physico-chemical conditions favour electron-transfer reactions in a biological media (Crans D.C., Zhang B., Gaidamauskas E., Keramidas A.D., Willsky G.R., 2010).

2.5. Vanadium bioavailability

Thorough understanding of the V bioavailability is necessary in evaluating potential toxicity of V to surrounding biota, especially concerning increased anthropogenic burdening of natural aquatic systems. Bioavailability is closely related to the process of remobilisation of V from the sediment to the water column and can be evaluated as the amount of V contained in the sediment fraction available for uptake to surrounding biota (Filgueiras, Lavilla and Bendicho, 2002). To completely understand the consequences of bioavailable V to surrounding biota, redox speciation of V in the mobile fraction is needed due to the varying toxic properties of V redox species (William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018; Shaheen *et al.*, 2019). Remobilisation process of V from the sediment to the water column, and consequently its bioavailability, is a result of several intertwined processes: redox changes of the sediment, V concentration, nature and strength of the binding to solid phase, changes of the particle surfaces in the sediment phase and changes in the ionic composition of V. Depending on the these conditions, V can be partitioned between several phases that are known to exist in the aquatic sediment (organic matter, oxyhydroxides of iron, aluminium and manganese, phyllosilicate minerals, carbonates and sulphides) (Filgueiras, Lavilla and Bendicho, 2002). Binding to each solid phase is governed by different mechanisms that control the strength of these interactions which usually include ion-exchange, outer and inner-sphere complexation i.e., adsorption, precipitation, or co-precipitation. Summary of the hypothesised chemical bioavailability of V species from the sediments is presented on Figure 4.

Discriminating V partitioning between different solid phases is usually conducted by means of sequential extraction procedures. These studies have a goal to determine which processes can affect immobilisation of the V in the sediment phase as well as the potential of V being remobilised to the water column. Exchangeable fraction is usually defined as the part of the sediment solid phase to which adsorbed metals are bound by weak electrostatic interaction and which can be easily released by ion-exchange processes. Generally, metals that are included in the exchangeable phase

are considered potentially bioavailable while other fractions are considered less mobile. The bioavailable fraction is usually represented by the carbonates in the sediment since this phase is characterised with weak binding which is easily affected by change of environmental conditions (especially pH) (Filgueiras, Lavilla and Bendicho, 2002; Cappuyns and Swennen, 2014). Organic and sulfidic solid factions are less mobile, as they can be potentially mobilised by strong change of redox conditions of the system. Lastly, binding to residual phase (silicates) can only be mobilised by weathering caused by long-term effects (Filgueiras, Lavilla and Bendicho, 2002).

Studies so far show that majority of V is usually contained in the reducible and residual phases of the sediment (Prohić and Kniewald, 1987; Nedrich *et al.*, 2018; William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018; Shaheen *et al.*, 2019). This observation is likely a result of reducing conditions that usually govern V chemical behaviour in the sediment and consequent predominance of reduced species which show greater affinity towards organic and inorganic solid phases (Shaheen *et al.*, 2019; Awan *et al.*, 2021). Sulphur biogeochemistry can also greatly influence the mobility of V, since reduction of V(+V) to V(+IV) is expected in sulphide-rich environments (Shaheen *et al.*, 2019). Although a range of sequential extraction methods have been developed to differentiate V binding to different sediment phases, it should be noted that in this case some authors use expression "speciation" in the interpretation of the results of sequential extraction procedure. However, this term is usually referred to the operational speciation, since distribution of V is based on its affinity towards each sediment phase with respect to the total V. Although used often, this expression can be potentially misleading since still V species distribution remains often unclear (Belazi *et al.*, 1995; Coetzee, 2006; Bo *et al.*, 2015). Especially, there is a significant lack of experimental data dealing with the V speciation in the bioavailable phase of aquatic sediments. Namely, speciation of V in aquatic sediment fractions using spectroscopic techniques (XANES) began only recently, with the study by Bennett *et al.* (2018) and Nedrich *et al.* (2018) (Nedrich *et al.*, 2018; William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018). While in the study of Bennet *et al.* (2018) it was found that V redox speciation in marine sediments is dominated with the mixture of V(+III) and V(+IV) species, authors have failed to determine exact host phase for V due to the shortcomings XANES analytical technique (addressed in more details in the section 2.6.3.) (William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018). Similarly, authors in the study of Nedrich *et al.* (2018) determined low bioavailability of V

in surface freshwater sediment, despite the high sediment concentrations. Established non-toxic behaviour was attributed to the reduced speciation (predominance of V(+III) and V(+IV) species), non-labile complexation, and sorption to the Al/Fe/Mn-oxyhdroxides (Nedrich *et al.*, 2018). Recently, Bian *et al.* (2022) provided experimental evidence for V speciation in ancient euxinic samples, showing that V(+IV)-S structure dominates V speciation in sulphide rich sediments (Bian *et al.*, 2022).

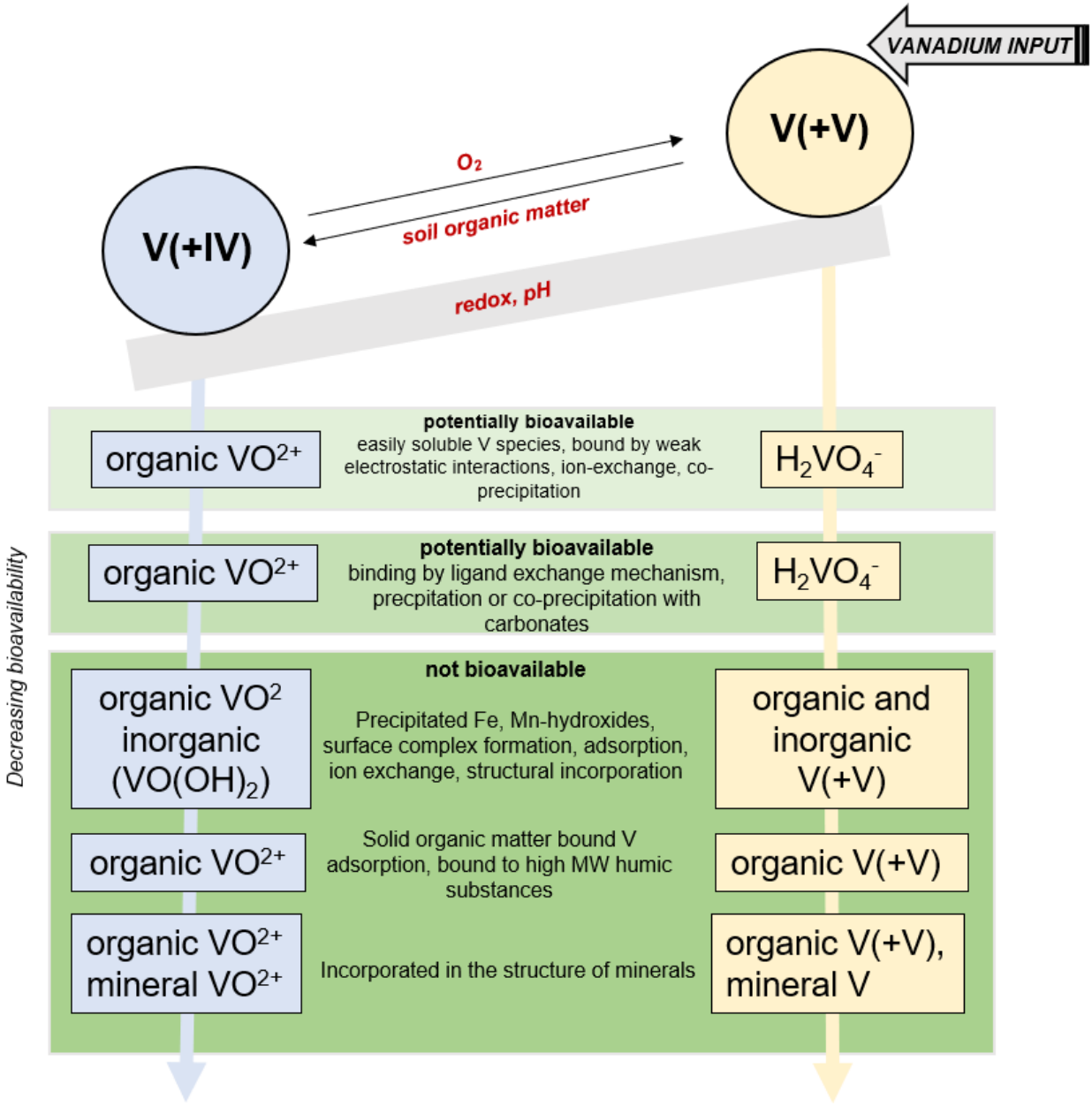

Figure 4. Vanadium chemical species bioavailability with indicated chemical processes and factors influencing it. The graphic was taken from Shaheen *et al.* (2019) and adapted for marine sediments (Shaheen *et al.*, 2019).

2.6. Chemical analysis of vanadium redox speciation in environmental samples

Nowadays, a variety of analytical methods have been developed with the goal of V redox speciation. These principally include a variety of pre-treatment procedures of varying complexity, followed with the usage of standard atomic spectroscopic techniques and separation methods coupled with sensitive detectors. Often, analytical methods must be further modified to obtain accurate redox speciation of V in complex and variable environmental samples (Hirose, 2006; Chen and Owens, 2008a). Chemical analysis of different V redox species in environmental samples can be usually performed in two ways (Hill, 1997; Pyrzyńska, 2006; Chen and Owens, 2008a):

- One step speciation analysis which is mainly performed by using analytical instrumentation which enables sample processing, separation of chemical species and their quantification in one system (e.g., electrochemical methods, spectrophotometric methods).
- Two step speciation analysis in which separation of analyte species is firstly performed by suitable sample processing procedures (e.g., extraction processes) or by suitable analytical instrumentation (e.g., chromatographic techniques) followed with their quantification by instrumentation that offers suitable detection limits for the processing of environmental samples (spectroscopic devices). The mentioned procedures can be performed separately, in the so-called "off-line" configuration, where often the separation of chemical species into individual fractions takes place in the sample processing procedure, while the quantification and determination of analytes in the fractions is performed in a separate procedure. However, the procedures for separation and quantification of chemical species V can be connected in a single system (so-called "on-line" configuration) by connecting mutually compatible analytical instruments, which is a more desirable performance of the analytical process. Namely, with such an approach, the risk of contamination or disruption of the equilibrium of V redox species in the sample is significantly reduced.

2.6.1. Sample pre-treatment

The sample processing procedure primarily depends on the type of analytical instrumentation used in the chemical analysis of the sample (Hill, 1997). Namely, standard analytical instrumentation is often not adapted to the complex matrix of environmental samples, which can in turn cause different types of interference during measurement, and consequently affect the accuracy of the analytical process. Also, when using most of the standard analytical instrumentation, it is often necessary to decrease the difference between the detection limits of the used analytical instrumentation and the actual concentrations of V in the sample by using preconcentration procedures in the sample pre-treatment step. Sample pre-treatment for redox speciation of V, often includes extraction processes which enable separation of V from the matrix of natural waters and/or preconcentration. Additionally, extraction processes can also be used for the separation of V redox species before the chemical analysis of the natural samples. They are usually connected with spectroscopic devices which enable quantification of analyte species, either in "on-line" or "off-line" configuration (Pyrzyńska and Wierzbicki, 2004b; Chen and Owens, 2008a). Complexation of redox species with suitable ligand which forms V complexes of known stability is often employed either as a part of extraction procedure or as an independent pre-treatment procedure. In this way, V redox species are converted into compounds of appropriate charge which enables or facilitates the chemical speciation analysis (Chen and Owens, 2008a; Gonzalvez *et al.*, 2009; Vieira *et al.*, 2009)

Liquid extraction (LLE) enables distribution of the analyte between two immiscible solvents. The choice of suitable solvent depends on the nature of the analyte, sample matrix and analytical method of choice for quantification of V redox species. Usually, V redox species are complexed with certain ligand to form selective complexes which enable its extraction from the solvent, usually with pH manipulation. Separated V redox species can be then directly determined with spectroscopic or chromatographic analytical techniques. Disadvantages of liquid extraction methods are mainly connected with the usage of costly, and non-ecological organic solvents. Also, the treatment of the natural sample usually contains complex pre-treatment steps (complexation, pH manipulation) and sample storage usually includes acidification, all of which can affect the redox equilibrium of V species in the processed natural sample (Chen and Owens, 2008a; Gonzalvez *et al.*, 2009; Vieira *et al.*, 2009).

Solid-phase extraction (SPE) are more often used extraction methods for V redox speciation, when compared to LLE. SPE have a high separation and pre-concentration efficiency V redox species, high recovery and lower consumption of organic solvents (Gonzalvez *et al.*, 2009; Vieira *et al.*, 2009). Most often, the separation of chemical species V takes place on ion-exchange or chelate resins (Chen and Owens, 2008a). The principle of V redox species separation is based on the competition of species of interest of a certain charge for sites on the resin of the opposite charge and the achievement of a chemical equilibrium with the stationary phase of the resin. Under certain elution conditions (most often the pH of the system is the control variable), redox species V are selectively separated and eluted from the column in individual fractions (Chen and Owens, 2008a). The quantification of chemical species V in the eluted fractions is often determined by spectroscopic instrumentation that has suitable detection limits for the analysis of analytes in natural samples ("off-line" configuration). However, due to the high risk of sample contamination and changes in oxidation states, the approach of combining extraction procedures is preferable with spectroscopic instrumentation realized within the flow injection (FI) system (Pyrzyńska and Wierzbicki, 2004a; Chen and Owens, 2008a).

In the Appendix I. (Table A1) an overview of the main developed methods of V speciation in natural samples based on extraction procedures is provided. The described extraction procedures are often high-risk for the preservation of V speciation, considering the complexity of the sample processing and its treatment. Also, considering other disadvantages of extraction techniques (high consumption of branch solvents, impossibility of monitoring possible contamination during the process, etc.), further development of extraction methods (single-drop microextraction, SDME and solid-phase microextraction, SPME) goes in the direction of reducing the working volume of organic solvent (1-3 µl), easier automation and greater control over the implementation of the procedure (Berton *et al.*, 2009; Gonzalvez *et al.*, 2009).

2.6.3. Analytical techniques for vanadium redox speciation

For liquid samples it is important to understand how physico-chemical parameters and possible presence of other forms (such as V complexes) can affect thermodynamic and chemical equilibrium of V species in certain sample (seawater, river water, lake water, drinking water and precipitation), and to adjust analytical technique accordingly (White and Levy, 2021). Commonly used methods

in the field of liquid samples processing are based on the electrochemical, chromatographic, and spectrometric analytical techniques.

Electrochemical methods in V redox speciation are mostly based on adsorpative cathodic stripping voltammetry (AdCSV) techniques. AdCSV are electroanalytical sensitive techniques, whose working principle is based on the formation of a surface-active complex of the target metal with a chosen ligand present in the electrolyte, and the resulting complex is accumulated on the working electrode. The produced analytical signal (current) is the result of the reduction of the adsorbed metal complex (Buffle and Tercier-Waeber, 2005). Speciation of V by AdCSV methods in natural samples often requires longer accumulation times to achieve adequate sensitivity of the employed technique. However, by adding bromate to the electrolyte, a better sensitivity of the analytical system for V redox species determination can be achieved (Bobrowski, Nowak and Zarebski, 2005). Also, V has a complex chemical behaviour in water samples as these chemical species are very sensitive to the addition of reagents to the sample (buffers, ligands, etc.) (Cornelis *et al.*, 2005). For example, Van der Berg *et al.* (1984) developed a voltametric method for determining V(+V) in natural samples on the hanging mercury drop electrode (HMDE) electrode in the form of a complex with catechol at pH = 6.9 (the samples were buffered using PIPES buffer) (van den Berg and Huang, 1984). However, the formation of V(+V) complexes with catechol can cause its reduction, which is especially pronounced in case of high excess of the added ligand and low pH values (Bruyère *et al.*, 2001). Also, pronounced difficulty in the development electrochemical methods is the overlap of the V signal with other metals (Li and Smart, 1996; Povar *et al.*, 2011). An overview of the methods in the Appendix I (Table A2) shows that electrochemical techniques mostly enable the direct determination of V(+V) and are less selective compared to other techniques. Considering the mentioned shortcomings, electrochemical methods are not often applied in the processing of the natural samples regardless of variety of electrochemical methods developed so far (Cornelis *et al.*, 2005).

Spectrometric methods used in V speciation studies include methods developed based on ultraviolet visible spectrophotometry (UV/Vis), atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma mass optical emission spectroscopy (ICP-OES) instrumentation.

Vanadium speciation using spectrophotometric methods often stand out as simple and economical choice among developed analytical techniques (Pyrzyńska, 2005; Chen and Owens, 2008a). The used methods can be divided in two categories: methods based on complex formation and catalytic methods (He, Wang and Yang, 2018). Spectrophotometric complexation methods are based on the use of suitable ligands that form chromogenic complexes with V chemical species. For successful spectrophotometric V speciation, used ligands used must meet the basic requirements of the process, i.e., the chemical complexation reaction must be fast, the resulting complexes of V chemical species must differ by a certain chemical property, and the complexes must have high UV absorptivity. Considering the high detection limits of the system (0.1 - 0.5 mg L^{-1}), direct spectrophotometric methods are often inappropriate when processing natural samples, and it is necessary to implement a suitable sample pre-treatment procedure in the analytical process (Pyrzyńska, 2005; He, Wang and Yang, 2018). In the Appendix I (Table A3), it is evident that spectrophotometric complexing methods serve for the determination of mainly V(+V), while only few enable simultaneous determination of V(+V) and V(+IV) redox species (often based on the use of masking reagents) (Balaji *et al.*, 1998; Pyrzyńska and Wierzbicki, 2005). Most of the catalytic spectrophotometric methods for determining V are based on oxidation-reduction reactions during which V changes its oxidation state in a cyclic process where the rate of decrease in the absorbance of organic compounds is proportional to the concentration of V (He, Wang and Yang, 2018). By comparison of the methods listed in the Appendix I. it is evident that catalytic spectrophotometric methods are more selective compared to methods based on complex formation. Also, acquired detection limits, compared to other analytical techniques, are relatively high which limits their use to the processing of specific natural samples (e.g., locations with pronounced anthropogenic influences and consequently high concentrations of V). Alternatively, implementation of appropriate pre-concentration methods in the pre-treatment procedure can circumvent this issue, but usually at the expense of higher contamination risk (Al-Tayar *et al.*, 2012; Uslu *et al.*, 2013).

Element-specific emission or absorption spectrometric techniques (AAS, AES, MS) if used separately, can only measure the total concentration of V, given that their working principle does not ensure the ability to distinguish between different oxidation states of V in the sample. However, since they have low detection limits (suitable for the quantification of V species in environmental samples), they are most often associated with other techniques that offer the possibility of

separating chemical V species on the basis of some chemical property (Michalke, 2002; Prange and Schaumlöffel, 2002). Such analytical procedures usually include extraction procedures (performed in the sample preparation procedure or connected to spectrometric instrumentation in a unique process - "on-line" configuration) or connection with other separation techniques (usually chromatographic). Each of the spectrometric techniques differs in its source of excitation and there are various variations. The advantages of using the described instrumentation in the analytical process are: monitoring the chemical composition of the reagents used in the process (easy detection of sample contamination), avoiding the need to separate the analyte from the sample, high selectivity according to a certain element that is determined, and the possibility of multi-element analysis (Das and Chakraborty, 1997; Marcinkowska and Barałkiewicz, 2016). The biggest shortcomings of these systems are manifested in the large influence of interferences associated with the matrix of natural samples, especially in seawater processing. Instrumentation such as ICP-AES or ICP-MS are most often used in the speciation of V in natural samples (Wrobel *et al.*, 2003; Chen and Owens, 2008a; Marcinkowska and Barałkiewicz, 2016). Usage of ICP-MS in the issue of V redox speciation analysis is experiencing the greatest development and application, given that it has very low detection limits, possibility of multi-element analysis and the possibility of isotopic analysis (Wrobel *et al.*, 2003; Chen and Owens, 2008a).

Chromatographic methods are increasingly used analytical methods of V speciation in natural seawater samples. Liquid Chromatography (LC) techniques associated with spectrometric devices with low detection limits are experiencing the greatest development in V speciation in natural systems. The reversed phase liquid chromatography (RPLC) and ion chromatography (IC) are the two most common forms of liquid chromatography that are used for the above purposes (Hill, 1997; Chen and Owens, 2008a). The separation of Vchemical species using RPLC techniques is based on a stationary phase of lower polarity than the mobile phase, where the retention mechanism depends on the relative hydrophobicity of the analyte (Michalke, 2002). Vanadium chemical species are separated mostly in the form of suitable complexes using the RPLC process. Complexation can be carried out during sample processing or on a chromatographic column during chemical analysis by adding a suitable ligand to the eluent (on-column complexation). Developed methods are based on the complexation of V chemical species with organic ligands which form organometallic complexes that can be separated by RPLC techniques (Appendix I, Table A4) (Liu S., Zhao M., 1992; Nagaosa and Kobayashi, 1995; Wann and Jiang, 1997).

Separation of V chemical species by IC techniques is based on the basic principle of ion exchange. Namely, the separation part of the instrumentation (ion-exchange column, i.e., stationary phase) has a negative or positive charge. V chemical species, with a charge opposite to the charge of the stationary phase, are retained on active sites, after which competition with ions from the eluent for active sites on the stationary phase takes place (Sarzanini and Bruzzoniti, 2001). Based on the described mechanism V redox species are separated based on the difference in charge and are eluted from the stationary phase. In most cases, V species are complexed with certain ligands to form complexes of a certain charge which enables the separation of V chemical species (Pyrzyńska and Wierzbicki, 2004a; Chen and Owens, 2008a). Ion Chromatography techniques are extremely compatible for connection with ICP-MS devices considering that, compared to other LC techniques, the content of organic solvents in the eluent is very low (Sarzanini and Bruzzoniti, 2001).

The main reason for the increasing development of LC techniques for the redox speciation of V in environmental samples is the possibility of easy connection with spectroscopic instrumentation in a unique analytical system ("on-line" configuration), which creates an analytical system with high separation power and low detection limits. Example of ion chromatography connected with ICP-MS instrumentation in a “on-line” configuration is shown on Figure 5. Also, the need for pre-concentration of V is avoided and the possibility of element-specific information and quick detection of contamination of the analytical system is offered (D. Pérez-Bendito and S. Rubio, no date; Hill, 1997; Wrobel *et al.*, 2003). The shortcomings of the described analytical systems are particularly noticeable in the processing of natural seawater samples or mixed systems (freshwater-marine) due to the pronounced matrix effects. A comparison of chromatographic methods with other analytical techniques (Appendix I, Table A4) shows the high selectivity, the possibility of avoiding high-risk procedures (such as preconcentration) and the low detection limit of these techniques.

Figure 5. Example of connected IC to HR ICP-MS instrumentation in a single analytical system ("on-line" configuration). This instrumentation was used in determination of V redox species in the water samples of Krka River estuary (Research paper I.), at Ruđer Bošković Institute (Zagreb, Croatia).

Capillary electrophoresis (CE) is a separation technique compliant to chromatographic techniques. These techniques have several advantages, mainly based on their high selectivity, efficiency, and rapid separation of chemical species. Usually, chelation with suitable ligands is used to follow metal ions mobility. Vanadium redox speciation is usually conducted either using on-capillary of pre-capillary complexation. Vanadium species are chelated usually with aminopolycarboxylic acids, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentacetic acid (DTPA), nitrilotriacetic acid (NTA) and N-2-hydroxyethylethylendiaminetriacetic acid (HEDTA), to form anionic complexes which are separated by CE with UV detection (Pyrzyńska and Wierzbicki, 2004a). In addition, the separation efficiency of the CE allows the study of simultaneous complexation equilibria including two or even more metal ions with the same ligand. Capillary electrophoresis is also useful for establishing the stoichiometries of various complexes. In particular, the changes in the mobility of the complexes allows to deduce their degree of protonation. In this respect, CE offers undeniable advantage over other classical methods (V. Sladkov *et al.*, 2018). Since, CE techniques usually come with a detector of high detection limit, processing of environmental samples for V redox speciation usually requires pre-treatment

procedures. This step can be avoided in case of connecting CE with spectrometric instrumentation (ICP-MS, ICP-OES) (Chen and Owens, 2008b). However, CE analysis of environmental samples still suffers from limitations, such as reliability, performance stability, time-consuming processing of environmental samples, incomplete complexation, etc. (Pyrzyńska and Wierzbicki, 2004a; Chen and Owens, 2008a). Especially, stability is highly affected by the critical influence of electrophoretic buffer composition on analyte mobility. As a result, the use of CE in real samples is still in its development. All the CE methods developed and applied for V redox speciation in environmental samples are listed in the Appendix I (Table A4).

Recent analytical advances enabled V redox speciation in solid samples as well. Different sediment samples can be either decomposed and sediment solution can be processed, or sample can be measured directly. Soil solution can be processed utilising the same techniques as other liquid samples mentioned above. Direct speciation of V in solids are mostly based on X-ray techniques. These techniques, such as XANES, XAS and EXAFS, proved as a powerful tool for evaluation of V speciation in solid samples (Shaheen *et al.*, 2019; White and Levy, 2021).

X-ray adsorption near edge structure spectroscopy (XANES) is an analytical technique used for determination of average oxidation state and coordination environment. This technique is highly used in the processing of sediment samples since minimal sample manipulation is enabled, and therefore the integrity of solid sample can be preserved. However, this technique suffers from certain limitation in the case of V redox speciation, especially in marine sediments. Namely, many marine sediments are dominated by crystalline quartz sand which can be strongly diffracting. Since V concentrations in the sediments are generally low, diffraction of crystalline phases can lead to the artefacts and non-linearities in the spectra. In addition, titanium is strong interference for V when using this technique due to the proximity of Ti K-beta fluorescence emission lines to the V-K-alpha emission lines of interest (William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018).

Synchrotron X-ray absorption spectroscopy (XAS) is a widely used technique for determining the local geometric and/or electronic structure of matter. A major advantage of XAS technique is that the measurement can be conducted with the whole soils, without drying it. Vanadium XAS measurements are typically conducted at the V K-edge, with a relatively low excitation energy (5465 eV) which may present practical limitations on the determination of redox states of V (Shaheen *et al.*, 2019).

Extended X-ray absorption fine structure (EXAFS) data can be used for the determination of the average molecular coordination environment of V soil components. Similar to the previous X-ray techniques, the use of V K-edge pre-peak suffer from interference, in this case Ba L_2 edge strongly interferes with the V K-edge EXAFS region (Shaheen *et al.*, 2019).

All listed X-ray techniques have significant constraints since they are costly, time consuming and interpretation of the data is not trivial as they represent the weighted sum of all the species present in the analysed sample. It is therefore difficult to quantify the species present in a small percentage in total V concentration in processed sample (Feldmann, Salaün and Lombi, 2009). For higher accuracy, comparison of V K-edge pre-intensities and peak positions of natural solid samples with those of known compounds is often included to assess oxidation state and local coordination environment. Data obtained on this manner can also be processed with principal component analysis to compare entire spectra of samples with standardised samples (White and Levy, 2021). Developed methods for V redox speciation in solid sediment samples are given in Appendix I (Table A5).

3. OBJECTIVE AND HYPOTHESIS OF THE WORK

The main objective of the research is the development and adaptation of specific analytical methods for V redox speciation in the sediment and water samples. Within the scope of the stated goal, the implementation of the described analytical methods during processing of natural samples of water column and sediment of the Krka River estuary is expected. Furthermore, the interaction of organic ligands and V redox species is planned to be investigated in the model solutions.

Hypothesis. The redox conditions of the system and the presence of organic ligands affect the distribution of V redox species, mobility and geochemical cycling in natural waters and sediments. More specifically, it is presumed that oxic conditions in the water column support predominance of V(+V) in the total dissolved V pool. Possible reduction and stabilisation of reduced species in the aqueous phase could be affected by oxygen deprivation and interaction of V species with natural organic ligands (carboxylic acid and sulphurised organic ligands) due to the established higher affinity of V(+IV) species towards complexation with simple organic ligand. Redox speciation in acid-extractable phase of sediments is dominated by reduced species which reflects physico-chemical conditions of sediment. Consequently, toxicity of V is presumably low.

4. RESEARCH PAPERS

4.1. Research paper I

Knežević, Lucija; Omanović, Dario; Bačić, Niko; Mandić, Jelena; Bura-Nakić, Elvira. Redox Speciation of Vanadium in Estuarine Waters Using Improved Methodology Based on Anion Exchange Chromatography Coupled to HR ICP-MS System. Molecules, 26 (2021), 9; 2436. 15 doi:10.3390/molecules26092436

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Article

Redox Speciation of Vanadium in Estuarine Waters Using Improved Methodology Based on Anion Exchange Chromatography Coupled to HR ICP-MS System

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Abstract: An improved methodology was developed for V redox speciation in estuarine waters using a hyphenated technique consisting of ion chromatograph (IC) with an anion exchange column and a high-resolution inductively coupled plasma mass spectrometer (HR ICP-MS). This approach enables the direct determination of V(V), whereas reduced species (mainly V(IV)) are calculated by subtracting V(V) concentrations from the measured total V concentration. Based on the "on-column" V(V) chelation mechanism by EDTA, with the eluent composed of 40 mmol L^{-1} ammonium bicarbonate, 40 mmol L^{-1} ammonium sulphate, 8 mmol L^{-1} ethylenediaminetetraacetic acid and 3% acetonitrile, the method was successfully used for analyses of V redox speciation in samples taken in the vertical salinity gradient of the highly stratified Krka River estuary. Due to the matrix effects causing different sensitivities, a standard addition method was used for V(V) quantification purposes. The limit of detection (LOD) was also found to be matrix related: 101.68 ng L^{-1} in the seawater and 30.56 µg L^{-1} in the freshwater. Performed stability tests showed that V redox speciation is preserved at least 7 days in un-treated samples, possibly due to the stabilization of V-reduced species with natural organic matter (NOM). The dominant V form in the analysed samples was V(V) with the reduced V(IV) accounting for up to 26% of the total dissolved pool. The concentration of V(IV) was found to correlate negatively with the oxygen concentration. Significant removal of dissolved V was detected in oxygen depleted zones possibly related to the particle scavenging.

Keywords: vanadium(V) redox speciation; ion chromatography; on-column complexation; Krka River estuary; high-salinity matrix

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1. Introduction

Vanadium (V) is a redox-sensitive trace metal, which occurs in three oxidation states (+III, +IV and +V) in the environment [1]. Given the high concentrations in igneous, sedimentary rocks and minerals (average crustal concentrations over 200 µg g^{-1}), V is also the second most abundant transition metal in seawater (around 35 nmol L^{-1}) [1,2]. In freshwater, dissolved V concentrations vary between 8.5 and 22.6 nmol L^{-1}, showing high dependence on the type of source rock and weathering type [3]. While vanadium in open ocean waters shows relatively conservative distribution, non-conservative behaviour is reported in coastal waters [4,5]. Due to the different chemical behaviour and toxicity depending on which species V takes the form of, speciation analysis is highly needed in order to evaluate the bioavailability and geochemical cycling of V in the environment [6]. The distribution of V redox species in natural waters is controlled by the pH, V concentration, redox potential, ionic strength of the system, chemical composition of natural organic matter and biological activity [1,2]. Thermodynamic calculations predict V(V) as a dominant species in well-oxidized marine environments, while V(IV) is stable in moderately

reducing environments [1]. Under the reducing conditions, $VO(OH)_3^-$ largely dominates V(IV) speciation (with the minor share of dimmer V(IV) species-$(VO)_2(OH)_5^-$), while in well-oxidizing environments, V(V) exists predominantly as a vanadate oxyanion ($H_2 VO_4^-$, HVO_4^{2-}) [1,7]. However, V(IV) has been previously reported to be present even in surface oxic waters due to its ability to form stable complexes with organic or inorganic ligands in natural waters [4,8,9].

Estuaries represent an ideal natural media for studying geochemical cycling of trace metals [10–12]. Due to the presence of various physical and chemical gradients as well as increased primary production (in comparison to open ocean), estuaries are a source of valuable information on how these factors can affect the distribution of vanadium species and its behaviour [10,13]. Although the weathering of silicate and other minerals is thought to be a dominant factor in controlling dissolved vanadium concentration in estuaries, other secondary factors have to be taken into account as well: adsorption effects, the nature of weathering processes, redox reactions, organic complexation and various anthropogenic inputs [14]. The biogeochemical cycle of V has recently been largely influenced by anthropogenic activity [2]. Xavier et al. (2020) established moderate contamination for V in the sediment samples of Cuñaní Estuary [15]. Furthermore, Ribeiro et al. (2018) determined V to be present as the 7th most abundant trace metal in Douro River estuary and established strong contamination by the anthropogenic activity [16]. Vanadium speciation in the Krka River estuary has been recently studied in sediment samples. The stated study showed that, although V is characterized by higher background levels, the anthropogenic input of V is generally considered low for sampled sites. The speciation in bioavailable sediment fraction shows the predominance of V(IV) species [17].

The three main difficulties identified in the redox speciation of V in marine waters are (i) the preservation of the original speciation, (ii) separation and individual identification of redox specie(s) and (iii) measurement interferences in a high matrix solution. Firstly, an issue of adequate preservation/storage of the sample has to be solved with a goal of avoiding changes of the original species distribution. Different procedures were proposed, which unfortunately are not adequate for all V redox species. While the acidification of the sample is recommended for V(V), the addition of chelating agent (EDTA) was used to maintain V(IV) stability [18–21]. A further step in V speciation analysis includes the choice of the V species separation method, mainly consisting of pre-concentration procedures due to the low concentration and complex environmental matrices [22–24]. Such techniques (chelation and extraction; precipitation and pre-concentration on ion exchange resins) often require large sample volumes due to their low detection limit and imply the usage of complexation ligands [21,25–27]. The off-line application of these methods is often under a high risk of sample contamination, while an on-line setup significantly increases analysis costs [23,24]. Most of the other on-line techniques (flow injection analysis; spectrophotometry coupled to devices of higher detection limit) require additional treatment of the samples, which is not desirable (addition of complexing ligands; lowering natural pH of the samples) due to the high risk of sample contamination or change in the present species [23]. Due to their simple and fast measurement, on-line separation chromatographic techniques (ion chromatography, IC; liquid chromatography, LC) are often hyphenated with analytical instrumentation of the lower detection limit, such as inductively coupled plasma-optical emission (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS) [22,28]. However, ICP-MS is more often used for vanadium determination, mainly because it enables element selective measurement with higher sensitivity, appropriate for the measurement of natural samples [23]. On the other hand, this analytical setup can be prone to matrix effects originating from environmental samples, especially those of high salinity [28–31]. Interactions of matrix ions with column, as well as interferences originating from spectrometric devices, can lead to peak broadening, suppression or enhancement of the analyte signal, co-elution effects, microgradient elution, etc. [23,28].

The published method for V speciation in the coke pore water samples and bacterial cultures by Li and Le using the HPLC-ICP-MS system on an anion exchange column served as a basis for V redox speciation in the samples of the Krka River estuary [18]. However, processed samples in the work of Li and Le were of very low salinity in comparison to the varying salinity samples of the Krka river estuary. To our knowledge, only one study using ion-pair reversed LC deals with the V redox speciation in high-salinity oceanic waters where the samples were pre-treated by the addition of EDTA prior to IC separation (diluted two-fold with the mobile phase) [32]. Consequently, the aims of this work are as follows: (i) improve analytical determination of vanadium species using IC on anion exchange column coupled HR ICP-MS taking into account matrix effects originating from estuarine samples of variable salinity; (ii) address species preservation and sample pre-treatment in order to avoid changes in original speciation; and (iii) application of the developed method for the determination of vanadium redox speciation in estuarine waters (the Krka River estuary, Croatia).

2. Results and Discussion

2.1. Methodology Improvements

2.1.1. Effect of Sample Matrix

Speciation analysis of V species is achieved by their complexation with EDTA, which enables the simple separation of the species in the form of corresponding V-EDTA negatively charged complexes using anion-exchange-based IC [33]. Figure 1a shows IC-HR-ICP-MS chromatograms of V(IV)-EDTA and V(V)-EDTA complexes prepared in Milli-Q water. The obtained elution times for two species are in agreement with previous reports [18,33,34]. While the IC-separation of V species in Milli-Q water provides the two peaks at different elution times, the chromatograms obtained in natural waters of different salinities are more complicated (Figure 1b). In both saline samples, the two well-separated peaks appeared, the first peak between 100 and 200 s and the second peak between 300 and 400 s. While the second peak corresponds to the elution of V(V), the first one is not related exclusively to V(IV), but is ascribed to the partial elution of V-species in the "void-volume" (this peak is hereafter termed as the "pre-peak").

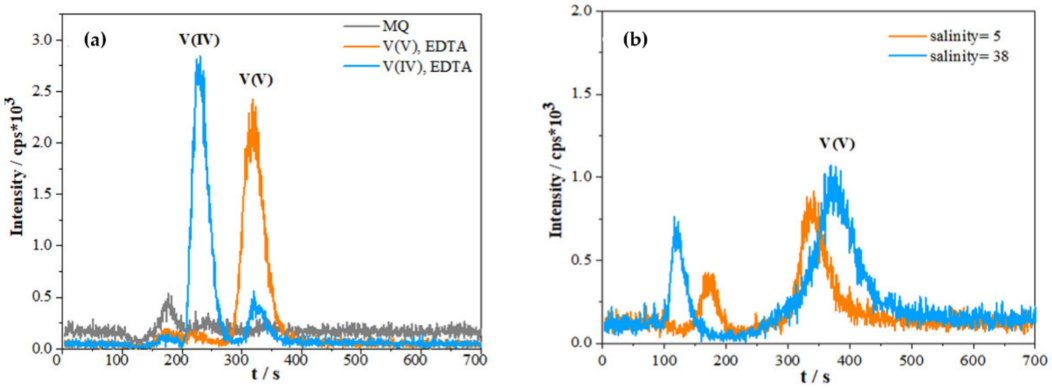

Figure 1. (**a**) Chromatograms of solutions containing 40 nmol L^{-1} V(V) (orange line) and 40 nmol L^{-1} V(IV) (blue line) in MQ water with the EDTA added in solution (3 mmol L^{-1}, pH = 7) compared to blank (MQ water, grey line) using IC–ICP–MS. (**b**) Comparison of chromatograms obtained by IC-HR ICP-MS at different salinities; total V and V(V) concentration measured in samples collected at "Vrnaža port" (VP) sampling station (orange line: environmental sample of salinity = 5, V_{tot} = 24.5 ± 1.4 nmol L^{-1}, V(V) = 21.8 ± 0.1 nmol L^{-1}; blue line: environmental sample of salinity = 38, V_{tot} = 37.0 ± 0.8 nmol L^{-1}, V(V) = 31.1 ± 3.34 nmol L^{-1}). All chromatograms (**a**,**b**) were measured using the following eluent composition: 40 mmol L^{-1} HCO_3^-, 40 mmol L^{-1} SO_4^{2-}, 8 mmol L^{-1} EDTA and 3% acetonitrile.

The appearance of a "pre-peak" in void volume using an anion-exchange column is a known issue in samples with high concentration of anions, such as marine waters [35–38]. In addition, the peak of V(V) and the peak within the void volume shift depending on the

ionic strength of the sample. The influence of the matrix composition (salinity) is clearly visible in two chromatograms in Figure 1b. It is likely that the high concentration of chloride anions originating from the samples interferes with the equilibria on the anion exchange column, since their concentration far exceeds the analyte concentration. Chloride anions are retained on the separation column and partially saturate the active sites of the column. Consequently, not all V species present in the sample successfully adsorb on the column, which leads to their partial elution within the void volume. The amount of vanadium successfully adsorbed on the anion exchange column depends on the concentration of interfering anion(s) [31]. Although in the investigated "Vrnaža port" (VP) samples, an appreciable amount of V(IV) was determined (see discussion later), the chromatographic peaks corresponding to V(IV) were not observed. As seen in Figure 1a, V(IV) elutes closer to the void volume in comparison to V(V). Possibly, the low concentration of the present V(IV), together with the high ionic strength of the investigated samples, causes the complete elution of V(IV) within the void volume. It should be highlighted that the "pre-peak" in the chromatograms in Figure 1b originates from the V, and not spectral interference caused by the formed ClO^+, since the medium resolution used for the measurement is sufficient to fully eliminate the spectral interference [35].

By comparing the chromatograms present in Figure 1b, an increase in the "pre-peak" in the measured samples of higher salinity and V concentration can be observed. However, despite the higher total V concentration (V_{tot}) in the sample of higher salinity, the peak attributed to V(V) is almost of the same size as the one at lower salinity. This is due to the lower efficiency of the V-EDTA species being retained in the column due to the interfering anion(s) and the lower sensitivity of HR ICP-MS caused by the ionization suppression of plasma in the heavier matrix [39]. Described behavior is the main reason for implementation of standard addition method for V(V) quantification instead of external calibration.

2.1.2. Species Preservation and Sample Pre-Treatment

The preservation of samples with the goal of V speciation studies is often based on the addition of a strong chelator (mostly EDTA) on-site and/or prior to analysis in order to stabilise reduced species (if present) against oxidation [18,20]. In order to identify if any changes in vanadium species occur in the sample solution after EDTA addition, a reported sample handling approach was tested in model solutions prior to the analysis of environmental samples [18,20,32,40]. Since predominant species in oxic water samples are expected to be mostly vanadate ions (V(V)), tested model solutions were prepared by using the V(V) standard [1,2]. Stability tests of vanadium species complexed with added EDTA in model solutions at different pH values are shown in Figure 2a,b (UV/Vis detector was used for measurement). It is apparent that the V(V)-EDTA complex shows significant instability at the two different pH values. Under both neutral and acidic conditions, a decrease in peak intensity for V(V) with the time was observed. Measured solutions at pH = 2 showed a reduction in V(V) in V(IV) after a 7-day period. On the other hand, chromatograms of solutions at pH = 7 showed slight changes in peak position after just 24 h, which could possibly be attributed to the slight increase in the pH of the solutions within the measurement time frame [32]. Under acidic conditions, the peak at 280 s elution time, which can be attributed solely to V(IV)-EDTA produced in the reduction processes between V(V) species and EDTA, showed a further increase. A higher reduction rate on lower pH values, in comparison with more alkaline pH, can be attributed to the more pronounced V(IV) stability against oxidation at the lower pH values. V(V) has been already shown to gradually convert to the V(IV) species in the presence of EDTA [41–44].

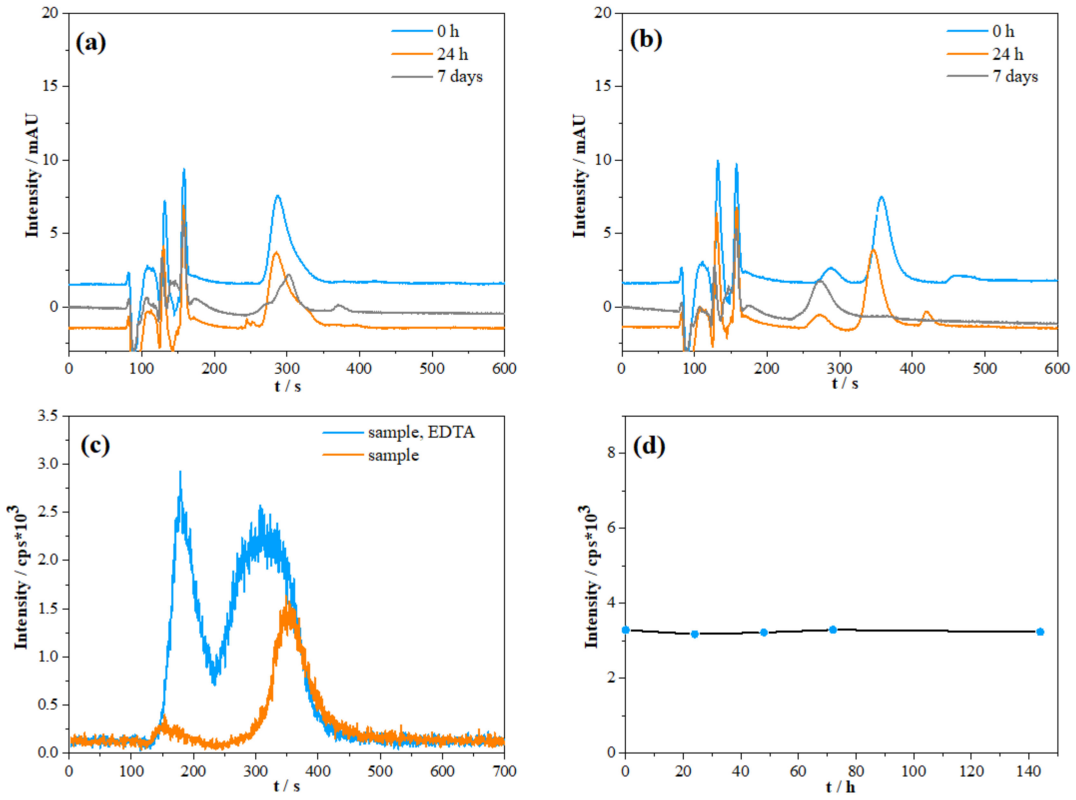

Figure 2. Temporal evolution of IC-UV/VIS chromatograms of V(V) (1 µmol L^{-1}) and EDTA (3 mmol L^{-1}) in two solutions of different pH values: (**a**) pH of 7 and (**b**) 2 (blue curve: solution measured right after preparation; orange curve: solution measured after 24 h; grey curve: solution measured after 7 days). (**c**) Comparison of different approaches to sample storage measured using IC–ICP–MS (orange curve: chromatogram of a sample filtered on-site and stored at +4 °C; blue curve: chromatogram of a same sample containing ligand (3 mmol L^{-1} EDTA) added on-site, after filtration. (**d**) Stability of V(V) (40 nmol L^{-1}) spiked in natural sample of the Krka River estuary during the time period of 144 h.

The same preservation approach was tested on natural samples of the Krka River estuary (Martinska (MA), sampling station). Figure 2c shows a comparison of chromatograms from differently stored (but otherwise the same) samples with the on-site addition of EDTA and without EDTA. It is obvious that sample storage using EDTA for the preservation of vanadium redox species is not adequate. The obtained chromatogram with EDTA addition, where severe peak broadening and an increase in "pre-peak" (peak at about 180 s) were observed, imply that a change in the original vanadium speciation is likely to occur. Contrarily, a well-shaped peak of V(V) was obtained in samples without EDTA addition. Furthermore, a prolonged stability test over a 6-day period with spiked estuarine samples (Figure 2d) revealed that V speciation under natural conditions was successfully preserved. Based on the conducted stability tests, it was decided that on-site filtration and storage of the samples at +4 °C would be the optimal sample handling strategy.

Following the mentioned non-preservation approach, an estuarine sample (Skradin Bridge (SB) site: with total dissolved V of 25.3 ± 1.0 nmol L^{-1}) was analysed twice within a period of 6 days: the first measurement performed after the sampling gave a V(V) concentration of 24.2 ± 0.5 nmol L^{-1}, whereas 22.9 ± 1.0 nmol L^{-1} was found in the same sample after 6 days. Assuming that the obtained difference in V(V) concentrations between these two measurements was withing the usual experimental error (<10%), it could be concluded that the redox speciation of V in an untreated sample over the period of at least 6 days is preserved. Our stability results are consistent with the results of other authors [45–47], suggesting the stabilization of V(IV) in oxic conditions by complexation with organic and/or inorganic ligands in natural samples [2–4,19,45]. It is thus presumed

that the chosen sample storage (without any chemical preservation) would not affect the speciation of vanadium species in the sample, even if reduced species are present.

2.1.3. Optimisation of Eluent Composition

An eluent consisting of 80 mmol L^{-1} NH_4 HCO_3, 2 mmol L^{-1} EDTA and 3% acetonitrile was suggested by Li and Le as optimal composition on valid separation of vanadium species and was used here as a starting composition for further optimisation [18]. As explained in the previous section, the addition of EDTA in the samples is not desirable due to the instability of the V(V)-EDTA. Several authors used EDTA in eluent solution to enhance the peak stability and to induce complexation of the vanadium species on the pre-column or on-column [40,48,49]. It was found that the on-column complexation with EDTA added only in the eluent would be sufficient and fast enough to separate and quantify vanadium species. The on-column formation of V(IV)-EDTA and V(V)-EDTA is enabled due to the high formation constants of V species with EDTA (K_f[V(IV)-EDTA = 18.80; K_f[V(V)-EDTA = 15.55) [48]. Chromatograms obtained with the model sample containing solely V(V) or both V(V) and EDTA, measured on IC-UV/Vis, are presented in Figure 3a. Retention times of eluted V(V) in both solutions are the same, suggesting that complete on-column complexation occurred in the case of the solution that did not contain EDTA.

Figure 3b presents two chromatograms which demonstrate the influence of different concentrations of EDTA in eluent solution on the separation of vanadium species in the seawater sample (Salinity = 38) using the IC-ICP-MS system. Compared to the previously suggested lower EDTA concentration [18], at a higher EDTA concentration, the V(V) peak is much better resolved due to the lower under-peak background, whereas the "pre-peak" intensity (signal from the void volume) is strongly diminished. A decrease in the "pre-peak" intensity with the usage of a higher concentration of EDTA in eluent can be linked with the parallel increase in the overall ionic strength of the eluent and promotion of V(V)-EDTA complexation on the anion-exchange column. By this modification, the concentration of V(V) eluting within the void volume decreased, and a more successful separation of V(V) on the anion exchange column was accomplished. In addition, lower background intensity on chromatograms measured on eluent containing a higher EDTA concentration was beneficial for analytical purposes, which led to more reliable results. Note that an increase in EDTA on concentrations above 8 mmol L^{-1} did not give further chromatogram enhancements. Consequently, the concentration of 8 mmol L^{-1} of EDTA in eluent was chosen to be optimal for the determination of vanadium species.

During the process of elution, eluents have to be of sufficient ionic strength to allow retained anions from the sample to be eluted. In the samples of high salinity where the concentration of matrix anions far exceeds the concentration of vanadium species, eluent ions have to efficiently remove retained matrix anions from the sample and allow the on-column anion exchange of V(V)-EDTA complexes. Thus, eluent composition was further optimized by adding ammonium sulphate to the eluent in order to increase the ionic strength of the eluent. A comparison of IC-ICP-MS chromatograms obtained with eluent composition suggested by Li and Le (2007) [18] (eluent 1) and our optimized eluent solution (eluent 2) is presented in Figure 3c. The most obvious differences are in retention times and "pre-peak" intensities.

The longer retention time obtained with eluent containing solely ammonium bicarbonate was partly caused by the lower pH compared to the bicarbonate–sulphate mixture (6.0 vs. 8.5). Additionally, the peak shift can be attributed to a slightly higher ionic strength of bicarbonate–sulphate eluent, allowing elution of the species at a lower retention time, which enables shorter analysis. The bicarbonate–sulphate mixture was also found to be more efficient in reducing "pre-peak" intensity, preventing a greater influence of the sample anion effects. The newly proposed eluent composition with pH = 8.5 is also more beneficial, since the higher pH values favour the formation of V-EDTA complexes. [18,40,48,49].

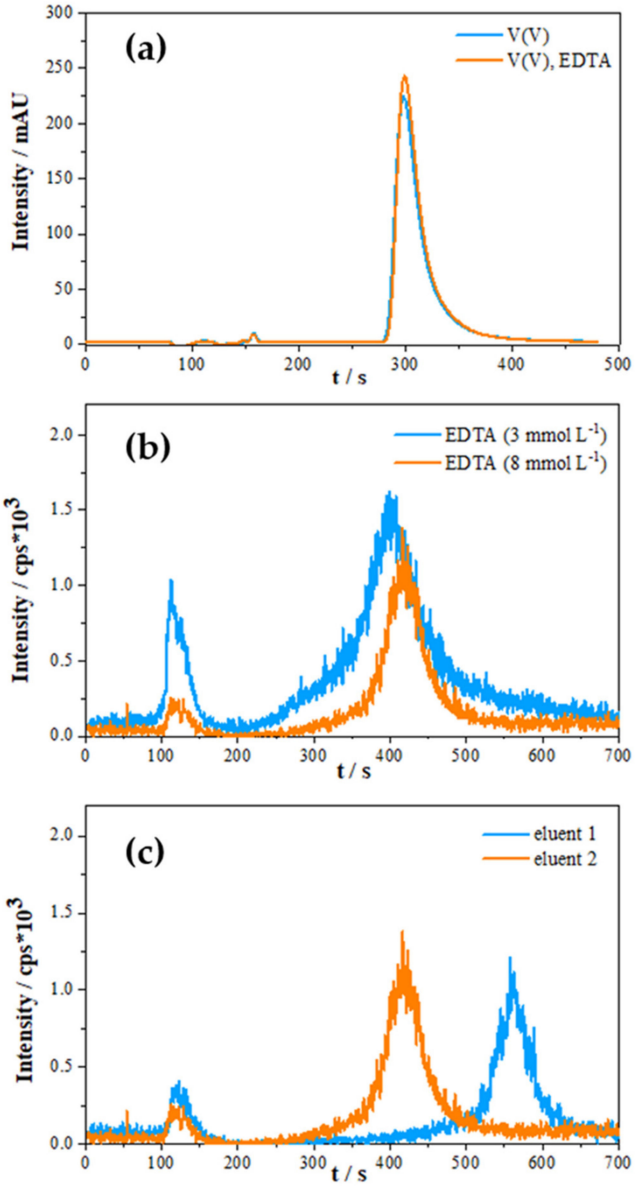

Figure 3. (**a**) Comparison of chromatograms for measured solutions using eluent composed from 40 mmol L^{-1} HCO_3^-, 40 mmol L^{-1} SO_4^{2-}, 8 mmol L^{-1} EDTA and 3% acetonitrile on IC-UV/Vis containing: V(V) (0.1 mmol L^{-1}) in MQ–blue line; V(V) (0.1 mmol L^{-1}) complexed withEDTA (1 mmol L^{-1}) in MQ– orange line; (**b**) comparison of IC–ICP–MS chromatograms in seawater sample (salinity= 38). Eluent containing 3 mmol L^{-1} EDTA, 40 mmol L^{-1} HCO_3^-, 40 mmol L^{-1} SO_4^{2-} and 3% acetonitrile (blue line) and 8 mmol L^{-1} EDTA, 40 mmol L^{-1} HCO_3^-, 40 mmol L^{-1} SO_4^{2-} and 3% acetonitrile (orange line) are used. (**c**) Eluents containing 80 mmol L^{-1} HCO_3^-, 8 mmol L^{-1} EDTA and 3% acetonitrile (blue line, eluent 1) or 40 mmol L^{-1} HCO_3^-, 40 mmol L^{-1} SO_4^{2-}, 8 mmol L^{-1} EDTA and 3% acetonitrile (orange line, eluent 2) are used.

Finally, a concentration of 40 mmol L^{-1} ammonium sulphate, 40 mmol L^{-1} ammonium bicarbonate, 8 mmol L^{-1} EDTA and 3% acetonitrile was selected as an optimal eluent composition, since this composition shortens the retention time of V(V), slightly increases V(V) peak intensity and decreases the void volume "pre-peak" intensity.

2.2. Distribution of V Redox Speciation in Krka River Estuary Samples

Respecting the optimised procedures for sampling storage and vanadium redox speciation, a selected set of estuarine samples covering the full salinity range, characterized by

different physico-chemical and anthropogenic conditions, was analysed. The dependence of total vanadium concentration on the salinity for the three sampling sites is shown in Figure 4. Concentrations of total vanadium in two end members (freshwater part and open sea) are also marked and connected with a line corresponding to the theoretical dilution line. Basically, a near-conservative behaviour was obtained for vanadium in the surface brackish layer in the absence of additional V input. A small positive deviation at low salinity was ascribed to the anthropogenic influence within the harbour area (VP site), whereas negative deviation at high salinities was observed for the most upstream site in the bottom seawater layer. This estuarine segment is characterized by a higher concentration of suspended particulate matter and a long residence time of the bottom seawater. Similar processes related to the particle scavenging, as well as to biogenic adsorption and/or biological uptake, are also found for the nearby Zrmanja River estuary [11].

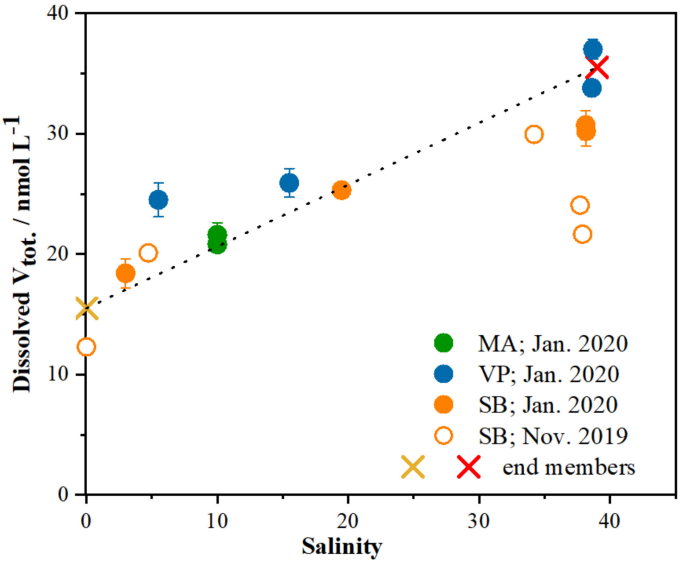

Figure 4. The relationship between the total dissolved vanadium concentration and salinity at the MA (green circles), VP (blue circles) and SB (full orange circles—January 2020; empty orange circles—November 2019) sites. The theoretical dilution line between the two end members, open sea (red cross) and the Krka River (light brown cross), is represented by the black line.

Vertical distributions of the total dissolved V and V(V) in the water column of the three sampling sites are presented in Figure 5, whereas numerical results are provided in Table S1. The increase in V concentration with the depth is in line with the above presented salinity dependency. As expected, the predominant V specie in all samples is V(V), accounting for more than 74% of the total V. The concentrations of reduced V(IV) species were higher in the deeper seawater layer than in the surface layer. Further examination revealed that the percentage of V(IV) was highly related to the oxygen concentration, as presented in Figure 6. Such dependence could be justified by different redox conditions and water chemistry, as detailed in Wang et al. [45].

In November, when the bottom seawater layer at SB site was highly depleted in oxygen, the concentration of the V(IV) was the highest, accounting for 26% of the total dissolved V. The development of hypoxia was found to be common in the autumn/winter period in this part of the estuary and is explained by the degradation of organic matter derived from high primary production in the summer period [12,50]. However, oxygen depletion does not seem to be the only factor affecting the distribution of the V redox species. Interestingly, concentrations of reduced species are higher on the VP sampling site compared to sampling site SB for the same concentration of dissolved oxygen. A possible contributing factor to the stability and increased share of reduced species at the VP location is linked with anthropogenic input characteristic for this part of the estuary [12,51,52].

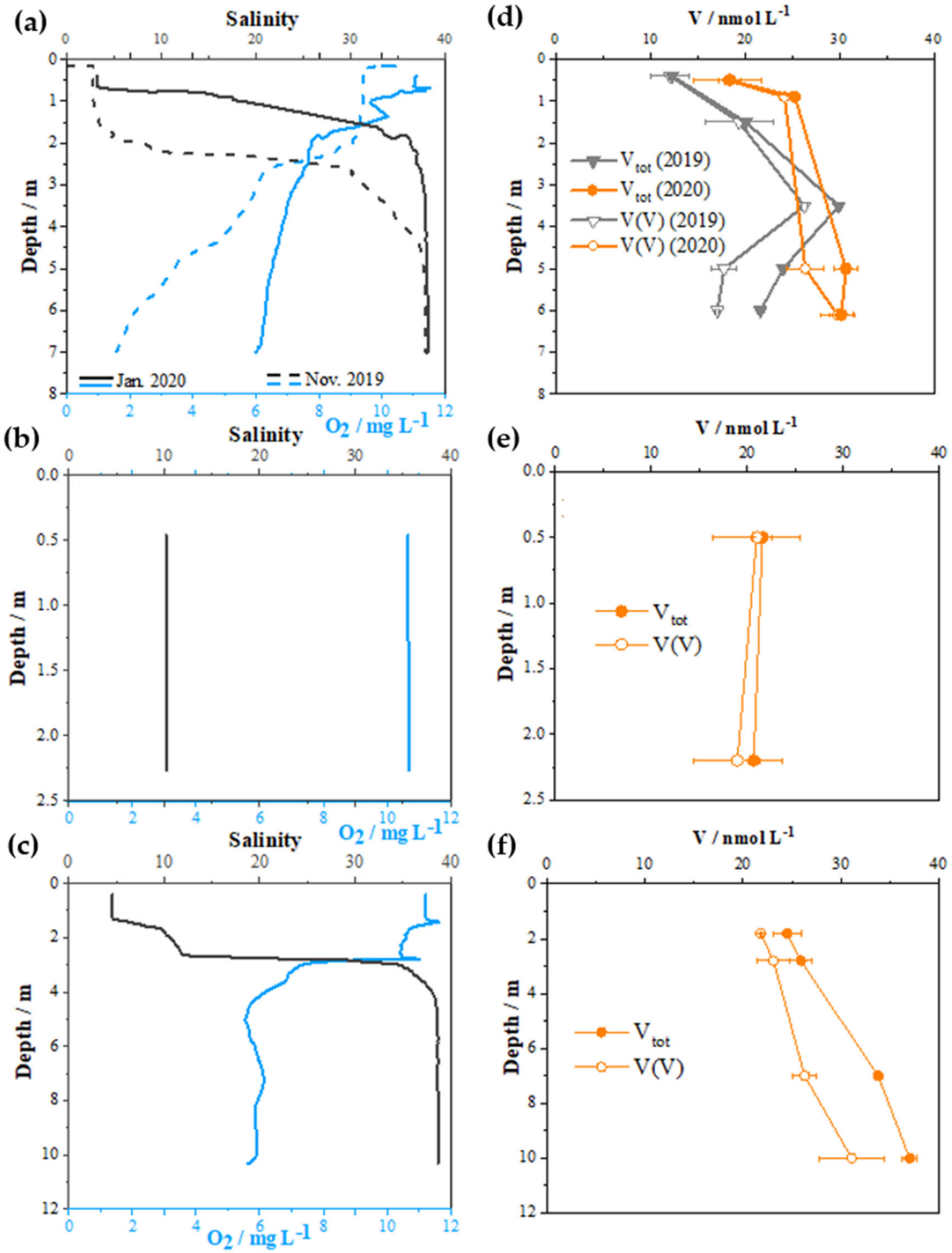

Figure 5. Vertical profiles of salinity and dissolved oxygen, and total dissolved V (full circles) and dissolved V(V) (empty circles) for SB (**a** and **d**), MA (**b** and **e**) and VP (**c** and **f**) sites. Dashed lines on panel (**a**) as well as grey symbols on panel (**d**) represent November 2019 sampling at the SB station, while the rest of the data represent January 2020.

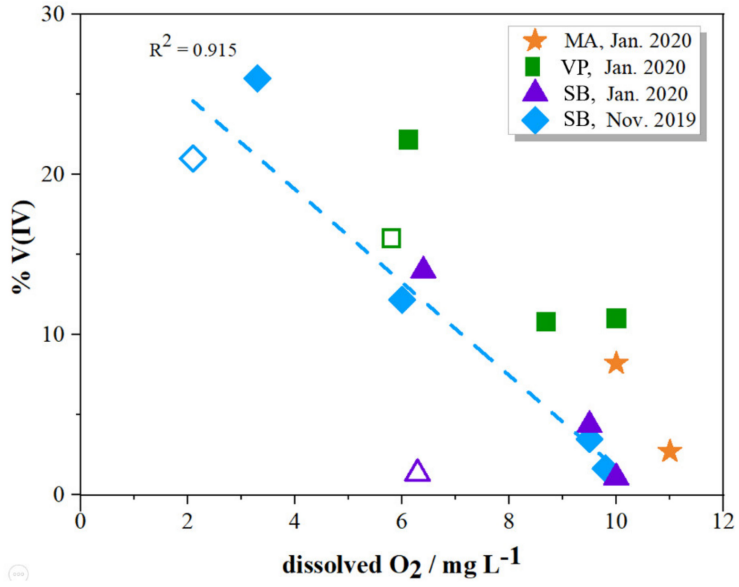

Figure 6. Percentage of reduced species in the water column of MA (orange markers), VP (green markers) and SB (purple markers—January 2020; blue markers—November 2019) sampling stations in relation to the dissolved oxygen concentration. Empty markers represent bottom seawater layer for each sampling station.

It is interesting to note that in January 2020, at the SB sampling site, V(IV) in the bottom layer was not observed. However at the bottom layer of VP sampling site V(IV) was detected (up to ~15% of total dissolved V), although the O_2 concentration between sampling sites differed only slightly (Figure 6, empty marker points). It is likely that the adsorption of V(IV) onto particles and colloids occurred, especially in the upstream parts of the estuary where the enrichment of particulate matter was reported [12].

In order to obtain information on the possible contributing factors of reduction mechanism of V(V) species in oxic water system, a study on the composition and the behaviour of the organic matter and particulate matter would be needed in further studies.

3. Materials and Methods

3.1. Study Site

The Krka River is a 49 km-long river with an average flow varying between 40 and 60 m^3 s^{-1}. The estuary starts below the waterfalls of Skradinski Buk and ends at the Šibenik channel, with a total length of 23.5 km [53] (Figure 7). Due to its low tidal range of 0.2 to 0.5 m and sheltered geographical position, the Krka River estuary belongs to a highly stratified type of estuaries [54]. The surface current is directed towards the sea, while the bottom current of seawater can be followed upstream to the Skradinski Buk [54]. Vertical gradient is characterized by three layers: a surface freshwater/brackish layer, a freshwater-seawater interface and a seawater layer. Halocline is usually positioned between 1.5 and 3 m, with the thickness of the halocline layer varying between a few cm up to 1 m [12,54]. Special characteristics make this sampling location ideal for evaluating the mobility and fate of trace metals and natural organic matter [12,53,55–57]. Trace metals have been previously studied, although mainly in reference to their total concentrations [12,51]. In addition, some of the recent studies on sediments of the Krka River estuary showed detectable anthropogenic input (due to the nautical tourism) in local restricted areas, although the estuary generally is not considered polluted [51,52,58].

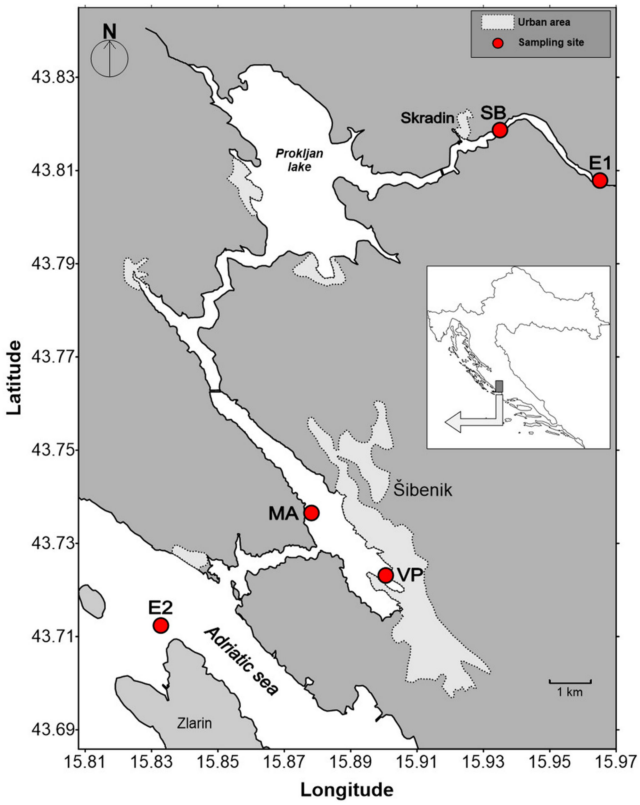

Figure 7. Map of the Krka River estuary with indicated sampling sites.

The sampling sites were selected to represent typical estuarine zones of varying anthropogenic input, physico-chemical gradients and biological activity [57,59]. Sampling was conducted on November 2019 and January of 2020 at three different locations along the Krka River estuary (Figure 7): Skradin bridge (denoted as SB), Martinska (denoted as MA) and Vrnaža port (denoted as VP), as well as two end-member locations: the Krka River water above the Skradinski Buk waterfalls (E1) and coastal seawater (E2). With the exception of two end members and the MA sampling station (where the brackish layer was sampled only), samples of the surface brackish layer, freshwater–seawater interface and seawater layer were collected at the remaining two sampling sites. Salinity of processed sampling sites varied from 4 to 38 salinity units (additionally shown in Section 2.2, Figure 4).

3.2. Equipment and Chemicals

For the determination of vanadium species, an ion chromatograph (Eco IC, Metrohm) with an anion exchange column (Metrosep A Supp 5–50/4.0, 50.0 mm length, 4.0 mm of inner diameter) was used. In the process of method development, two different instrumental systems for the determination of V were used: the 944 Professional UV/Vis Detector Vario and a high-resolution inductively coupled plasma mass spectrometer (HR ICP-MS, Element 2, Thermo). Both were operated at a flow rate of 0.3 mL min^{-1}, controlled by the IC system. HR ICP-MS was operated at *m/z* = 50.942 mass detection since it is the dominant naturally occurring V isotope (99.76%). In order to avoid isobaric interferences (the dominant one being $^{35}Cl^{16}O^{+}$ due to the high salinity of the samples), ICP-MS measurements were heldat medium resolution (M/ΔM = 4000). The temperature of the anion exchange column was ambient (~22 °C, air conditioned), and the sample injection volume was 100 µL. Details on other operating conditions of the HR ICP-MS system are available in the ESI material.

Vanadium (IV) stock standard solutions of 0.02 mol L^{-1} were prepared by dissolving 0.50 g of $VOSO_4 \times 5H_2O$ (VWR BDH Prolabo 132 Chemicals) in 100 mL of MQ water (18.2 MΩ cm, Milipore, USA). A stock solution of V(V) (0.02 mol L^{-1}) was prepared by dissolving 0.23 g of ammonium metavanadate (VWR BDH Prolabo Chemicals) in 2 mL

concentrated HNO_3 (KEMIKA d.d.), then diluting to 100 mL with MQ water (18.2 MΩ cm, Milipore, USA). The used chemicals for eluent preparation were: acetonitrile (VWR BDH Prolabo Chemicals), ethylenediaminetetraacetic acid (EDTA) (VWR BDH Prolabo Chemicals), ammonium hydrogen carbonate (NH_4 HCO_3) (VWR BDH Prolabo Chemicals) and ammonium sulphate ($(NH_4)_2$ SO_4) (VWR BDH Prolabo Chemicals). Indium standard solution for V_{tot} measurements was prepared by the dilution of 1000 mg L^{-1} AAS standard (Fluka).

Bottles for sampling and sample storage (PFA-perfluoroalkoxy, Nalgene) were previously cleaned with 10% HNO_3 of analytical reagent grade, rinsed thoroughly with Milli-Q water (18.2 MΩ cm, Millipore, USA) and filled with Milli-Q water until use. Upon sampling, the sampling bottles were rinsed with the sample.

After collection, the final samples were filtered with previously pre-cleaned 0.22 μm pore size filters (cellulose-acetate, Minisart, Sartorius; precleaned in HNO_3 and rinsed with Milli-Q water) and stored at natural pH at +4 °C until analysis. Vertical profiles of physical and chemical parameters (salinity, temperature, pH, dissolved oxygen and chlorophyll a) were measured in situ, using an EXO2 multiparameter CTD probe (YSI).

3.3. Determination of V_{tot} and V Redox Species

V_{tot} was measured using HR ICP-MS analytical instrumentation. Quantification was performed by using external matrix matching calibration. Briefly, the stock solutions with increasing V concentrations (0, 0.1, 1 and 10 μg L^{-1}) for external calibration were prepared in a 10× diluted CASS-5 (nearshore seawater reference material for trace metals, NRC, Canada) certified sample. The internal In standard (10 ppb) was added to both calibration solutions and sample solutions in order to minimise the matrix effect affecting the accuracy of the V_{tot} measurement. The same certified sample was also used for the quality control (QC) and measured as every 5th sample in the sequence. The obtained V concentrations agreed within 10% with the certified value. Working standards, as well as blank solutions, were prepared with the addition of 2% high-purity HNO_3. For total V concentration measurements, samples were diluted 10× with 2% high-purity HNO_3.

Vanadium speciation on natural samples was conducted using anion exchange ion chromatography coupled to HR ICP-MS. Filtrated samples of Krka river were directly processed on stated analytical instrumentation, and successful separation of V(V) species was achieved. Instead of calibration curves suggested by Li and Le, in our work, for the accurate quantification of V(V), we used a standard addition method (peak heights of the chromatograms were used as an analytical signal). V(IV) species quantification was achieved by the subtraction of determined V(V) species from the total V measured. The limit of detection was found to be 2 nmol L^{-1} in the seawater and 0.6 nmol L^{-1} in the freshwater, while recoveries were within 5%.

4. Conclusions

An improved method for vanadium redox speciation using anion exchange ionic chromatography (IC) coupled with high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS) was described and applied to the environmental samples of the Krka River estuary. By modification and optimization of eluent composition (40 mmol L^{-1} HCO_3^-, 40 mmol L^{-1} SO_4^{2-}, 8 mmol L^{-1} EDTA and 3% acetonitrile), eluent pH values and sample pre-treatment approach V(V) species were successfully separated even in the samples of high salinity. Due to the complex matrix of natural samples, V(V) species were determined using a standard addition method, whereas reduced species (mostly V(IV)) were determined by subtracting the V(V) concentration from the total V. Preservation and storage tests showed that unlike the previously suggested EDTA addition to samples upon the sampling, the speciation was preserved for at least 7 days without any ligand additions, if samples were kept at natural pH and at 4 °C.

Conservative behaviour of V species was found in the salinity gradient of the surface estuarine layer. It was found that V(V) species were predominant redox species in all

samples. However, a high share of V(IV) (up to 26%) was found in the samples taken from the oxygen-depleted water layers at the upper part of the estuary. Additionally, higher concentrations of reduced species were detected at the sampling locations linked with anthropogenic input. It was shown that the stability of reduced species is preserved even in the oxic conditions, suggesting the interaction of vanadium species with organic or inorganic ligands present in the water column of sampling stations.

Supplementary Materials: The following are available online, Table S1. Numerical values of total dissolved V(V) concentration and calculated percentage within the Krka River estuary samples. Table S2. Operating conditions of High Resolution Inductively Coupled Plasma Mass Spectrometry (HR ICP-MS).

Author Contributions: L.K., Formal analysis, Methodology, Data Validation and Writing—Original Draft. D.O., Formal analysis, Data validation and Writing—Review and Editing. N.B., Formal analysis. J.M., Formal analysis. E.B.-N., Conceptualization, Formal analysis, Funding acquisition, Supervision and Writing—Review and Editing. All authors have read and agreed to the published version of the manuscript.

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4.2. Research paper II

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SEDIMENTS AS A DYNAMIC NATURAL RESOURCE – FROM CATCHMENT TO OPEN SEA

Ion-exchange chromatography as a tool for investigating vanadium speciation in sediments: preliminary studies

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Abstract

Purpose We conducted this investigation in order to develop a simple method for the determination of bioavailable vanadium and its speciation within sediments.

Materials and methods The developed method is based on the determination of acid-extractable vanadium, as this fraction is presumably bioavailable, and its subsequent speciation using IC–UV/Vis (ionic chromatography with an ultraviolet-visible detector). A published procedure for vanadium speciation in pore water was further optimized in order to separate V(IV) and V(V) species using column EDTA derivatization on sediment samples. The analytical approach was applied in order to assess acid-extractable vanadium and its speciation within estuary sediments of the Krka River at the Dalmatian coast of Croatia.

Results and discussion The results imply that the majority of vanadium within estuary sediments is in the form of less soluble fraction while the acid-extractable fraction comprises approximately 40% of the total vanadium present. In contrast, open sea sediment (station K4) was completely dominated by the acid-extractable vanadium. The extracted vanadium is in the form of V (IV).

Conclusions Ion-exchange chromatography with an UV/Vis detector is a promising analytical method for vanadium speciation, however, further optimization of the conditions (improvement of the sequential extraction procedure) is needed in order to assess speciation also within other vanadium sedimentary fractions.

Keywords Extractable sediment fraction · Ion chromatography · Krka River estuary · Speciation · Trace metals · Vanadium chemistry

1 Introduction

Vanadium, V, is a transition metal and is one of the most abundant elements in Earth's crust (among the 20) with the average content in the upper part of Earth's crust of 60 μg g^{-1}, reaching concentrations over 200 μg g^{-1} within bulk and lower continental crust (Taylor and McLennan 1995). It is the second most abundant transition metal in seawater with its concentration only being exceeded by molybdenum (~ 35 nmol dm^{-3} and 110 nmol dm^{-3}, respectively— e.g., Emerson and Husted 1991 and references therein). The prevailing vanadium valence states in nature are vanadium(III), vanadium(IV), and vanadium(V) of which the latter two are the most soluble (Wanty and Goldhaber 1992) making the determination of vanadium speciation a challenging task. Under acidic conditions most V species display cationic character, such as the vanadyl (VO^{2+}) and vanadate (VO_2^+) ions, while under less acidic to mildly alkaline conditions the vanadium species are hydrolyzed, ultimately forming in the case of vanadium(V) the anionic species HVO_4^{2-} or $H_2VO_4^-$ (e.g., Huang et al. 2015). The vanadium(V) speciation in natural water is affected by several factors (for example pH, ionic strength, and ligand forming species), including the total vanadium concentration, due to the formation of stable vanadium polymers at concentrations above 100 μmol dm^{-3} (e.g., Brookins 1988; Huang et al. 2015).

Vanadium concentration within sedimentary rocks echoes primarily the abundance of detrital Fe oxides, clay minerals, hydrous oxides of Fe and Mn, and organic matter. The lowest average V content is characteristic for pure carbonate (up to the 15 μg g^{-1}), with higher values in black shale, basalts, and clay (reaching ca. 200 μg g^{-1}—Levinson 1974; Ketris and

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Yudovich 2009). The high V concentration within black shale is reflecting both the affinity of the element for organic sorption sites and its relative immobility under reducing conditions due to the important role of redox regime (Anbar and Knoll 2002). That is, V remains mobile under oxidizing conditions but precipitates just above the sulfate/sulfide redox zone (Brookins 1988).

Almost all reported results concerning the direct determination of vanadium species in the solid phase of environmental materials are from XAS (X-ray absorption spectroscopy) and XANES (X-ray absorption near-edge structure) (e.g., Gustafsson 2019). However, the ability of ion chromatography (IC) to quantify different metal oxidation states and stable metal complexes in sediment extracts has advantages when choosing a suitable analytical approach (Jackson 2000).

For oxic environments, such as the unsaturated zone of soils, vanadate(V) is usually the most stable oxidation state. In these environments, where the surface reactions with iron(III) and aluminum(III) compounds dominate, Larsson et al. (2015a, b, 2017) studied vanadium speciation by use of XANES spectroscopy in a number of Swedish forested and agricultural soils. The vanadyl(IV) was predominating in the mor layer (the organic surface horizon) while deeper down in the soil profile the vanadium appeared as vanadate(V). Only two studies were found that used XANES spectroscopy for V speciation in reduced natural samples showing the predominance of reduced V (III and IV) (Bennett et al. 2018; Nedrich et al. 2018).

Vanadium as a trace metal present in the Krka River estuary has been studied only once by Prohić and Kniewald (1987). In this paper, the authors addressed bulk concentrations and percentage of vanadium in extractable and less extractable fraction. However, the oxidation state of vanadium in investigated fractions was not addressed. In order to understand the biological and geochemical cycling of vanadium, it is imperative to describe and measure transformations of the vanadium among a range of chemical species that it takes form of. Consequently, information on diagenetic processes for vanadium accumulation in the sediment can be made (Bennett et al. 2018). Moreover, usage of only bulk concentrations is an insufficient indicator of a mechanical pathway of specific trace elements and their accumulation time in sediment (Loring 1979). Present vanadium species in easily extractable fraction of sediment can originate from weathering or from anthropogenic input and they are accessible to surrounding biota. Therefore, extractable fraction represents the important part of the geochemical cycling of vanadium species between sediment and water column in the Krka River estuary. Taking into account vanadium chemistry, with V(V) being the most toxic and soluble species, chemical speciation of easily extracted fraction of sediment is needed.

To fully understand the geochemistry of vanadium, and to be able to predict its mobility and bioavailability, reliable information from speciation studies are of importance. Also, accumulation of vanadium in sediment can represent valid proxy for human and biological activity from local to global scale (Anbar and Knoll 2002). Within this paper, we introduce the use of ion-exchange chromatography with a UV/Vis detector as a tool for determining vanadium species in sediments. The method development and preliminary results of vanadium speciation within estuary sediment samples of the Krka River will be presented.

2 Materials and methods

2.1 Instrumentation

The used system was METROHM ECO IC; Ion chromatograph with "944 Professional UV/Vis Detector Vario" and anion exchange column. The IC-UV/Vis operated at a flow rate of 0.700 mL min^{-1}. The column temperature was ambient (~ 22 °C) and the sample injection volume was 20 μL. Used anion exchange column is Metrosepp A Supp 5-50/4.0, with 50.0 mm length and 4.0 mm of inner diameter.

2.2 Reagents

Stock standard solutions of 1.0 g dm^{-3} V(IV) were prepared by dissolving 0.50 g of $VOSO_4x5H_2O$ (VWR BDH Prolabo Chemicals) in 100 mL of distilled and deionized water. Stock solution of V(V) (1.0 g dm^{-3}) was prepared by dissolving 0.23 g of ammonium metavanadate (WR BDH Prolabo Chemicals) in 2 mL concentrated HNO_3 (T.T.T. d.o.o.), then diluting to 100 mL with distilled and deionized water. Concentrated HCl (Fisher Chemical) was used for adjusting the pH of solutions.

The IC eluent was prepared using acetonitrile (3%) (VWR BDH Prolabo Chemicals), ethylenediaminetetraacetic acid (EDTA) (2×10^{-3} mol dm^{-3}) (VWR BDH Prolabo Chemicals) and ammonium hydrogen carbonate (NH_4HCO_3) (8×10^{-2} mol dm^{-3}) (VWR BDH Prolabo Chemicals). After preparation, HCl was used in order to adjust the pH of the eluent to a value of 6.

2.3 Methods

Sediment was collected at four locations, three within the Krka River estuary and one in the open sea (Fig. 1). Gravity corer (Uwitec) equipped with Plexiglas tubes (φ = 6 cm) was used for sampling. Only the top 5 cm were retrieved and used for analysis. After the sampling, sediment samples were deep-frozen (− 18 °C), freeze-dried, and sieved to < 2 mm.

Total vanadium concentrations, that were used for comparison, were determined in acid digested samples by using High Resolution Inductively Coupled Plasma Mass Spectrometer (HR ICP-MS, Element 2, Thermo). Sediment samples were

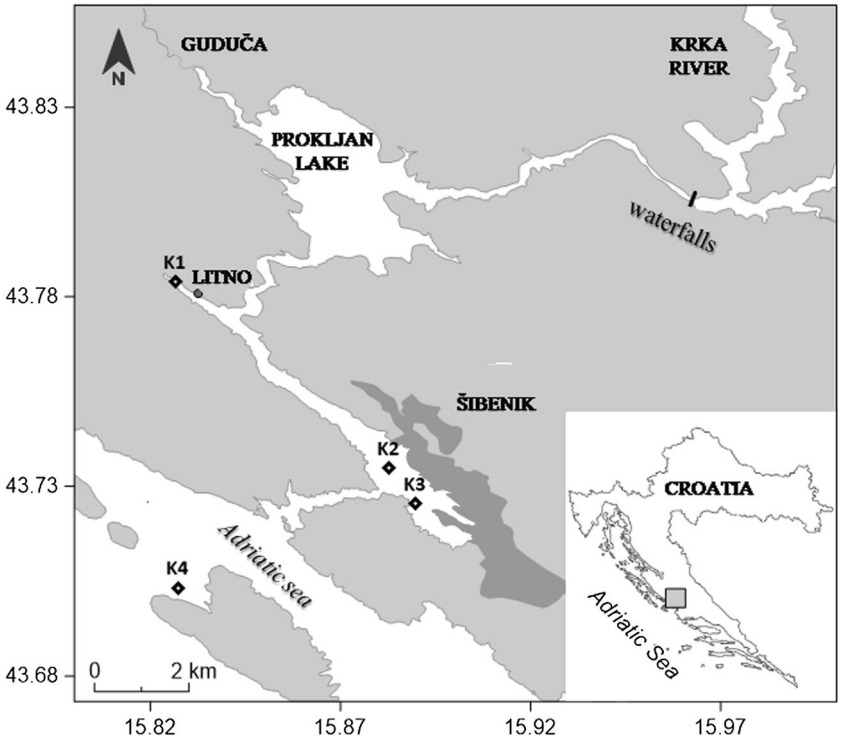

Fig. 1 The sampling area of the Krka River estuary with defined sampling stations marked: K1 (Litno-sampling site), K2, K3, and K4 (open sea sampling site).

digested as follows: 100 mg of sediment was weighed and placed in 50 mL Teflon (PTFE) bombs together with 10 mL of aqua regia (HNO_3:HCl 1:3, Fisher Scientific® Trace Analysis grade) and placed in microwave oven (ANTON PAAR® Multiwave 300). After digestion, samples were filtered and diluted to 100 mL for analysis. A certified material PACS-2 (National Research Council of Canada) was used for validation of the analysis.

For the chromatographic determination of easily extractable vanadium, 1.0 g of each sediment sample was weighed in and 10 mL of deionized and distilled water containing 0.12 mol dm^{-3} HCl was added to each sample. Prepared samples were left to dissolve in water for approximately 40 min with occasional mixing. Afterward, they were filtrated through filters with 0.2-μm filter pore size.

Prepared samples were then measured using ion chromatography and an anion exchange column with "944 Professional UV/Vis Detector Vario," with wavelength set at 280 nm.

3 Results

3.1 Method development

Although the use of synchrotron methods (XAS and XANES) is dominant in the determination of trace metals in sediment, in this case, it is found to be an unsatisfactory approach. One of the main disadvantages is the fact that XANES spectra present a weighted sum of all the species present in the analyzed sample, i.e., speciation of trace metals is very difficult task using stated methods (Feldmann et al. 2009). Bearing in mind that sediment represents a complex mixture of chemical species, by applying this method insufficient results would be obtained. As it was the main goal of this research to determine bioavailable vanadium species in extractable sediment fraction, ion chromatography was found to be suitable approach. This method proved to be sensitive enough for determination of trace levels of vanadium in a complex matrix such as sediment. With the usage of ion chromatography conclusions on the transport mechanism and toxicity of vanadium in sediment can also be made (Cardellicchio et al. 1999). For determination of vanadium species in reducible, organic/sulfidic and residual fraction, it is suggested optimization of sequential extraction process.

The method for determination of vanadium species was previously published and used for speciation in pore water (Li and Le 2007). Speciation analysis of V(IV) and V(V) in model solutions was achieved by measuring negatively charged complexed vanadium species with EDTA. During the complexation reaction with EDTA on the anion exchange column, V(IV) and V(V) form complexes of different charge $[VO(EDTA)]^{2-}$ and $[VO_2(EDTA)]^{3-}$, respectively. Charge difference between formed complexes of V(IV)-EDTA and V(V)-EDTA is the main principle of the separation of V(IV) and V(V) species on the anion exchange column, where the stationary phase is positively charged and the negatively charged $[VO(EDTA)]^{2-}$ and $[VO_2(EDTA)]^{3-}$ species are attracted to it (Li and Le 2007). Figure 2a shows a chromatogram of vanadium species in mixed model solution of V(IV)

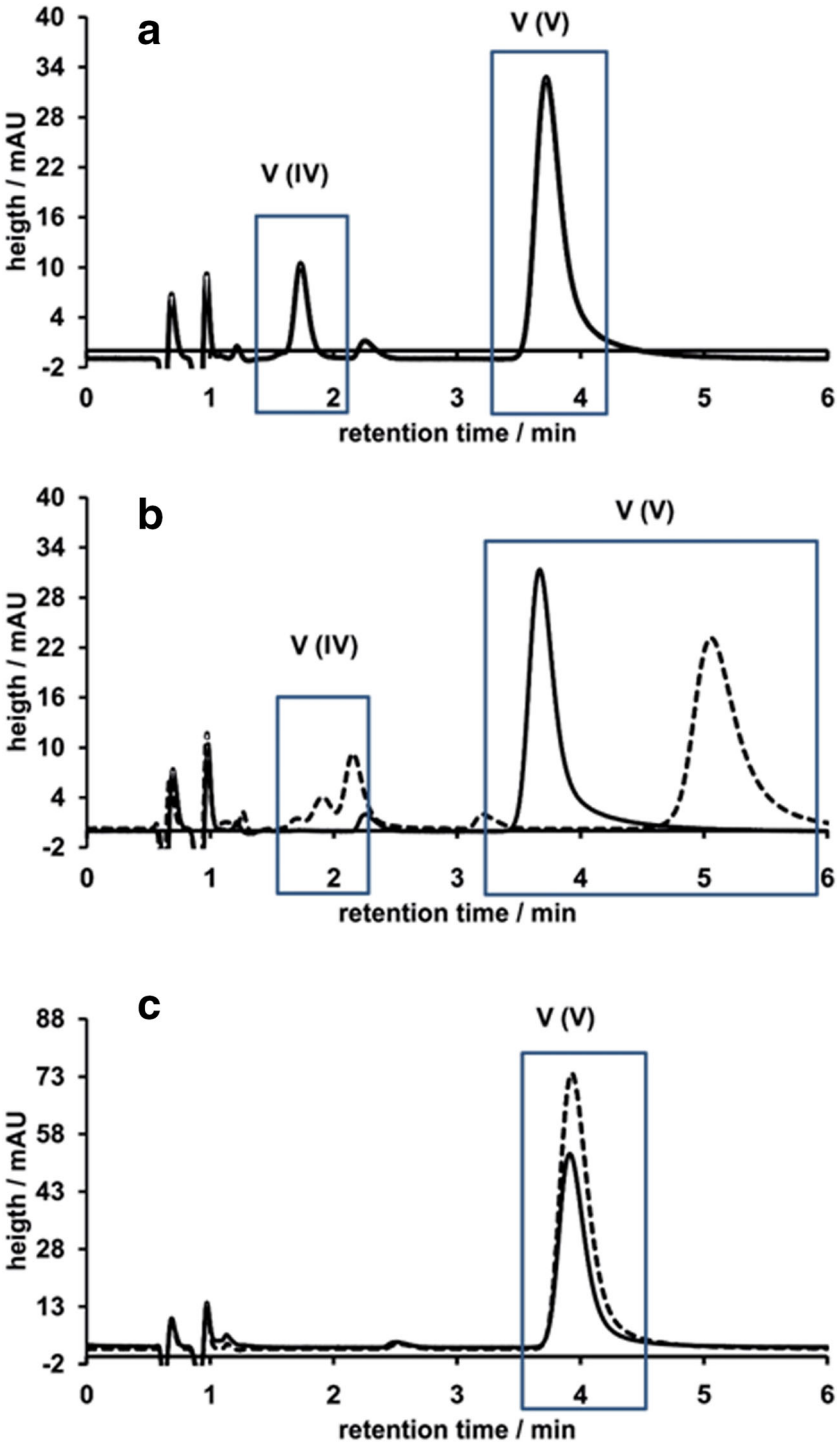

Fig. 2 **a** Model mixed chromatograms of V(IV)-ETDA and V(V)-EDTA complexes using anion exchange chromatography separation and UV/Vis detector; speciation was achieved by measuring V(IV) and V(V) model solutions (1×10^{-5} mol dm^{-3}) while having EDTA in eluent (2×10^{-3} mol dm^{-3}); pH (solution) = 3.0. **b** Comparison of model chromatograms of V(V) (1×10^{-4} mol dm^{-3})-EDTA complex with EDTA (1×10^{-4} mol dm^{-3}) in the solution (pH = 6.0) (dashed line) and V(V) (1×10^{-5} mol dm^{-3}) without EDTA in the solution (pH = 3.0) (solid line) using anion exchange chromatography separation and UV/Vis detector. **c** Model chromatograms of V(V)-ETDA complex using anion exchange chromatography separation and UV/Vis detector; speciation was achieved by measuring V(V) model solution (1×10^{-4} mol dm^{-3}) while having EDTA in eluent (2×10^{-3} mol dm^{-3}) and in the solution (1×10^{-4} mol dm^{-3}) at the pH of 2 (dashed line) and of 6 (solid line)

and V(V), based on anion exchange chromatographic separation and UV/Vis detection.

Although the stated method suggested that complexation of V with EDTA in the solution stabilizes formed complexes on the column, this step was avoided. As can be seen in Fig. 2b, when using EDTA in the solution and eluent, the peaks become more unstable. It is found that EDTA shows the tendency to reduce the V(V) species after the equilibrium of the complexation reaction was established (Kanamori et al. 1999). When comparing the kinetics of the complexation reaction between vanadium species and EDTA on different pH values, in both cases it is shown that on lower pH values complexation reactions are faster than on higher (alkaline) values. Moreover, V(IV) on lower pH values is present as stable VO^{2+} ion and does not oxidize to V(V) species, while on pH values higher than approximately 5.0, it can oxidize to

V(V) within a few minutes. Because of the described effects, it was decided that the concentration of EDTA in eluent (2×10^{-3} mol dm^{-3}) would be sufficient and that complexation reaction of vanadium species with EDTA directly on column would be fast enough when the pH of measured solutions is held at acidic values (Fig. 2c). Furthermore, any transformations of vanadium species with EDTA that might occur on column are reproducible and therefore the deviations are easily calculated. Using this optimization of the method, the same peak resolution and sensitivity was accomplished as when using EDTA in the solution, but without the presence of the stated instabilities of vanadium species.

Figure 2b also shows the effect of eluent and pH of the solution on the chromatograms. In the case when EDTA was used in solution to determine the V(V)-EDTA complex, the eluent was acidified using HCl to pH 6.0 (dashed line), which clearly extended the retention time. Considering that the acidified eluent did not improve the peak height and stability and even extended the retention time, this step was avoided when treating the sediment samples. Moreover, Fig. 2b shows a comparison between used EDTA in the solution, where pH of the solution was kept at 6.0, and a solution where EDTA was not present as a reagent where the pH of the solution was reduced on 3.0. It can be seen that lowering pH accelerates complexation reaction and increases peak height and stability.

Figures 3 a and b show the measured detection limit for V(IV) and V(V) species in model solutions using the described method with UV/Vis detector. Detection limit for V(IV) and V(V) species was found to be on approximate value of 5×10^{-6} mol dm^{-3}; i.e., good sensitivity was accomplished and determination of vanadium species in acid extractable sediment sample was possible with the used analytical approach.

3.2 Discussion

The Krka River and its estuary is located in the central part of the Eastern Adriatic, in the karst region of Croatia. Since Krka River drainage area is mainly composed of carbonate rocks and it has tufa barriers along the stream, the Krka River itself brings small quantities of suspended terrigenous material to the estuary. Main suppliers of the terrigenous material to the Krka River estuary are small tributary Guduča River and Litno Spring, whose catchment areas are composed mainly of flysch and flysch-like deposits (Prohić and Juračić 1989; Juračić and Prohić 1991; Cukrov and Barišić 2006). Although this type of deposits are subordinate to carbonates in the Krka River estuary area, they play an important role in distribution of some trace elements in the estuary (Prohić and Kniewald 1987; Cukrov et al. 2009).

Figure 4 depicts chromatogram obtained after applying leaching using the 0.12 mol dm^{-3} solution of HCl, revealing the presence of vanadyl species within all investigated samples. Having in mind used leaching conditions (mild concentrated HCl, 40 min) the recovered vanadyl species mostly represent the easily exchangeable fraction of the total vanadium present. Using peak height and the calibration curve for the vanadyl ion (open symbols in Fig. 3b) the determined concentration of such manner recovered vanadyl species are represented in Table 1 and were compared with the total vanadium concentration determined using the ICP-MS.

The sediment at the station K1 is under the influence of submerged freshwater inflow, bringing detrital driven suspended matter and is characterized with the highest Al content (50933 μg g^{-1}) from all investigated sediments. On the other hand, station K4 is not under the influence of the Krka Riverplume and certainly the sedimentary clay fraction is the lowest with the Al content of 18257 μg g^{-1}. The total vanadium concentration is following the observed pattern, i.e., the stations under the Krka River plume influence are characterized by the higher V as well as Al concentration (stations K1, K2, and K3). However, the percent of acid-extractable vanadium has opposite behavior, i.e., highest percent of extracted vanadium (almost 100% of the total vanadium present) is observed within the sample with the lowest total vanadium concentration (station K4).

Previous studies also have reported changes in a type of sediment, from Prokljan Lake seawards. For instance, total carbonate content in the estuarine sediments increases from 55.1% in Prokljan Lake, across 70.8% in the lower part of the estuary to 91.7% in the open sea (Juračić and Prohić 1991). Contrariwise, the share of fine fraction (< 32 μm) diminishes from Prokljan Lake (94%), via lower part of the estuary (29.1%) to the open sea (4.4%) (Juračić and Prohić 1991). These differences can be attributed to the input of terrigenous material, meaning that in Prokljan Lake sediment is a mixture of marine carbonates and terrigenous material, while in the lower part of the estuary marine biogenocarbonate sedimentation prevails (Cukrov et al. 2009).

As it can be seen, similar results are obtained on stated sampling sites in comparison with sampling sites from Krka River estuary from previous studies (Prohić and Kniewald 1987). Although previous studies had their sampling stations dominantly at high freshwater influence without comparison with open sea values, it can be seen that values for total concentration of vanadium and its carbonate fraction are somewhat alike. It is highly probable that the reducible fraction, organic/sulfidic fraction, and residual fraction of vanadium concentration in Krka River estuary cannot be determined using the described chromatographic method, therefore sequential extraction is necessary.

Sequential extraction studies of different soil samples revealed that V is recovered mainly from the less soluble fractions with strong sorption properties what is in accordance with our preliminary results. Previous

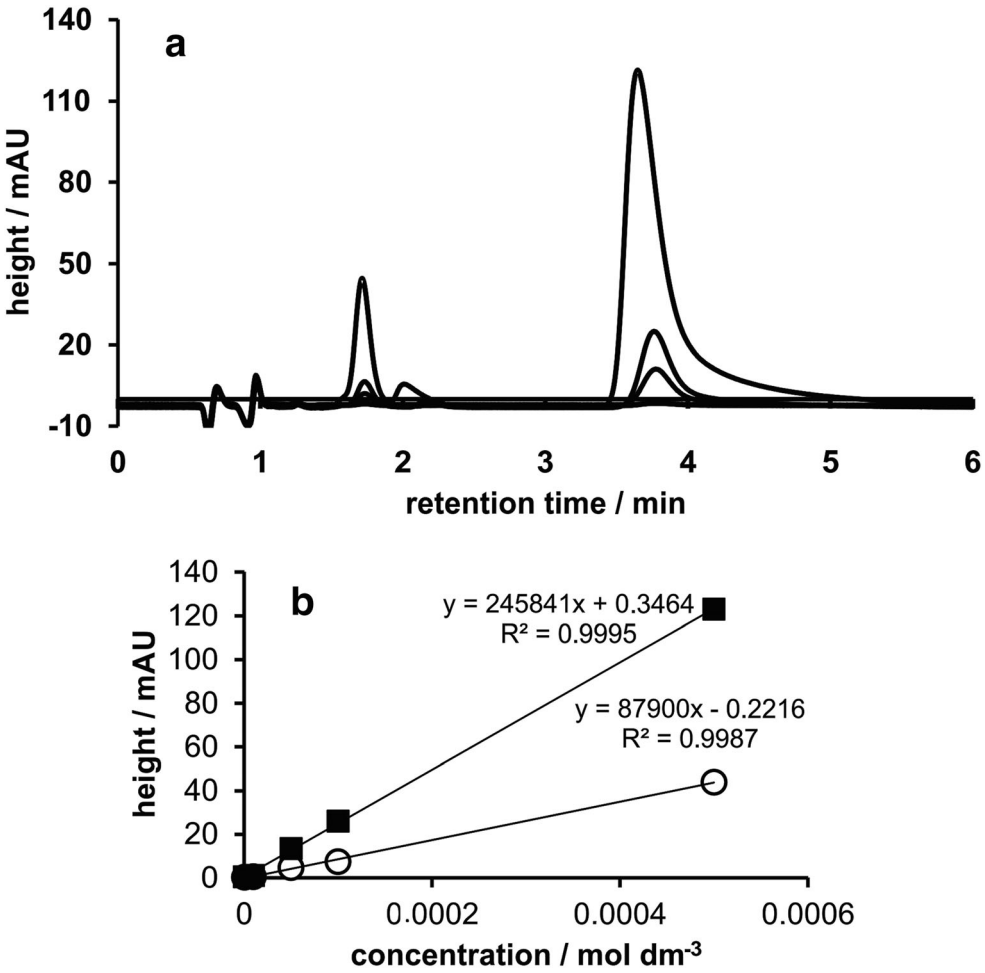

Fig. 3 **a** Chromatograms of measured detection limit for V(IV)—EDTA and V(V)—EDTA complexes using anion exchange chromatography with UV/Vis detector. Concentrations of V(IV) and V(V) in the solutions were 5×10^{-4} mol dm^{-3}, 1×10^{-4} mol dm^{-3}, 5×10^{-5} mol dm^{-3}, 1×10^{-5} mol dm^{-3}, and 5×10^{-6} mol dm^{-3}, respectively. **b** Calibration plot graphs for V(IV) and V(V) in model solutions. Measured detection limit for V(IV) and V(V) species was found to be on approximate value of 5×10^{-6} mol dm^{-3}

research on vanadium speciation in sediment fractions suggests that vanadium is present as more reduced species in less extractable fractions due to the reduced solubility and precipitation tendencies of species at lower oxidation states (Shaw et al.1990). With vanadium species being pH and Eh dependent, their transport into the sediment is controlled with lower Eh value. In the process of geochemical cycling, vanadium species are being released into the water column by oxidation into the more soluble species. Source of reduced species is thought to be detrital flux (Shaw et al. 1990). It seems very likely that Krka River plume is bringing detrital highly weathered material, i.e., clay minerals where vanadium most probably is strongly adsorbed. Also, the exchangeable fraction gives an indication of the metal bound on the particle surfaces as well as the amount of metal bound to the acid-soluble salts such as carbonates (Filgueiras et al. 2002). Also some of the Fe and Mn oxides can be recovered in this stage.

Although determining source of naturally occurring trace elements in the sediment is not straightforward because of the dependence upon multiple factors (changes in the chemistry of the source region, physical and chemical conditions during weathering, transport, deposition, diagenesis, chemistry, and the movement of the groundwater), bulk concentrations in the pre-industrial era are commonly used as the background level in determination of input source of trace metals (Prohić and Juračić 1989). The literature states that vanadium distribution in the Krka River estuary is characterized by higher background levels in surrounding rocks, influenced by flysch areas and Lake Visovac sediment, with its bulk concentration measured from 60 to 105 μg g^{-1} (Prohić and Kniewald 1987). With measured bulk concentrations being in agreement with previous studies, it can be concluded that anthropogenic input of vanadium throughout sampling stations in Krka River estuary is not significant. Despite relatively high vanadium concentrations in the sediment, bioavailability of vanadium is found to be relatively low and the majority of the vanadium species are present in less soluble fractions.

Generally, heavy metals in the exchangeable and acid-soluble fractions are considered readily and potentially bioavailable, while the reducible and oxidizable fractions are relatively stable under normal soil conditions (Filgueiras et al.

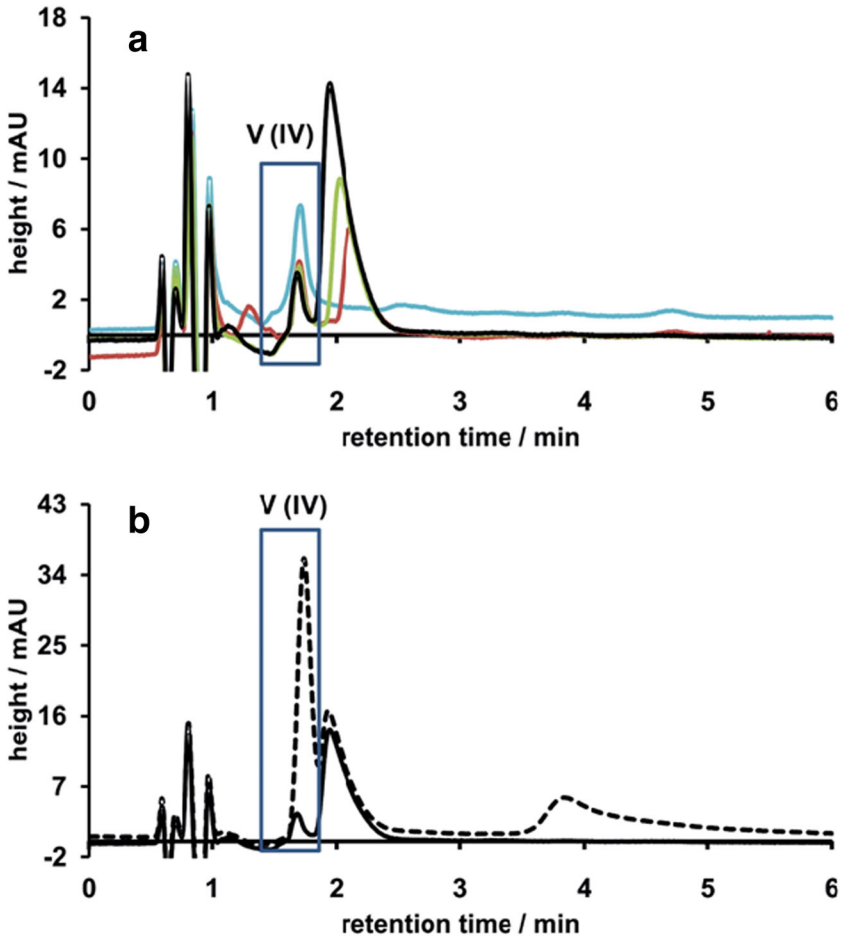

Fig. 4 **a** Chromatograms of selected sediment samples: station K1 (blue curve), K2 (red curve), K3 (green curve), and K4 (black curve). **b** Chromatograms of K4 sediment sample before (solid curve) and after standard V(IV) addition (dashed curve)

2002). Interestingly, the studies of Wisawapipat and Kretzschmar (2017) on highly weathered soils by XAEFS revealed vanadate adsorbed to goethite, ferrihydrite, gibbsite, and/or Fe(III)–natural organic matter complexes and vanadyl in the structure of goethite may be present but cannot unequivocally be distinguished by XANES spectroscopy. These authors also demonstrated that kaolinite and Fe oxides can effectively sequester V in highly weathered soils by mechanisms of adsorption and structural incorporation, and are relevant to other Fe-oxide-rich environments under acidic and oxic conditions.

Table 1 Vanadium concentration determined using ICP-MS and IC with UV/Vis detector. Values for each kind of measurement compared for each sampling site in Krka River estuary; K1, K2, K3, and K4. Each sampling station is characterized with Al content

Sediment sample	V/μg g^{-1} (ICP-MS)	V/μg g^{-1} (IC)	Al/μg g^{-1}
K1	101.83	41.61	50933.4
K2	60.64	28.76	28516.6
K3	59.62	23.86	30076.4
K4	41.73	41.00	182572

4 Conclusions

Generally, the excess of trace metals exists in different chemical forms that tend to accumulate at exchangeable sites of various mineral components of sediment. Furthermore, the problem of bioavailability and remobilization of different chemical forms cannot be solved by trace metal analysis of the bulk sample alone because of the wrong implication that all chemical forms have equal impact on the environment (Prohić and Kniewald 1987).

Preliminary studies on the developed ion-exchange chromatographic method show that this method is sufficient for the analysis of vanadium speciation. However, the method cannot be applied for the determination of vanadium speciation in less soluble fractions where it is suspected, from previous studies, that the vanadium residue is adsorbed. For a thorough determination of vanadium speciation in sediment samples the method requires improvement by employing a sequential extraction procedure. Furthermore, for determining fractions of sediment that cannot be seen chromatographically, transfer of the developed method to ICP-MS is needed in order to offer complete information on vanadium speciation in sediment.

This can lead to new conclusions with respect to the chemistry of trace metals in freshwater and marine sediments.

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4.3. Research paper III

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Chromatographic and spectrophometric studies of vanadate (+V) reduction by 3–mercaptopropionic acid

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ABSTRACT

The reduction of vanadate (+V) in the presence of 3-mercaptopropionic acid was studied using a chromatographic method for the determination of vanadate (+V) versus vanadyl (+IV) species. Ion chromatography was combined with spectrophotometric investigation of the absorption properties of the solution. The chromatographic method for the separation of vanadate (+V) and vanadyl (+IV) was carried out with an anion exchange column. In the initial reaction mixture containing vanadate (+V) and 3-mercaptopropionic acid, ethylenediaminetetraacetic acid – EDTA was added in an excessive amount relative to the concentration of reactants in the solution. After the ligand exchange reaction, the added EDTA terminates the reduction, allowing redox speciation in the solutions. A strong pH dependence of the reduction rates in the investigated solution was observed. The vanadate reduction seems to proceed in 2 steps: 1) formation of the intermediate vanadate (+V)-thioester; 2) reduction reaction and formation of the vanadyl (+IV)-thiol complex. The obtained results strongly suggest that the reaction of vanadate (+V)-thioester formation is proton catalyzed. It was observed that the overall reduction rates are pH dependent due to the complex vanadate (+V) solution speciation and changes in the ionic form of 3-mercaptopropionic acid.

1. Introduction

The aquatic behavior of vanadium compounds in the presence of biologically active thiols has attracted the attention of many researchers as vanadium participates in numerous biochemical reactions in living organisms [1]. Vanadium chemistry under physiological conditions is very complex due to the facile transformation between vanadium species of different oxidation states, especially between vanadate (+V) and vanadyl (+IV) species [2]. Moreover, vanadium forms hydrolysis products in all oxidation states, which further complicates vanadium chemistry [3–5]. Vanadate is known for its inhibition of sulphur-containing enzymes, of which the tyrosine phosphatase proteins are of great importance as they regulate important intercellular processes such as mitoses, T-cell activation and insulin control [1,6,7].

Vanadate's ability to mimic phosphate in the various biological fluids enables it to participate in the great number of enzymatic reactions [11,12]. While vanadate and phosphate show similar structure, vanadate can attain stable, five-coordinated form that is, in the case of phosphate, only a transitional state. Phosphate substitution with vanadate leads to inhibition of enzymes such as phosphatases and kinases due to the vanadate's ability to from stable complexes in different oxidation states under physiological conditions. Unlike phosphate, vanadate is easily reduced to vanadyl, and this conversion between these two forms of vanadium plays an important role in regulating physiological processes [11,12,14].

The anti-diabetic activity of vanadate compounds has been observed by many authors, with the insulin-mimicking property being well documented. Vanadate has been shown to lower blood glucose levels, but only in subjects with elevated blood glucose levels, while no effect was observed in subjects with normal glucose level in the blood [3,5,12]. Although vanadate was known to be less potent inhibitor than molybdate and tungstate, anti-diabetic effects are more enhanced by vanadate, suggesting that coordination of vanadate is also an important factor for inhibition activity as well as the type of the chelating agents [13–15]. While five-coordinated vanadate complexes are the most potent phosphatase inhibitors, other vanadate forms and compounds also show inhibitory effects on various enzyme systems [16,17].

In the recent study by Feng at al, [18] an attempt was made to prepare tyrosil vanadate ester with high inhibitory activity and high selectivity. For this purpose, vanadate compound was stabilized by

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incorporation into the membrane-permeable graphene quantum dots, resulting in the formation of a vanadate complex with high selectivity for protein tyrosine phosphatase (PTP1B) compared to T-cell protein tyrosine phosphatase (TCPTP). While the production of stable and highly selective inhibitors is challenging, they show significant potential for application in biochemistry and biotechnology [18].

Although some authors suggest that vanadate is completely reduced to vanadyl in cells with cell glutathione, recent studies show that the reaction yield of vanadate reduction is lower than previously suspected [1,2]. It has been observed that vanadate reduction with glutathione does not occur under physiological conditions in the absence of vanadyl-complexing compounds, which is in contrast to the reaction in vanadate-cysteine systems where cysteine is readily oxidized under physiological conditions [1,2]. It was also suggested that important step of vanadate reduction in solutions containing organsulfur compounds (thiol) is formation of vanadate–thioester as intermediate specie. Many studies have confirmed that vanadate complexes with thiol-containing ligands can be stable, allowing their characterization [1,2,10,19]. Under physiological conditions, the reduction of vanadate occurs slowly, allowing sufficient time for the formation of thio complexes with vanadate [2,8]. Although many authors have investigated vanadium chemistry in biological systems, the exact mechanism has remained unclear due to the conflicting informations that have been reported [2,8]. With the aim of resolving these contradictions and providing a detailed characterization of vanadate reactions with sulphur-containing compounds, the behavior of vanadate in the presence of 2-mercaptoethanol was investigated under physiological conditions [2]. The obtained results suggest that thiols are readily oxidized with vanadate, but the reaction yield depends on the redox potential for both reactions: vanadate reduction and thiol oxidation; as well as on the reactions of polymerization and complex formation [2]. The formation of vanadate complexes with thiols may prevent reduction if vanadate complexes are more stable than vanadyl complexes. Similarly, in the absence of complex formation between vanadate and thiol, the reduction reaction is thermodynamically favored in a solution with high vanadate/vanadyl concentration ratio [2]. In this study [2] it was confirmed that vanadate can form complexes with thiols without undergoing the reduction to the vanadyl form, although reduction occurs under different conditions. In the reaction with 2-mercaptoethanol under neutral or alkaline conditions, vanadate was observed to form stable complexes with 2-mercaptoethanol without prior reduction. The formed vanadate complexes show a stoichiometry of 2:2. Contrary to that, at lower pH values and high 2-mercaptoethanol concentrations, vanadate is reduced with 2-mercaptoethanol, resulting in the formation of vanadyl complexes that show a stoichiometry of 1:2. These observations confirm that the oxidation state of vanadium and the pH of the system strongly influence the course of the reaction and the structure of the final products [2]. Further evidence for the stable complex formation between vanadate and sulphur-containing compounds under physiological conditions was provided in the study of Brattacharyya et al. [1], in which it was shown that although vanadate can be rapidly reduced by the thiol groups present in dithiothreitol, this reduction proceeds very slowly at high vanadate concentrations, especially at high pH values (> 7). Instead of a rapid vanadate reduction, a complexation reaction was observed, leading to the formation of the product with V_2L_2 stoichiometry [1]. The formation of vanadate thioester was further confirmed by spectrophotometric measurements, while EPR measurements also confirmed the formation of the vanadate intermediate complex, as no consumption of free thiol was observed, indicating that the reduction reaction had not yet occurred. After reduction, the vanadyl ion forms thio complexes when thiols are present in excessive amounts [8,20].

The reduction of vanadium with thiols under physiological conditions has been studied mainly by spectrophotometry, nuclear magnetic resonance - NMR and electron paramagnetic resonance (EPR) [1,2,8,21] while techniques such as X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) are used for the structural characterization of intermediate species and final products [1,20]. While spectroscopic techniques, such as atomic absorbance spectroscopy (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) [3,23] inductively coupled plasma mass spectrometry (ICP-MS) [24] and X-ray fluorescence spectrometry (XRF), can only be used to measure total vanadium; techniques such as capillary electrophoresis (CE), liquid chromatography (LC) and high performance liquid chromatography (HPLC) have been used to distinguish between vanadium atoms of different oxidation states [3,23,25].

In the current study, the vanadium chemistry in the systems containing 3-mercaptopropionic acid was investigated at different pH values by spectrophotometry at different reaction times. In addition, time-dependent measurements of the reaction products were investigated for the first time by ion chromatography in the solution where EDTA was added at different times during the reaction course to prevent further reactions, allowing observation of the formation of intermediate species at different reaction times.

2. Materials and methods

2.1. Materials

For the exact determination of the distribution of vanadium species in the investigated solutions, an ion chromatograph (Eco IC, Metrohm) with a Metrosep A Supp 5–50/4.0 anion exchange column, 50.0 mm length, 4.0 mm inner diameter) was used in combination with the 944 Professional UV/Vis Detector. The chromatographic system was operated at a flow rate of 0.30 ml min^{-1}, a sample injection volume of 100 µl and the ambient temperature of the anion exchange column (~22 °C, air-conditioned). In addition, the absorbance characteristics of the solutions in which the reduction of vanadate occurs were followed using the Analytik Jena Specord 200 plus spectrophotometer.

A stock solution of vanadate (0.02 mol dm^{-3}) was prepared by dissolving 0.23 g of ammonium metavanadate (VWR BDH Prolabo Chemicals) in 2 ml of concentrated nitric acid (HNO_3) (KEMIKA d.d.) and then diluted to 100 ml with MQ water (18.2 MW cm, Milipore, USA).

The chemicals used for eluent preparation were as follows: Acetonitrile (VWR BDH Prolabo Chemicals), ethylenediaminetetraacetic acid (EDTA) (VWR BDH Prolabo Chemicals) and ammonium hydrogen carbonate (NH_4HCO_3) (VWR BDH Prolabo Chemicals). The final concentration of NH_4HCO_3 and EDTA in the eluent was 40 mmol dm^{-3} and 8 mmol dm^{-3}, respectively. The eluent also contained 3% of acetonitrile.

Mercaptopropionic acid solution with a concentration of 1 mol dm^{-3} was prepared by dissolving 5.3 g of 3-mercaptopropionic acid (SIGMA - ALDRICH) in 50 ml of MQ water. The 1 mol dm^{-3} borate buffer was prepared by dissolving 6.183 g of orthoboric acid (VWR BDH Prolabo Chemicals) in 100 ml of MQ water. The pH of the borate buffer was adjusted by addition of small amounts of 1 mol dm^{-3} sodium hydroxide (NaOH) until pH of 8.5 was reached. Model solutions of 50 ml were prepared by adding 5 ml of borate buffer and 1 ml of 3-mercaptopropionic acid. The pH of the solution was adjusted by adding concentrated hydrochloric acid (HCl) to the solution prior the vanadate addition.

2.2. Reduction of vanadate (+V) by thiols

The route implemented to measure the reduction of vanadate by thiols contained the thiol (3-mercaptopropionic acid) at a final concentration of 0.02 mol dm^{-3} and vanadate at a concentration of 0.009 mol dm^{-3}. The thiol/vanadate ratio was approximately 2:1 and the total volume was 50 ml. Borate buffer of 0.01 mol dm^{-3} concentration was added to the reaction medium to avoid pH changes during the reaction course. The reduction kinetics studies were carried out in a pH range of 3–6.5 and the pH of the solution was achieved by addition of concentrated HCl.

The reactions were initiated by adding an aliquot of the vanadate

stock solution to the buffered 3-mercaptopropionic acid solution at room temperature. After mixing the reagents, the reduction reaction was monitored with the spectrophotometer in the time frame from 3 to 6 h. This assay was also used for the ion chromatographic determination of vanadate and vanadyl formed during the reduction. The chromatographic determination of vanadate and vanadyl was performed within 24 h after mixing the solution aliquot with EDTA. For this purpose, 1 ml of solution aliquot was mixed with 1 ml of 0.1 mol dm^{-3} EDTA and diluted with MQ to the final volume of 10 ml. The analysis of vanadate and vanadyl is performed by their complexation with EDTA, which allows the rapid and simple separation of the oxidation states of vanadium +V and + IV in the form of corresponding V-EDTA complexes, which are negatively charged, enabling the use of anion-exchange based ion chromatography [22,25,26]. The difference in determined V (+V)-EDTA and V (+IV)-EDTA peak intensities is within the usual experimental error (<10%). [26].

3. Results and discussion

3.1. Ligand exchange reaction of EDTA with the 3–mercaptopropionic acid vanadate (+V) and vanadyl (+IV) complexes

In the existing literature, vanadate/vanadyl interactions with thiols in aqueous media have been mainly studied using EPR (electron paramagnetic resonance) spectrometry or NMR (nuclear magnetic resonance) techniques in combination with spectrophotometric measurements [2,8,22]. The possibility of using ion chromatography in combination with an anion exchange column to study vanadate/thiol interactions in aqueous solution as a cost-effective alternative to the EPR/NMR technique was investigated. Anion exchange chromatography is used when the molecule(s) of interest is negatively charged. Although the available studies on the structure of vanadate and vanadyl complexes with thiols suggest the formation of negatively charged compounds, the possibility of using EDTA as a chelating agent that efficiently traps the formed vanadyl and the remaining vanadate (if reduction occurs) in the present vanadate and thiol system (3-mercaptopropionic acid) was examined. In the study by Macara et al. [9] EDTA was effectively used to scavenge vanadyl formed after vanadate was reduced by glutathione at a solution pH of 7.5. In order to understand the inhibitory role of vanadate on the enzymatic activity of (Na + K)-ATPase, a detailed study of the reduction mechanism was carried out in vivo using human erythrocytes as model tissue. These experiments were complicated by the rapid air oxidation of vanadyl at the studied solution pH. Macara et al. [9] also reported that at this pH, much of the vanadyl is present as vanadyl hydroxide, which has no detectable EPR signal [9]. However, when 0,05 mol dm^{-3} EDTA was added to the vanadate-glutathione reaction mixture to scavenge any vanadyl ions formed, an EPR signal characteristic for the vanadium (IV)-EDTA complex was observed [6].

Fig. 1 shows time-resolved chromatographic curves after addition of 0.01 mol dm^{-3} EDTA to the solution. The reaction solution (pH = 4.2) contained 0.009 mol dm^{-3} of vanadate and 0.02 mol dm^{-3} of 3-mercaptopropionic acid, respectively. In order to study the course of the ligand exchange reaction (EDTA versus 3-mercaptopropionic acid) subsamples were taken 30 min and 120 min after mixing vanadate and 3-mercaptopropionic acid, and the changes in chromatographic peaks intensities over time were observed. The changes of signal intensities of V(+V)-EDTA and V(+IV)-EDTA complexes, separated using an anion exchange column, were highest within the first hour after EDTA addition. Afterward, a slow progress of the reaction is observed, while the exchange completion is reached within a few hours. Taking into account the high stability constants of the V(+V)-EDTA and V(+IV)-EDTA complexes and the high excess (almost 10-fold) of EDTA over 3-mercaptopropionic acid, it is evident that EDTA is slowly outcompeting 3-mercaptopropionic acid as a chelating agent for the vanadate and vanadyl species present in the investigated system [27]. Considering the high standard

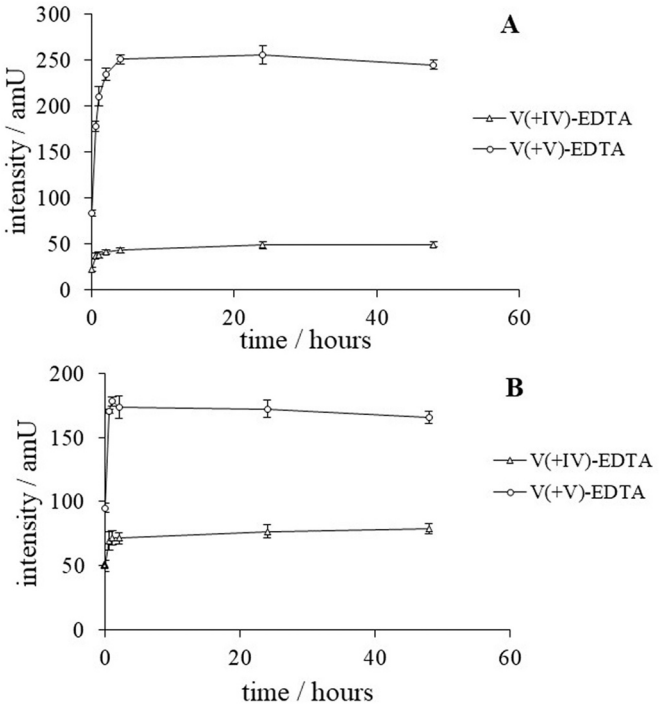

Fig. 1. Time-resolved chromatographic determination of the vanadium oxidation states (+V and + IV) in subsamples of the reaction solution. The subsamples for chromatographic analysis were taken after A) 30 min and B) 120 min of reaction. 0.01 mol dm^{-3} EDTA was present in the subsamples and the course of the ligand exchange reaction (EDTA against 3-mercaptopropionic acid) was observed for 48 h.

redox potential of the $V^{V}O_2(edta)]^{3-}/[V^{IV}O(edta)]^{2-}$ couple [28], we also investigated the possibility of 3-mercaptopropionic acid oxidation in the $V^{V}O_2(EDTA)]^{3-}/[V^{IV}O(EDTA)]^{2-}$ system where the reverse experiment is performed (Supplementary material, Fig. 1). The 3-mercaptopropionic acid was added to the solution after the formation of the vanadate complex with EDTA. As it is shown, no changes in the reaction system were observed during the 48 h, confirming the stability of V(+V) EDTA under present experimental conditions (10-fold excess of EDTA over the 3-mercaptopropionic acid).".

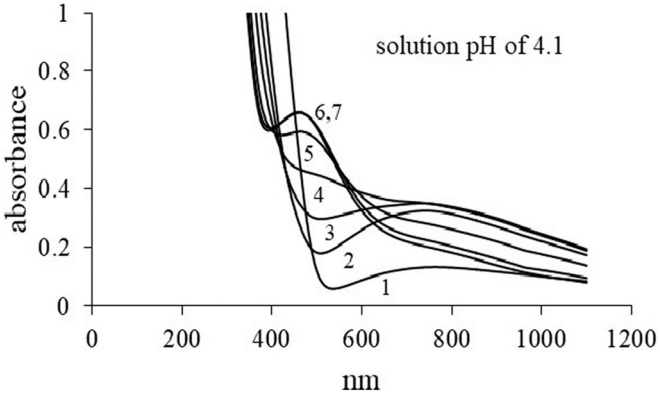

Fig. 2. Time dependent absorbance spectra (190–1100 nm wavelenght range) during the vanadate (0.009 mol dm^{-3}) reduction by 3–mercaptopropionic acid (0.02 mol dm^{-3}): 1) 2 min; 2) 20 min; 3) 30 min, 4) 40 min; 5) 60 min, 6) 90 min and 7) 120 min after mixing the reactants.

3.2. Vanadate (+V) reduction by 3–mercaptopropionic acid – Reconciling the spectrophotometric and chromatographic measurements

Fig. 2 shows the time-dependent recordings of the spectra (wavelength range 190–1100 nm) of the sample containing vanadate and 3-mercaptopropionic acid at a solution pH of 4.1. The spectrophotometric curves recorded within the first 30 min of the reaction course are characterized by a pronounced absorption in the range from 650 to 800 nm (with an absorption maximum at approximately 750 nm). The absorption in this wavelength range increases during the first 30 min of the reaction course, while thereafter a decrease in absorption in this wavelength range is observed with a simultaneous increase in absorption in the range from 420 to 550 nm. According to the experimental observations during vanadate reduction by cysteine, it can be assumed that the first step in vanadate reduction is the formation of an intermediate compound consisting of vanadate and thiol in a molar ratio of 1:1 (i.e. a vanadate thioester). This complex exhibits absorption maxima at 750 nm [8]. Interestingly, Legrum also proposed formation of purple cis/trans vanadyl complex with the cysteine after the decomposition of vanadate–thioester [8]. The determination of the oxidation states of (+V) versus (+IV) vanadium in the investigated reaction solution by chromatographic measurements, accompanied with the approach described earlier (Materials and Methods section), confirms mechanism proposed by Legrum. [8].

Time-dependent chromatographic curves with resolved vanadate and vanadyl peaks are shown in Fig. 3. The subsamples for the chromatographic determination of the vanadate (+V) and vanadyl (+IV) in the reaction solutions were taken in the same time frame as the subsamples for the spectrophotometric measurements (Fig. 2). In the subsamples, the observed vanadate peak intensity decreases which is accompanied by an increase in the vanadyl peak, confirming the vanadate reduction in test solutions. The combination of the EPR determinations of the vanadyl species with the spectrophotometric measurements of vanadate and cysteine-containing solution are in good agreement with the observations presented here [8]. As already mentioned, in investigated vanadate and cysteine solutions, the absorbance peak (with the maximum absorbance at 750 nm) followed by formation and decomposition of an intermediate compound (vanadate–thioester) is observed [8].

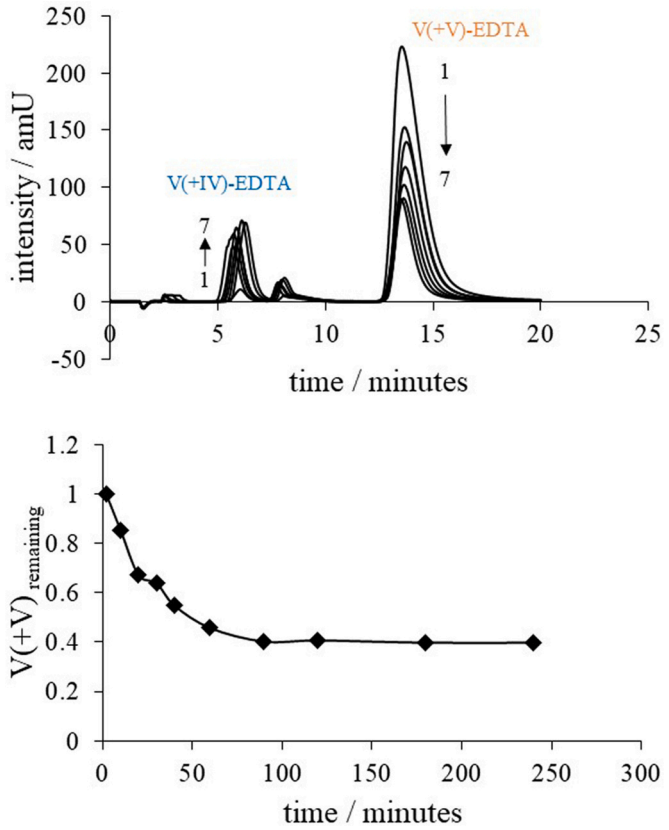

Fig. 3. A) Time dependent vanadate and vanadyl peak intensities determined using ion chromatography. Solution reactants concentration and solution pH are same as on the Fig. 2: 1) 2 min; 2) 20 min; 3) 30 min, 4) 40 min; 5) 60 min, 6) 90 min and 7) 120 min after mixing the reactants. B) Time dependent vanadate peak intensity decrease, determined using ionic chromatography. V $(+V)_{remaining}$ is defined as the ratio of initial intensity of vanadate chromatographic signal at the $t = 0$ versus intensity of vanadate chromatographic signal at the $t = 2$, 20, 30, 40 60, 90 and 120 min of reaction course.

The formation and decomposition of an intermediate compound was accompanied by the increase in the concentration of vanadyl determined by the EPR measurements [8]. A similar conclusion can be drawn from the combination of spectrophotometric and chromatographic measurements in this study (Figs. 2 and 3). The decrease of absorbance in the 650–800 nm range is followed by an increase of the intensity of the vanadyl signal as determined by chromatographic measurements (Figs. 2 and 3). After approximately 100 min of reaction time, equilibrium is reached, which is visible in the chromatographic and spectrophotometric curves. It can be assumed that the observed absorbance increase in the range of 420–550 nm belongs to the postulated cis/trans vanadyl-thiol complex [8].

3.3. pH dependant vanadate (+V)–thioester formation and vanadate (+V) reduction

The formation of chromate (+VI)–thioester during the reaction of chromate with organic and inorganic acids, cysteine, penicillamine, gluthatione, cysteamine, thiourea, hydrogen thiocyanate and hydrogen thiosulfate under acidic conditions was observed [29,30–34] The chromate thioester is formed by the ligand exchange reaction [34]. It has been shown that the ligand substitution reactions of chromate with thiols involve attack of the protonated thiol on either chromate or hydrogen chromate with proton transfer as the rate-determining step [34]. Loss of water and formation of the chromate thioester at higher pH (> 3) is facilitated by proton transfer from a bonded carboxylic acid group to hydrogen chromate. In the absence of a proton donor (oksonium ion - H_30^+, carboxylic group -COOH, ammonium ion - NH_4^+), the reaction proceeds very slowly because hydroxide is the leaving group [34]. Fig. 4 shows the time-resolved spectrophotometric curves recorded after 120 min of reaction progress in solutions with different pH and the same initial concentrations of vanadate and 3-mercaptopropionic acid (0.0009 and 0.002 mol dm^{-3}, respectively). The spectrophotometric curves recorded at a solution pH below 5.0 are characterized by an increased absorbance in the range of 420–550 nm, indicating a pronounced and almost complete vanadate reduction and the formation of a vanadyl-thiol complex. At a solution pH above 5.0, the formation of an intermediate compound (vanadate-thioester) is observed after 120 min, indicating slower reaction kinetics. The observed changes in the absorption properties of the investigated solutions are also accompanied by the changes in the vanadate and vanadyl concentrations determined by the chromatographic method already described (see Fig. 5). The observed pH dependence of the vanadate reduction and the transient vanadate thioester formation can be attributed to the solution properties of the thiol (3-mercaptopropionic acid) as well as the vanadate. The same behavior has already been observed in reaction solutions containing chromate and thiols [28–34]. Further proposed mechanism is simplified due to the more complex speciation of the vanadate in solution, as it will be explained. Above a solution pH of approximately 4.5, the carboxylic group of 3-mercaptopropionic acid is dissociated (dissociation constant - pKa = 4.57) and prevents protonation of the vanadate hydroxide group via the ligand exchange mechanism [34]. At a solution pH below 5, the first step, formation of the vanadate thioester, is thought

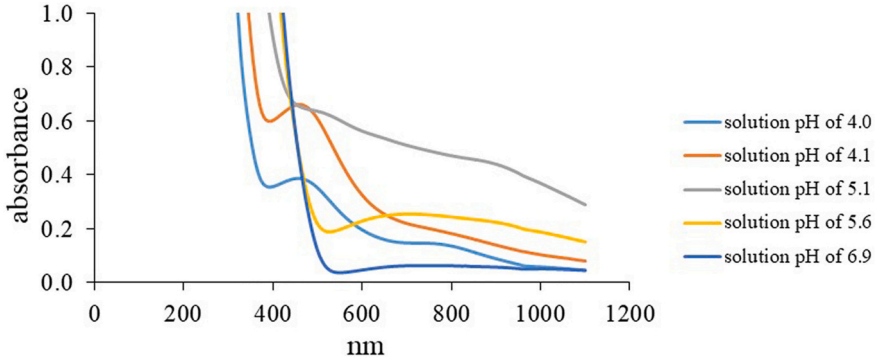

Fig. 4. Time resolved (after the 120 min of reaction course) spectrophotometric curves (190–1100 nm wavelenght range) recorded at the different solution pH. Reactant concentrations are labeled on the Fig. 2.

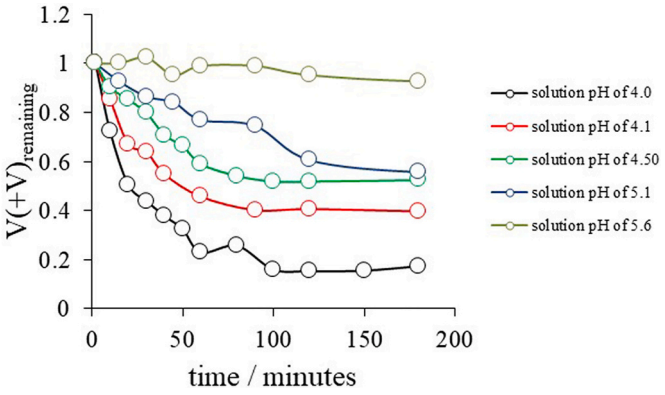

Fig. 5. Time resolved vanadate peak intensity decrease determined by ionic chromatography (for method details on chromatographic separation of vanadium (+V) and (+IV) see Material and methods section). Reactant concentrations are labeled on the Fig. 2.

to be facilitated by proton transfer from the non-dissociated carboxylic group of 3-mercaptopropionic acid to the vanadate hydroxide group. However, at the solution pH, distribution of investigated vanadate species is more complicated than that of chromate. Specifically, in the investigated vanadate concentration range, solution speciation is dominated by the decavanadate ($HV_{10}O_{28}^{5-}$, Fig. 6) when the solution pH ranged from 3.5 to 4.5 while at the solution pH of 5.0 the monomeric hydrolyzed vanadate ($H_2VO_4^-$, Fig. 6) predominates with the significant shares of vanadate tetramer ($V_4O_{12}^{4}$, Fig. 6)$^-$ and vanadate dimer ($H_2V_2O_7^{2-}$, Fig. 6). At the solution pH <3.5 the decavanadate

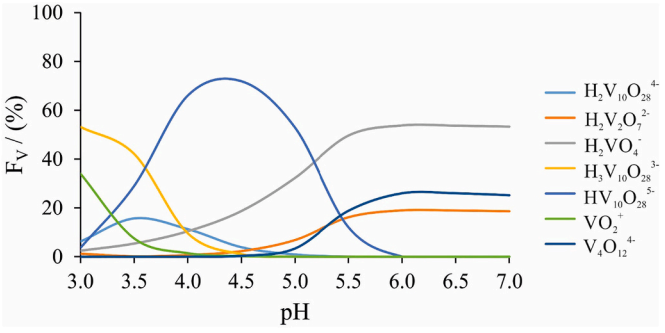

Fig. 6. Vanadate solution speciation at different solution pH is performed using MINTEQ program. Input vanadate concentration is 0.001 mol dm^{-3} (I = 0.1 mol dm^{-3}). The solution vanadate species contributing only with more than 10% of the total vanadate concentration present are depicted. The constants used are given in Cruywagen, 1999. [35].

($H_3V_{10}O_{28}^{3-}$, Fig. 6) and non-hydrolyzed vanadate (VO_2^+, Fig. 6) are dominating species in the solution.

3.4. Kinetic considerations

The interpretation of the kinetic data and derivation of equilibrium constants require detailed knowledge of the reactant species as well as of the reaction products. The concentration of vanadate in the current study was such that the predominant reactant solution species can be resolved (as shown on the Fig. 6) throughout the used acidity range. For 3-mercaptopropionic acid, values of dissociation constant (pKa) corresponding to the loss of a proton from thiol group (− SH) and carboxylic group (− COOH) are >10 and > 4, and 20, respectively [34]. The reaction products at the equilibrium (Figs. 3A, B and 5) are most probably an intermediate vanadate–thioester as well as vanadyl complex with 3–mercaptopropionic acid. Available studies are implying 1:1 and 1:2 stoichiometries in the case of vanadate and vanadyl complexes with the cysteine and glutathione, respectively [8]. Possible complexity of investigated system is pointed out in the study by Crans et al. where additional thiol-containing vanadate and vanadyl complexes of different stoichiometry are observed [2]. The definition of reaction products only on the basis of solution absorbance measurements presented in this study is extremely challenging although the definition of reactant species in currently investigated system is possible. Therefore, the study case was simplified. Considering the simplest possible case, which is the vanadate reduction at the solution pH of <2, the valid reaction expression can be written as follows:

$$2VO_2^+ + 2RSH + 2H^+ = 2VO^{2+} + RSSR + 2H_2O \quad (1)$$

where RSH represents organosulfur compound and RSSR is organic disulfide.

Assuming that the system is buffered (i.e. that the pH remains the same during the course of the reaction), the full set of ordinary differential equations (rate equation) can be stated as follows:

$$\frac{dc_{VO_2^+}}{dt} = -2k_f c_{VO_2^+}^2 c_{RSH}^2 c_{H^+}^2 + 2k_b c_{VO^{2+}}^2 c_{RSSR}$$

$$\frac{dc_{RSH}}{dt} = -2k_f c_{VO_2^+}^2 c_{RSH}^2 c_{H^+}^2 + 2k_b c_{VO^{2+}}^2 c_{RSSR}$$

$$\frac{dc_{VO^{2+}}}{dt} = 2k_f c_{VO_2^+}^2 c_{RSH}^2 c_{H^+}^2 - 2k_b c_{VO^{2+}}^2 c_{RSSR}$$

$$\frac{dc_{RSSR}}{dt} = 2k_f c_{VO_2^+}^2 c_{RSH}^2 c_{H^+}^2 - 2k_b c_{VO^{2+}}^2 c_{RSSR}$$

where k_f represents rate constant for the forward reaction and k_b is the rate constant for backward reaction.

For the numerical solution of this system of ordinary differential

equations (ODEs), a mathematical code was developed by assuming the constant H^+ concentration throughout the experiment and initial disulfide (RSSR) concentration of zero (the description of ODEs system is given in Supplementary material). Concentrations of VO_2^+ and RSH at the beginning of reaction are known from the experimental setting. The relative concentration of VO_2^+ was monitored by ion-chromatography (IC) and direct proportionality between signal height and concentration was assumed. Thus, if IC-measured intensity was extrapolated to $t = 0$, the IC-measured data can be converted to concentrations.

Fig. 7 shows fits of acceptable quality at different levels of pH. Rate coefficients for forward reaction (k_f) and backward reaction (k_b); and the equilibrium constant ($K = k_f/k_b$) are shown in Table 1.

The experimental data can only be modeled if k_f and k_b change when the pH of the solution is increased, implying a more complicated and pH-dependent reaction mechanism. The observation of the decrease in vanadate concentration as a function of solution pH (Fig. 8) provides further insight into the reaction kinetics. The observed nonlinearity between the decrease in vanadate concentration and solution pH is consistent with the estimated rate coefficients presented in Table 1, suggesting nonlinear, pH-dependent reaction kinetics. Connet and Wetterhahn had already shown that the reaction rates of ligand substitution for chromate systems with various organic reagents such as cysteamine, cysteine, cysteine-ethyl ester, homocysteine, 3-mercaptopropionic acid, *N*-acetylcysteine and thioglycolic acid [34] are pH dependent. More specifically, constant rate analysis revealed a complex reaction scheme in which the measured second-order rate constant showed pH-dependent behavior. In the pH range of 2–10, the reaction of chromate with 3-mercaptopropionic acid could be described by the reaction of chromate and thiocarboxylic compounds having one, two, or three of the three possible groups (oxo, sulfhydryl and carboxylate) protonated [34]. In the case of vanadate, the situation could be even more complicated due to the complex speciation of the vanadate in solution (Fig. 6). Significantly different reaction kinetics in the pH range of 4–5 (Fig. 8 and Table 1) can be assigned to the changes of the solution speciation of both reactants, i.e. predominance of the decavanadate ($HV_{10}O_{28}^{5-}$) and deprotonation of carboxylic group of 3-mercaptopropionic acid.

Table 1
Estimated rate coefficients and equilibrium constants.

pH	$k_f/dm^{15}\ mol^{-5}\ s^{-1}$	$k_b/10^9\ dm^6\ mol^{-2}\ s^{-1}$	$K/10^9\ dm^6\ mol^{-3}$
4.06	11.8	0.502	23.5
4.11	7.43	4.19	1.77
4.50	24.7	5.65	4.37
5.10	171.9	4.56	37.7
5.64	173.4	24.0	7.22

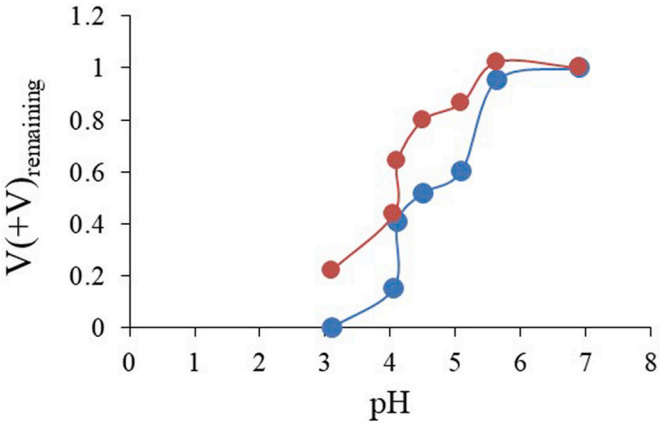

Fig. 8. Vanadate (+V) reduction percentage versus pH. Red circles: after 30 min of reaction course. Blue circles: after 120 min of reaction course. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

The reduction of vanadate (+V) in the presence of 3-mercaptopropionic acid was studied by spectrophotometry and ion chromatography. The chromatographic assay provided information on the redox speciation of vanadium (+V versus +IV) in the test solutions. The

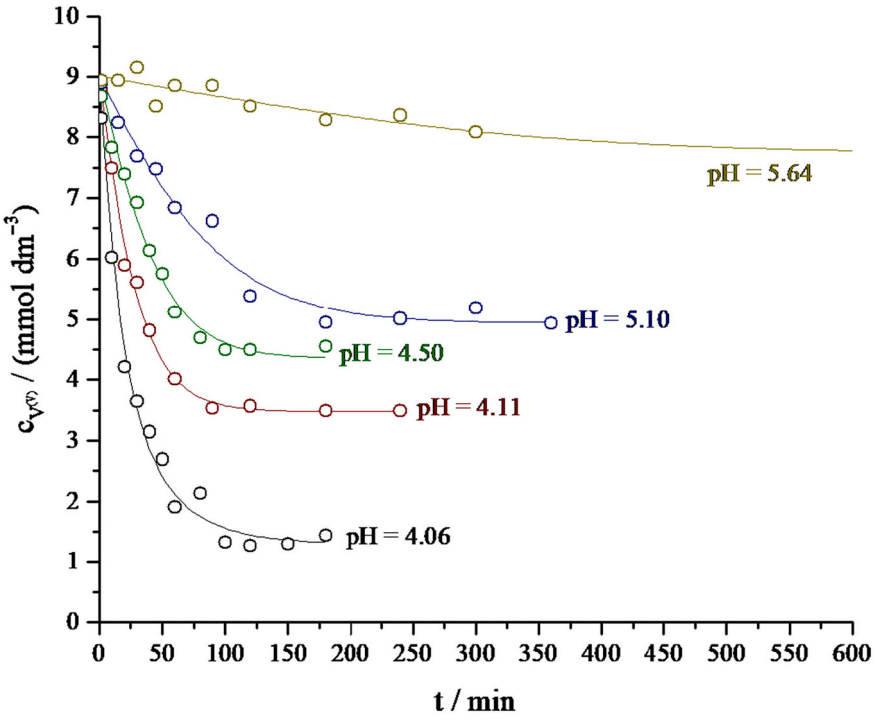

Fig. 7. Kinetic curves (cVO_2+ versus t data) fitted by the model.

separation of vanadate (+V) and vanadyl (+IV) was carried out successfully using existing chromatographic equipment with an anion exchange column. In the reaction mixture, originally containing only vanadate (+V) and 3-mercaptopropionic acid, EDTA was added in excessive amount in relation to the original solution reactants. A ligand exchange reaction occurred between the added EDTA and the 3-mercaptopropionic acid which terminated the course of the reduction/complexation reaction. Formed V(+V)-EDTA and V(+IV)-EDTA are successfully separated using an anion exchange column. The coupling of the spectrometry with the chromatographic analyses proved to be advantageous, allowing a profound insight into the course of the reaction. In particular, a pH-dependent vanadate (+V)-thioester formation was observed. The overall reduction reaction also shows a strongly pH-dependent behavior. It is suggested that the formation of vanadate (+V)-thioester is the rate-determining step. The product of the reduction reaction, i.e. vanadyl (+IV), enters into a complexation reaction with unreacted 3-mercaptopropionic acid. The presence of complexes of vanadate (+V) and vanadyl (+IV) with 3-mercaptopropionic acid is confirmed by spectrophotometric measurements. Vanadate (+V)-thioester shows absorption maxima at about 750 nm, while vanadyl (+IV) complex with 3-mercaptopropionic acid shows absorption maxima in the wavelength range from 420 to 550 nm. The reduction rates showed a pH-dependent behavior, indicating complex reaction kinetics depending on both: the complex speciation of vanadate (+V) in the solution and the changes in the ionic form of 3-mercaptopropionic acid.

CRediT authorship contribution statement

Elvira Bura-Nakić: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – original draft. **Lucija Knežević:** Formal analysis. **Jelena Mandić:** Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinorgbio.2022.111747.

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5. DISCUSSION

5.1. Vanadium(+IV) and V(+V) complexation by succinic acid studied by affinity capillary electrophoresis

Obtained results in the research paper I. indicate V(+IV) stabilisation in oxic water column of Krka River estuary, despite unfavourable thermodynamic predictions. This hypothesis is supported with other studies performed in oxic natural waters, where a non-negligible portion of V(+IV) in the total V concentrations is often encountered (Wang and Sañudo-Wilhelmy, 2008; Wang and Sañudo Wilhelmy, 2009; Shi, Mangal and Guéguen, 2016). Among a variety of possible geochemical processes, complex formation with natural organic matter (NOM) present in the aqueous phase may play an important role in the distribution of V redox species since formation of such complexes could potentially cause significant changes in their migration properties in the environment (Larsson M., Baken S., Gustafsson J.P., Hadialhejazi G., 2013). However, lack of experimental data on complexation mechanism of V(+IV) and V(+V) species with natural occurring ligands still prevents from obtaining complete biogeochemical pathway of V in natural aqueous systems (Linnik and Linnik, 2018; Gustafsson, 2019). Therefore, it was decided to experimentally study complexation of V(+IV) and V(+V) species with simple organic ligand (succinic acid) in model aqueous solutions. Previous studies indicate that V species seem to bond to NOM through oxygen atoms with the affinity towards binding sites of salicylate type (Mangrich and Vugman, 1988). Thus, succinic acid was chosen as a simple model for structurally more complex NOM (Shelke and Jahagirdar, 1977; V. Sladkov *et al.*, 2018). Additional motive for choosing succinic acid as a complexing ligand, is a significant lack of experimental data on complex equilibria with V species (Shelke and Jahagirdar, 1977; Narasimhulu and Seshaiah, 1980; Sakurai *et al.*, 1980; Pessoa *et al.*, 1998). Only a few papers dealing with stability constants for V(+IV)-succinic acid system, while for V(+V) complexes there is no reports on thermodynamic constants so far (Shelke and Jahagirdar, 1977; Narasimhulu and Seshaiah, 1980; Pessoa *et al.*, 1998). For the purpose of investigation of complex stability between V(+IV) and V(+V) species with succinic acid, affinity capillary electrophoresis was chosen as suitable analytical instrumentation (Sladkov, 2010, 2013). All the information on the used materials and methods are explained in detail in Appendix II. Stated research has been published recently (Knežević *et al.*, 2023).

5.1.1. Electrophoretic mobility of V(+IV) and V(+V) species in the presence of succinic acid

Electropherograms obtained by measurement of sample which contained analyte species, injected either simultaneously or separately, in the presence of different concentrations of succinic acid in BGE at pH=2 at an ionic strength 0.1 mol L^{-1} ($HClO_4$-$NaClO_4$) are presented in Figure 6.

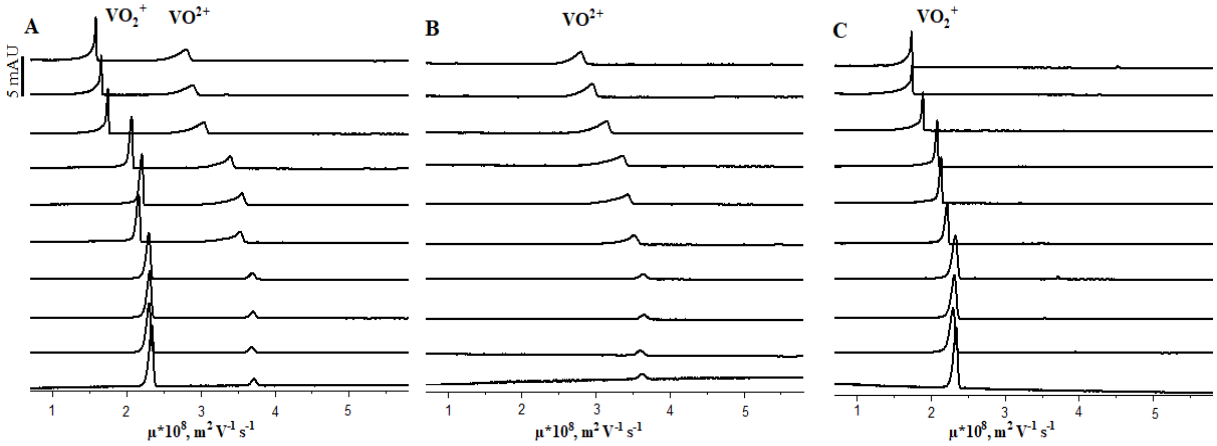

Figure 6. Electropherograms of 5×10^{-4} M V(+IV) and $1x10^{-3}$ M V(+V), in the presence of different amounts of succinic acid in BGE. Ligand concentrations in BGE from bottom to the top: 0, 1×10^{-3}, 6×10^{-3}, 8×10^{-3}, 0.04, 0.08, 0.1, 0.2, 0.3 and 0.4 M. A – simultaneous injection of two analytes. B – injection only V(+IV). C – injection only V(+IV). pH=2, I=0.1 M ($NaClO_4$ – $HClO_4$). Detection wavelength: 200 nm, T=25°C. Other conditions are listed in the Appendix II.

The charge differences between V(+IV) (present as VO^{2+}) and V (+V) (present as VO_2^+) in aqueous solution on used experimental conditions enables easy separation and satisfactory selectivity of analytical system with respect to V species (Larsson M., Baken S., Gustafsson J.P., Hadialhejazi G., 2013). Obtained electropherograms show decrease of mobility with the increase of succinic acid concentration in BGE. This indicates that we deal with kinetically labile complexes (Krylov, 2007; Sladkov, 2016). The equilibrium is sufficiently fast, and the time required for equilibrium is negligible compared with the separation time. Vanadium(+IV) mobility is larger (migration time is lower) than for V(+V). The observed mobility corresponds to the sum of mobilities of coexisting charged species formed with the ligand under study as expressed in the Equation 1 (V. Sladkov *et al.*, 2018) :

$$\mu_{\text{obs}} = \sum_i \alpha_i \, \mu_i \qquad (1)$$

In Eq.1, μ_i stands for the intrinsic electrophoretic mobility and α_i for the molar fraction of individual species (i) present in the migration band. The decrease of the global charge of the species when increasing the concentration of succinic acid (migration time increases), suggests the formation of a less charged complex species with increasing of succinic acid concentration.

5.1.2. Stoichiometry of formed complex species

Prior to calculation of stability constants of formed complexed between the V species and succinic acid, it is crucial to evaluate stoichiometry of the newly formed compound. Vanadium(+IV) and (+V) species may from protonated or deprotonated complexes with succinic acid. Generally, formation of protonated complexes of transition metals and succinic acid are known to form in aqueous solution (Morphy *et al.*, 1990; V. Sladkov *et al.*, 2018). However, apart from the study by Costa Pessoa *et al.* (1998), mostly deprotonated V(+IV)-succinic acid complexes have been reported in the earlier studies (Shelke and Jahagirdar, 1977; Narasimhulu and Seshaiah, 1980; Pessoa *et al.*, 1998). Since ionisation characteristics of a certain complex affect its net charge, by using ACE it is possible to discriminate between protonated or deprotonated species of formed complexes. Generally, the identification of the interacting species of used ligand requires the variation of possible interacting species with determined mobility values of metal species (Sladkov, Fourest and Mercier, 2009; Sladkov, 2010). As all succinic species are present in the dynamic equilibria at used conditions, it is necessary to vary the concentration of all succinic species present in the aqueous solution (equations 2,3 and 4). Generally, succinic acid protonation equilibria in aqueous solution are (Reuben and Fiat, 1967).

$H_2L \leftrightarrows HL^- + H^+$, pK_1=4.00 (25°C, I=0.1 M) (2)

$HL^- \leftrightarrows L^{2-} + H^+$, pK_2=5.24 (25°C, I=0.1 M) (3)

Thus, the total succinic acid concentration is:

$C(L) = [H_2L] + [HL^-] + [L^{2-}]$ (4),

where C(L) is the total succinic acid concentration, and $[H_2L]$, $[HL^-]$ and $[L^{2-}]$ the concentrations of the different forms of succinic acid at equilibrium. Therefore, V(+IV) and V(+V) mobility values, measured at different pH values were plotted as a function of the total succinic acid, $[H_2L]$,

[HL^-] and [L^{2-}] (Figure 7). Correlation of the curves observed on Figure 7C, indicate that dominantly [HL^-] species affect mobility of both V(+IV) and V(+V). However, slightly decreased mobility is observed at pH=2.4 for V(+V) species. Namely, 15% of V(+V) species at used conditions hydrolyses which could generate formation of less charged species (Appendix II). However, the contribution of hydrolysed species is considered low, which indicates that the formation of mainly protonated complexes of V(+IV) and V(+V) with succinic ligand is occurring in the aqueous solution on used experimental conditions.

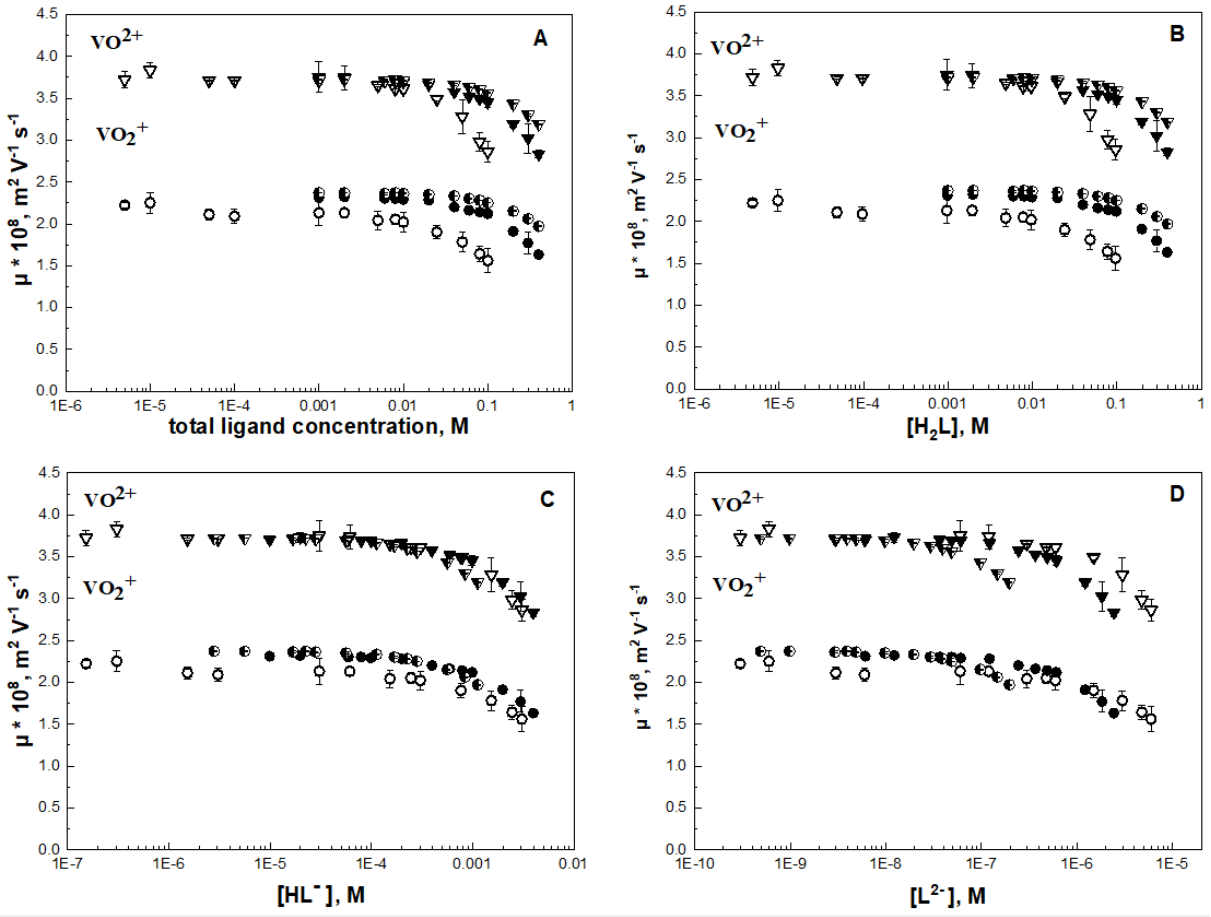

Figure 7. Mobilities of V(+IV) (triangles) and V(+V) (circles) at pH=1.5 (half-full markers) pH=2 (full markers) pH=2.5 (empty markers) as a function of total ligand concentration, [H_2L], [HL^-] and [L^{2-}]. Simultaneous injection of two analytes. All other conditions listed as in the Figure 6.

5.1.3. Chemical equilibria and stability

The equilibria of the V species with succinate ligand (denoted as L^{2-}) can be expressed as:

V(+IV)

$$VO^{2+} + nL^{2-} + mH^{+} \leftrightarrows VOL_nH_m^{2-2n+m} \quad (5)$$

with the corresponding stability constant β_{1nm}

$$\beta_{1nm} = \frac{[VOL_nH_m^{2-2n+m}]}{[VO^{2+}][L^{2-}]^n[H^+]^m} \quad (6)$$

V(+V)

$$VO_2^+ + nL^{2-} + mH^+ \leftrightarrows VO_2L_nH_m^{1-2n+m} \quad (7)$$

with β_{1nm}

$$\beta_{1nm} = \frac{[VO_2L_nH_m^{1-2n+m}]}{[VO_2^+][L^{2-}]^n[H^+]^m} \quad (8)$$

Equilibrium constants values can be obtained by fitting experimental mobilities (Tables A6-A8) using general equation (6). Considering only the equilibria with $[HL^-]$ species, characterized as the main interacting ligand specie, different models were explored: formation of one 1:1:1 complex, simultaneous formation of two complexes (1:1:1 and 1:2:2), formation of one 1:2:2 complex, simultaneous formation of two complexes (1:1:1 and 1:2:1). A non-linear fitting (NNLS) method is chosen over a linear regression method (Bowser and Chen, 1999; Šolínová *et al.*, 2016). In this case, the molar fraction of species to be introduced in Equation (1), which can be written as

V(+IV)

$$\alpha_{1nm} = \frac{[VOH_nL_m^{2+n+2m}]}{C(V(IV))} = \frac{\beta_{1nm}[VO^{2+}][H^+]^n[L^-]^m}{[VO^{2+}] + \Sigma m\beta_{1nm}[VO^{2+}][H^+]^n[L^-]^m} \quad (9)$$

V(+V)

$$\alpha_{1nm} = \frac{[VO_2H_nL_m^{1+n+2m}]}{C(V(V))} = \frac{\beta_{1nm}[VO_2^+][H^+]^n[L^-]^m}{[VO_2^+] + \Sigma m\beta_{1nm}[VO_2^+][H^+]^n[L^-]^m} \quad (10)$$

We note that the product $\alpha_{111}\mu_{111}$ (eq. 3) is well defined but each individual term cannot be determined precisely in used mathematical equation. In this case, it was necessary to make a reasonable guess of the μ_{111} value and keep it constant in the modelling of experimental data. This approach was successfully applied in similar case (Vladimir Sladkov *et al.*, 2018). Obtained stability constants were extrapolated to zero ionic strength with the use the Davies equation (Equation 11) (G. Adamson, 1993). The Davies equation for the activity coefficient (γ) of an ion i of charge z_i is, at 25°C:

$$\log 10\gamma_i = -0.5102\ z_i 2(I_m^{1/2}/\ (1 + I_m^{1/2}) - 0.3I_m) \quad (11)$$

The equation works fairly well up to ionic strengths (I_m) of 0.1 mol kg^{-1}. Numerical values of obtained stability constants at different pH values for V(+IV) and V(+V) complexes with succinic acid are given in Table 1, along with calculated average values. In the case of V(+IV), model describing the formation of two complexes (1:1:1) and (1:2:2) gave best fit to the experimental results. Specifically, obtained value for $\log\beta_{111}$ is very close to the one proposed by Costa Pessoa *et al.* (1998) (Pessoa *et al.*, 1998). For the second constant (1:2:2) the error is much larger, since on used experimental conditions the concentration of these species is low. Conversely, in the case of V(+V), only one complex (1:1:1) was found. It should be noted that experimental results obtained by injecting analyte separately and simultaneously were processed, and the obtained values are practically the same which provides further confidentiality in obtained results.

Table 1. *Stability constant values obtained for V(+IV)-succinic acid and V(+V)-succinic acid. I=0.1 M (NaClO4-HClO4), excepting the last line (I=0M). T=298K.*

	V(+IV)				V(+V)	
	$\log\beta_{111}$		$\log\beta_{122}$		$\log\beta_{111}$	
N / pH	1	2	1	2	1	2
2.5	7.2 ± 0.2	7.5 ± 0.2	14.6 ± 0.1	13.7 ± 0.6		
2.0	7.3 ± 0.2	7.4 ± 0.1	14.2 ± 0.2	13.6 ± 0.5	7.3 ± 0.4	7.3 ± 0.4
1.5		7.6 ± 0.1		14.4 ± 0.5		7.4 ± 0.4
Average*	7.3 ± 0.6	7.5 ± 0.2	14 ± 2	14 ± 1	7.3 ± 0.4	7.4 ± 0.6
Average**	7.4 ± 0.2		14.1 ± 0.5		7.3 ± 0.1	
Average (I=0)	8.3 ± 0.2		15.6 ± 0.5		7.9 ± 0.1	

N- number of analytes injected
* only for the same number of analytes injected and different pH values
** for all measurements

5.1.4. Comparison with the Literature data

To fully comprehend obtained stability constants values and compare them with literature data for complexes formed by different metal ions with succinate, a linear free energy relationship (LFER) approach is applied. This approach is commonly used in the literature to compare the affinity of a given ligand for a series of metal cations (M^{z+}). The effect of the negatively charged oxygen-donor group of the ligand on complex stability appears to depend on the acidity of metal ion considered, i.e., the affinity of the metal ion for the negative O-donor ligand, the OH- ion (Hancock and Martell, 1989). Thus, the stability constant of metal ion complexes with a ligand, containing oxygen-donor group, correlates with the formation constant K_{MOH} on a log-log scale, indicating that the stability depends primarily on the Lewis acidity of the metal ion, as it is expected for an electrostatic interaction. Moreover, a similar binding mode can also be inferred from such linear plots.

Figure 8. shows a LFER plot where $\log\beta_{111}$ is plotted as a function of log K_{MOH}, for different metal ions, including the $\log\beta_{111}$ values for V(+IV) and V(+V) measured by ACE in this study. The

stability constant values for other metal ions at 0.1 M ionic strength and 25 °C were taken from the literature (Appendix II, Table A9). A good linear trend is observed for all cations, except for V(+V). The logβ_{111} for V(+V) is lower, than predicted from the LFER correlation. This suggests that V(+V) complexation is not only governed by electrostatic interactions, but that there might be a significant contribution of a steric factor. Indeed, the $VO_2(H2O)_4^+$ displays a very distorted octahedral configuration with a O=V=O bond angle of ca. 105° (Krakowiak, Lundberg and Persson, 2012). This can create difficulties in the electrostatic interaction with the ligand and weaken the complex stability. Most probably, for the same reason, the oxalate complexes of V(+IV) are more stable than for those of V(+V) (Bruyère *et al.*, 2001).

In the results obtained in the research paper I, it was evident that the possibility of soluble organic complexes with V(+IV) species could affect its stabilisation in oxic conditions. Successful description of stability of V(+IV) and V(+V) with succinic acid supports proposed hypothesis on V(+IV) stabilisation with organic ligands in oxic aqueous system. Moreover, study provides a valuable contribution to the general knowledge of V(+IV) and V(+V) chemistry with respect to the simple organic ligands.

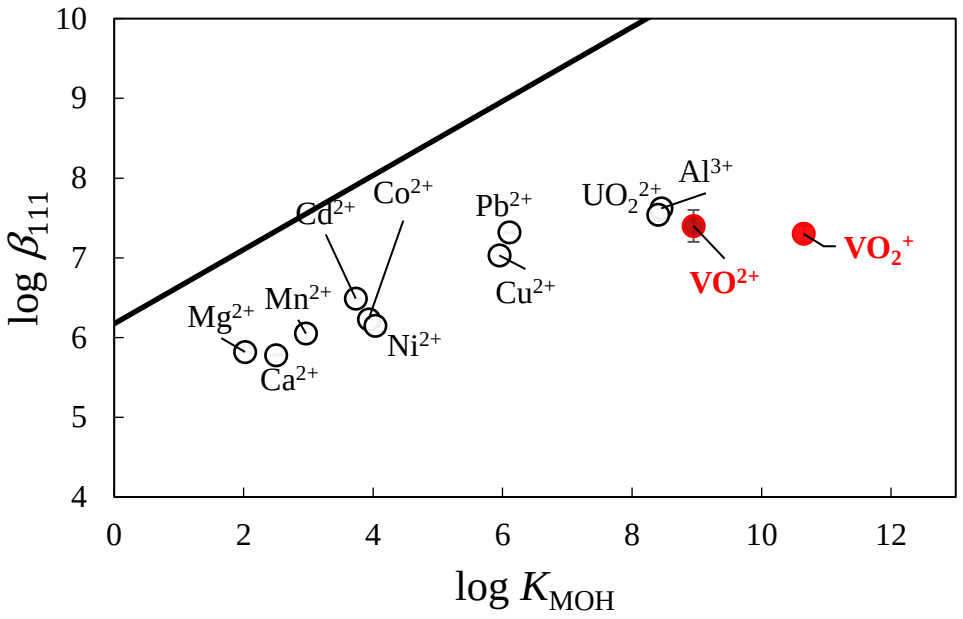

Figure 8. Plot of the stability constants (log β_{111}) of different metal ions with succinic acid as a function of their first metal hydrolysis constants (log K_{MOH}).

5.2. Variability of acid-extractable V(+IV) in the surface sediments of Krka River estuary

Study of V speciation in the acid-extractable phase of Krka River estuarine sediment presented in the research paper II. has been further extended. Developed experimental approach from the research paper II. was applied on surface sediment samples of Krka River estuary at the sampling location shown in Figure 9. Importantly, study now covers surface sediment samples taken from locations under the direct terrigenic input of the main provider of such material for Krka River (Guduča River), and sites where majority of the suspended material is being sedimented (Prokljan Lake). In addition, critical sites under anthropogenic influence are studied as well, regarding modern and former pollutant sources of the Krka River estuary (Former electrode and ferroalloy factory, Šibenik port, and Marina shipyard) (Cukrov, 2021).

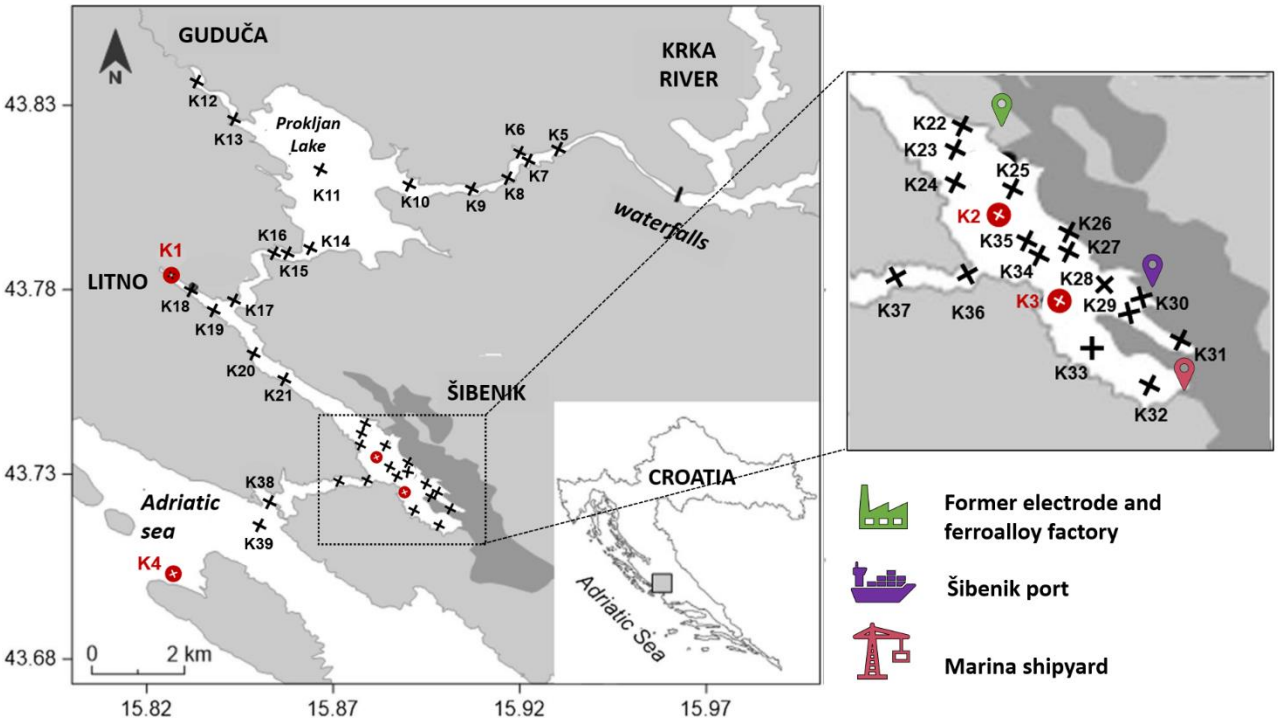

Figure 9. Map of Krka River estuary with indicated study sites and main sources of pollution (K5-K39). Sampled station in the research paper II. are marked red (K1-K4).

As it was the case in research paper II., V_{TOT} concentrations follow Al concentrations pattern in the processed surface sediment samples. Established linearity of V_{TOT} and Al (Figure 10) indicates lithogenic origin of V in the Krka River estuary. This is supported with earlier studies, where V concentrations seem to be influenced by terrigenic components which were eroded from Eocene flysch terrains (Cukrov *et al.*, 2008).

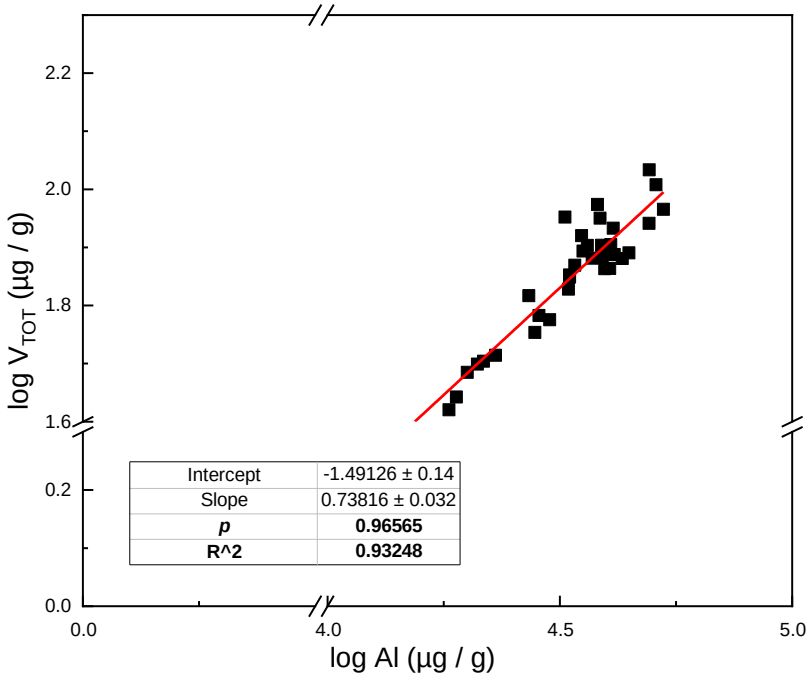

Figure 10. Linearity of V_{tot} and Al concentrations for sampling stations in Krka River estuary with indicated linearity parameters.

In general, normalisation of trace metals to common lithogenic elements (Al, Fe or Li) is used to recognise concentration elevations of TM due to the possible anthropogenic input, and to eliminate possible lithogenic variability (Cukrov, 2021). Normalised concentration are used for the calculation of enrichment factor (EF) of studied TM in sediment samples based on the Equation 12., where *X* is a lithogenic element for normalization; *(Me/X)sample* is the metal/X ratio in the sample of interest; and *(Me/X)background* is the natural background value of the metal/X ratio (Cukrov *et al.*, 2020).

$$EF = \frac{\frac{Me}{X}sample}{\frac{Me}{X}background} \quad (12)$$

Based on the EF values, anthropogenic enrichment of a certain TM can be classified into 5 categories; EF<2, deficiency to low enrichment; EF= 2-5, moderate enrichment; EF=5-20, significant enrichment; EF=20-40, very high enrichment; EF >40, extremely high enrichment (Sutherland, 2000). Because of excellent correlation of total V and Al in the surface sediment samples observed in the extended study (Figure 10), Al was chosen for normalisation of V_{TOT} concentrations to geological background. Values for natural background for V and Al were taken from Cukrov *et al.* (2021) (Cukrov, 2021). It should be noted that the use of Al for normalisation of trace metals to geological background in Krka River estuary is often avoided due to the possible localised anthropogenic input of Al in the past (boxite deposits in the catchment area and aluminum industry in Šibenik) (Prohić and Kniewald, 1987; Cukrov *et al.*, 2020). However, it is highly unlikely that anthropogenic Al could be present in the surface estuarine sediment (0-5 cm) due to the characteristic sedimentation rates along Krka River estuary (< 5 mm/year) (Cukrov, Barišić and Juračić, 2007).

Along with established positive correlation of V_{TOT} to Al, V speciation study in the acid-extractable fraction of additional samples, further supports results obtained in the research paper II. Specifically, it was established that V is dominantly present in the form of V(+IV) species in the mobilized phase, as observed from the chromatograms shown on Figure 11.

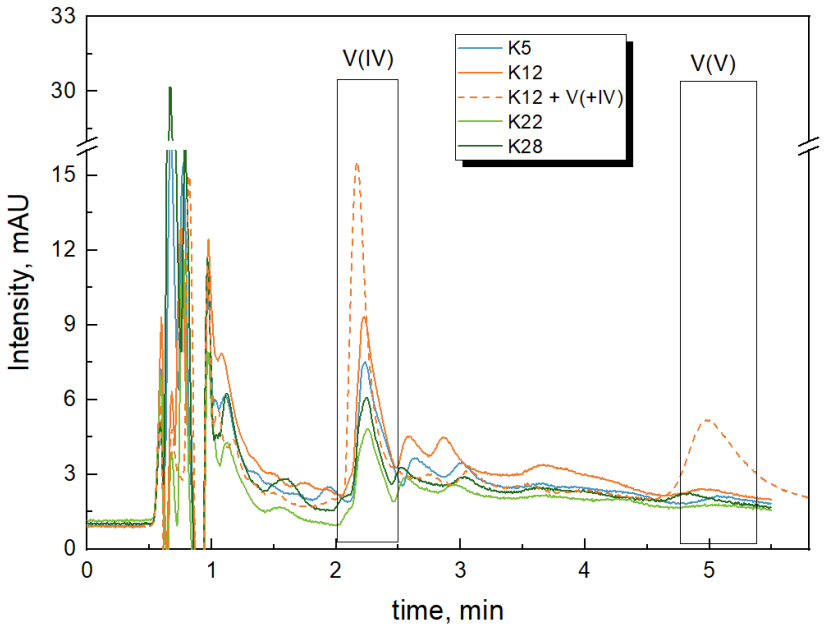

Figure 11. Chromatograms of selected sediment samples: station K5 (blue full line), K12 (orange full line), K12 after standard addition of V(+IV) (orange dashed line), K22 (light green full line), K28 (dark green full line).

Extracted concentrations of V from the mobile fraction with respect to total V concentrations were used to calculate risk assessment code (RAC), defined as the exchangeable/bioavailable metal fraction (BF) in the total V concentration (Equation 13). Values were interpreted as; <1%, no risk to the aquatic environment; 1-10%, low risk; 11-30%, medium risk; 31-50%, high risk; >50%, high risk to the aquatic environment (should be considered dangerous) (Bo *et al.*, 2015; Abdallah, 2017).

$$RAC = \frac{BF}{V_{TOT}} \; x \; 100 \qquad (13)$$

Calculated EF and RAC values for all Krka River estuary surface samples are plotted on the Figure 12. Values determined in the research paper II., are plotted as well to obtain cohesive outlook on the obtained results.

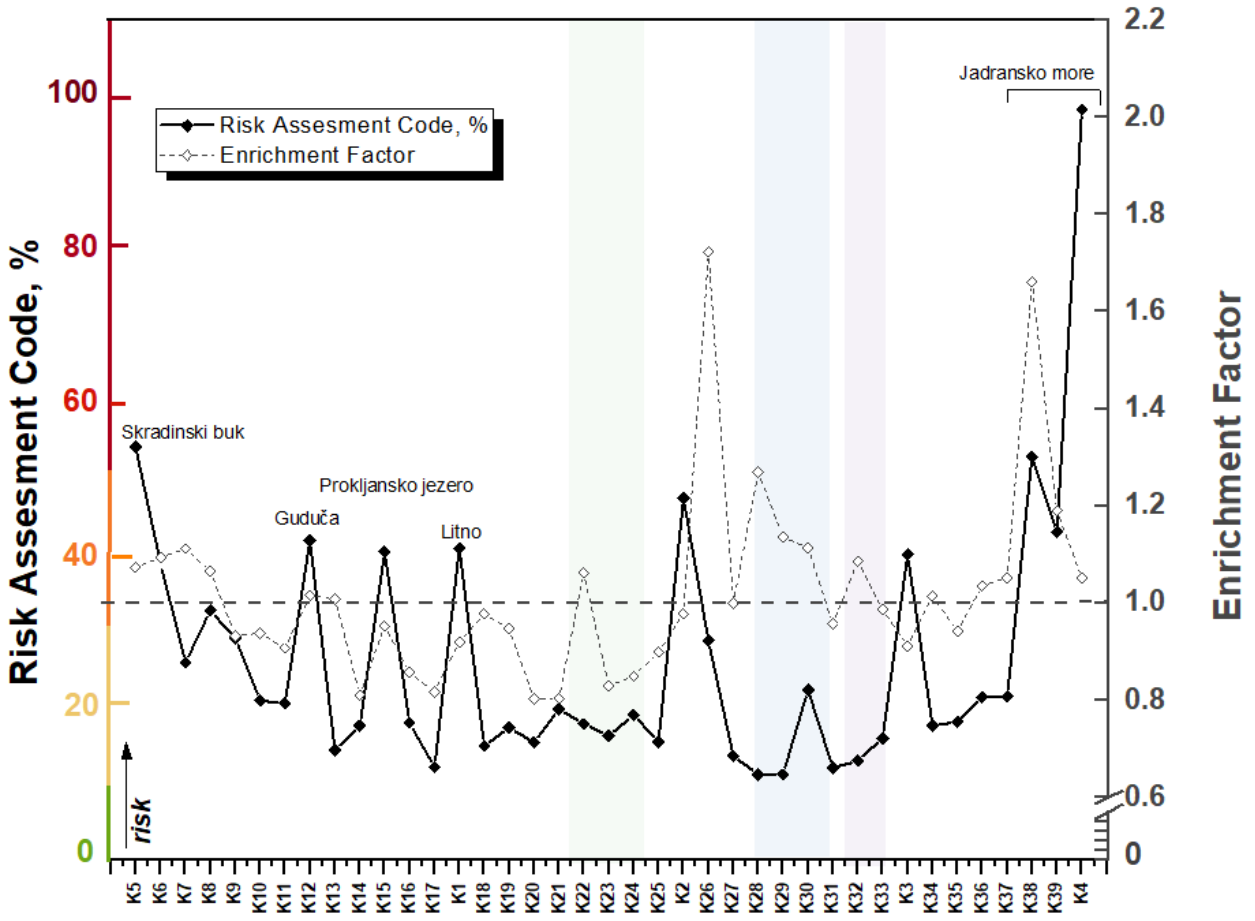

Figure 12. Comparison of measured Enrichment Factor (EF) and Risk Assesment Code (RAC) of V in the extractable phase of surface sediment samples of Krka River estuary. Anthropogenic influences on sediments are highlighted in green (former electrode and ferroalloy factory), violet (Šibenik port) and blue (Marina shipyard).

Generally, the Krka River estuary can be divided on the upper and lower part of the estuary, based on the amount of the retained suspended material and its sedimentation rates. Specifically, in the upper part of the estuary, sediment is a mixture of marine carbonates and terrigenous material (K5-K21) with sedimentation rates ranging from 2 mm/year (the head of the estuary) to 4-5 mm/year (Prokljan lake) (Cukrov, 2021). In the head of the estuary (location K5), slight enrichment of total V is observed, while determined bioavailable V(+IV) concentrations amounted over 50% of total V concentration. By comparing results on V speciation in the water column at the head of the estuary (research paper I., sampling station denoted as "SB"), depletion of oxygen caused up to 26% share of V(+IV) species in the total V content. In addition, removal of V from the bottom water layer was observed also. The water column and surface sediments were both sampled at autumn period, thus we can assume probable hypoxic events occurring in the water column, as visible in research paper I. (Legović, Petricioli and Žutić, 1991). Since V(+IV) species have greater affinity towards organic ligands and particles, they could be more easily removed from the water column which would cause slight enrichment of V observed at these stations. The upper part of the estuary is dominated by clay type material, which is significant binding phase for V (and trace metals in general) and contributes to the mineralogical composition of these sediments (Prohić and Kniewald, 1987). Although V(+IV) is known to easily substitute Al(+III) or Si(+IV) ions in the clay mineral structure, it is more likely that V is associated with the clay minerals through weal electrostatic binding. Thus, outer-sphere complexation with clay minerals most likely affects determined high V mobility at the head of the estuary (Gehring *et al.*, 1993).

Going downstream from the head of the estuary (locations K1; K6 - K21), generally low risk of V remobilisation can be noticed, except for locations K12, K15 and K1. Specifically, these locations are under direct influence of the Guduča River, Prokljan Lake and Litno Spring, respectively. These sites are also characterized by highest concentrations of terrigenous particulate material in the Krka River estuary. Especially, Guduča River catchment area has no tufa barrier and is mainly composed of flysch or flysch-like deposits. Thus, Guduča River is the main supplier of particulate material to the Krka River through weathering process, which is mostly deposited and retained in the Prokljan Lake (Cukrov, 2021). This is further supported with highest sedimentation rates measured in the estuary, amounting up to 4-5 mm/year in Prokljan Lake (Cukrov, Barišić and Juračić, 2007). Juračić (1987) assigns retention of terrigenous material in Prokljan Lake to its sheltered position, low-energy estuarine type of circulation, physico-chemical and biological influence (Juračić,

1987). However, overestimation of measured acid-extractable V on these sampling sites is also possible, due to the operational conditions. Specifically, earlier studies show that majority of organic material in this part of estuary has terrestrial origin, so mild acidic extraction conditions could possibly affect extraction of V bound to fulvic acid compounds (which are known as acid soluble) (Gaffney, Marley and Clark, 1996; Marcinek *et al.*, 2020). However, on rest of the locations in this part of the estuary, extractability of V remained low. Observed V mobility trend in this part of the estuary can potentially reflect irregular input and type of detrital material into the estuary, as observed by Prohić *et al*. (1987) (Prohić and Kniewald, 1987).

The sediment in the lower part of the estuary (K22-K39) is mainly affected by autogenous biogenic sedimentation. It is characterized by coarse carbonate fraction of recent and subrecent biogenic origin, as indicated by presence of aragonite and Mg-calcite. The particles of abrasive origin (grains of limestone and dolomite) are present in lesser extent, while the amount of fine fraction of terrigenous origin is very low (Cukrov, 2021). Since this part of the estuary is characterised with higher carbonate fraction and very low sedimentation rates (1 mm/year), we can assume that acid-extractable V is mainly bound to carbonate fraction as it was stated in the research paper II (K2-K4). Results obtained in the research paper II., are further supported with high mobility of V measured in the additional samples with lowest V total concentrations, located on the exit of Krka River estuary (K38), with lower influence of Krka River plume. Total concentrations of V and Al, as well as acid-extractable V(+IV) concentrations are listed in the Appendix III (Table A10). These locations are also lowest with Al content (and consequently low clay fraction), which points to V binding to carbonate fraction.

On the stations directly under anthropogenic input, slight enrichment in total V concentrations with respect to Al has been observed. Although not-considered polluted with V, there is clearly an input of V in the estuary, consequent to human activities. At the same time, these locations are characterized by lowest concentrations of acid-extractable V, with respect to its total concentration. This implies that anthropogenic burdening of the sediment with V impacts its binding to less mobile phases of the sediment, which is in strong agreement with previous studies (Prohić and Kniewald, 1987; Abdallah, 2017; William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018; Shaheen *et al.*, 2019). This hypothesis is further supported by the results on V redox speciation in the overlaying water column in Vrnaža port (Scientic article 1, sampling station denoted as “VP”). Stabilisation of V(+IV) species in the oxic water column was

noticed and ascribed to the possible organic complexation. Additionally, these species can be more easily removed from the water column due to their higher reactivity and could eventually bind to less mobile phases of sediment (Gustafsson, 2019). Vanadium is known to have especially high affinity towards organic/sulphidic sediment fraction, especially under the anthropogenic input which brings high content of organic matter (Abdallah, 2017). The highest concentration of acid-extractable V on anthropogenically influenced points was present in the Šibenik port (K30-20% of the total V content). The Šibenik port is a considerable source of contamination of Krka River estuary, especially phosphates, due to the transhipment activities of phosphate ore and fertilizers (Cukrov, 2021). Usually, the phosphate ore used to manufacture fertilizers contain various amounts of accompanying elements, including V, which can become even more concentrated during production process (Cappuyns and Swennen, 2014). Strong relationship between the V enrichment and formation of phosphorus nodules was observed in earlier studies (Wu *et al.*, 2021). Phosphorus, as well as V, can be loosely bound to exchangeable phase or incorporated with carbonates which would explain exceptional V mobility on stated sampling location compared to other locations under anthropogenic influence (Ruttenberg, 1992).

Overall, calculated enrichment factor shows that there is no recorded V pollution in surface sediment of the Krka River estuary. Importantly, it is established that estuarine sediment under the anthropogenic influence does not pose risk for surrounding biota and can be considered of general low risk of V toxicity and/or contamination. However, it was noticed that slight enrichment of V_{TOT} is, almost in all cases, accompanied with lower mobility of V across surface sediment of the Krka River estuary. Enrichment of V_{TOT} likely impacts V binding to more reducible phases of the sediment, thus exerting opposite trend of mobilised V with respect to V_{TOT} / Al. It is presumed that binding of the V to the less mobile phases of the sediment is influenced by the V detrital character and anthropogenic input in the sampled points (Shaheen *et al.*, 2019). Vanadium shows affinity for high molecular weight-compounds which impacts its binding to organic phases and reducible phases of sediment (Awan *et al.*, 2021). Enhancement of V mobility is mostly visible in carbonate sediments and sediments under the direct input of terrigenous material. In addition, speciation studies showed that V is dominantly present in the form of V(+IV) which further support results obtained in the research paper II. Due to the established lower toxicity of V(+IV) compared to V(+V), it can be concluded that even in case of high remobilised concentrations, toxic risks to the surrounding biota are still considered low. However, it should be noted that redox conversion of V

species, especially +IV and +V, is rather easy and it depends on various physico-chemical parameters of sediment and overlaying waters (Cappuyns and Swennen, 2014). Especially, oxic conditions of overlaying water column could support fast oxidation of free V(+IV) species. In this case, remobilisation of V(+IV) could potentially lead to its oxidation and conversion to toxic species.

5.3. Investigation of relevant thiol compounds (L-cysteine, thioacetic acid and ethanethiol) with V(+V) and V(+IV) using combined spectroscopy and chromatography

Following results obtained in the research paper III., extension of the study was performed to investigate other biologically and environmentally important thiol compounds (L-cysteine, Thioacetic acid (TAA) and Ethanethiol) and their reducing ability towards V(+V). Described research has been published recently (Knežević and Bura-Nakić, 2023).

Structrual characteristic of studied thiols are shown on Figure 13. *L-cysteine* is a biogenic sulfur-containing amino acid in which sulfur atom in the side chain is involved in the formation of a reactive sulfhydryl (-SH) group and the resulting thiolate is one of the most reactive functional groups in proteins (Figure 13A) (Bulaj, Kortemme and Goldenberg, 1998; Yin *et al.*, 2016; Sameem, Khan and Niaz, 2018). This amino acid contains overall three dissociable protons (pK_{COOH}= 1.91, $pK_{NH_3^+}$= 8.16 and pK_{SH}= 10.25) that can participate in the interaction with V(+V) in solution (Silva *et al.*, 2006).

Thioacetic acid (TAA) is a thiocarboxylic acid and plays an important role in energy transfer reactions in prebiotic metabolism (Figure 13B). Due to the reactivity of thiol group, it is used as an efficient acetylating agent for amino acids and reactant in the synthesis of organic compounds (Sanden *et al.*, 2020).

Ethanethiol is sulphur-containing analogue of organic alcohol and second in the homologous series of mercaptans (Figure 13C) (Roberts and Friend, 1988; Jou, Mather and Schmidt, 2021). Ethanethiol is emitted from natural sources through ocean emissions from marine phytoplankton and from anthropogenic sources as one of the industrial organosulfur pollutants. Importantly, ethanethiol was detected as a dominant pollutant in sediment and water environment burdened with high anthropogenic input (Saltzman, 1989). In its structure, ethanethiol contains R-SH group at the

end of the alkyl chain and owns highest pKa values out of all studied thiols ($pK_a \sim 10.6$) (Kortum, Vogel and Andrussow, no date).

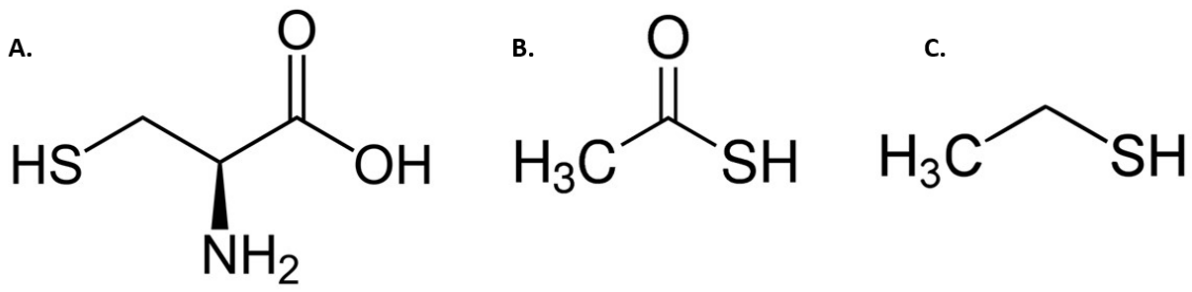

Figure 13. Structural properties of thiol compounds used in this study. A. L-cysteine B. Thioacetic acid (TAA) C. Ethanethiol.

Results obtained in the research paper III., strongly suggest that the reaction of vanadate(+V)-thioester intermediate formation, that precedes formation of V(+IV)-thiol complex, is proton catalysed. Similarly, earlier studies indicate that formation of stable bidentate V(+V) complexes with dithiothreitol requires the presence of an unblocked sulphur together with an additional functional group (Nxumalo F and Tracey, 1998). In contrast, Hsu *et al.* (2006) found the formation of non-oxo V(+IV) complexes and rejected the potential formation of V(+V) complexes with other ligands present in thiol compounds (Hsu H., Su C., Gopal N.O., Wu C., Chu W., Tsai Y., Chang Y., Liu Y., Kuo T., 2006). Therefore, there are still a lot of inconsistencies regarding exact reaction mechanism of V(+V) species and biologically important thiols. The question of the exact V(+V) reduction mechanism, specifically is the reduction of V(+V) proton catalysed, has important biological implications because it is directly related to the mechanism of V(+V) toxicity decrease in biological medium.

The goal of the extended study on V(+V) reduction in the presence of biologically and environmentally relevant thiol compounds is to provide further insight on how additional proton groups in L-cysteine compared to TAA and ethanethiol can affect V(+V) reduction. Comparing the interaction of V(+V) with polydentate (3-mercaptopropionic acid, L-cysteine) and monodentate thio-ligands (TAA and ethanethiol) will help to clarify the role of proton donor groups (COOH, NH_3^+) in the reaction mechanism between V(+V) and thiol compounds. At the same time, comparison of the V(+V) interaction with thiocarboxyl and thioalcohol can be used to assess the affinity of stated thioligands for V(+V). Moreover, the data on V(+V) interaction with thioacetic

acids and ethanethiol studied are very valuable because, to our knowledge, they were collected for the first time.

In this study analytical approach, published in research paper III., was followed. All the other details on methodology and measurement conditions are available in Appendix IV.

5.3.1. Chromatographic measurements of V(+V) reduction in the presence of thiols

The stability of V(+V) in the presence of L-cysteine, thioacetic acid and ethanethiol was studied as a function of the wide range of the solution pH (from pH=2.2 up to 8.5). In the measured time frame and at all pH values, L-cysteine proved to be the strongest reducing agent for V(+V), followed by TAA and finally ethanethiol (Figure 14). Exceptional high reduction in case of TAA and ethanethiol compared to L-cysteine was only noticed at pH=2.2. However, noticed discrepancy in the highly acidic medium was probably affected by possible precipitation occurring in the solution. The presence of both TAA and ethanethiol, in the solution probably favoured the formation of $V_2O_{4(s)}$, which precipitated easily from the solution. This would then be especially pronounced in acidic medium, due to the facile protonation process (Martins, 2000). For thioacetic acid, reduction was observed up to pH=4 which coincides with deprotonation of thiocarboxylic group ($pK_a \sim 3.5$). Ethanethiol proved as least successful at reducing V(+V), where reduction wasn't noticed above pH=3. In case of ethanethiol, additional pH value of 10.5 was tested to examine whether high deprotonation pK_a values affect V(+V) reduction. The chromatographic experiments didn't show any reduction taking place in the solution over a course of 24 hours (data not shown).

Obviously, the structure of the investigated thiols has a significant effect on the V(+V) redox conversion in aqueous solution. In the case of L-cysteine and TAA, the reduction reaction is pH dependant. However, the reaction with ethanethiol does not seem to follow the same pattern. Since only L-cysteine has additional functional groups, obtained data strongly support the hypothesis that V(+V) reduction is proton catalyzed. In the case of TAA and ethanethiol, we cannot exclude the possibility of a favoured complexation of V(+V), as suggested in the study by Crans *et al.*(2010), as well as a slower reduction occurring outside the measured time frame (Crans D.C., Zhang B., Gaidamauskas E., Keramidas A.D., Willsky G.R., 2010). When we compare monodentate

thioligands, the data suggest that the thiocarboxyl functional group is more reactive towards V(+V) compared to thioalcohol.

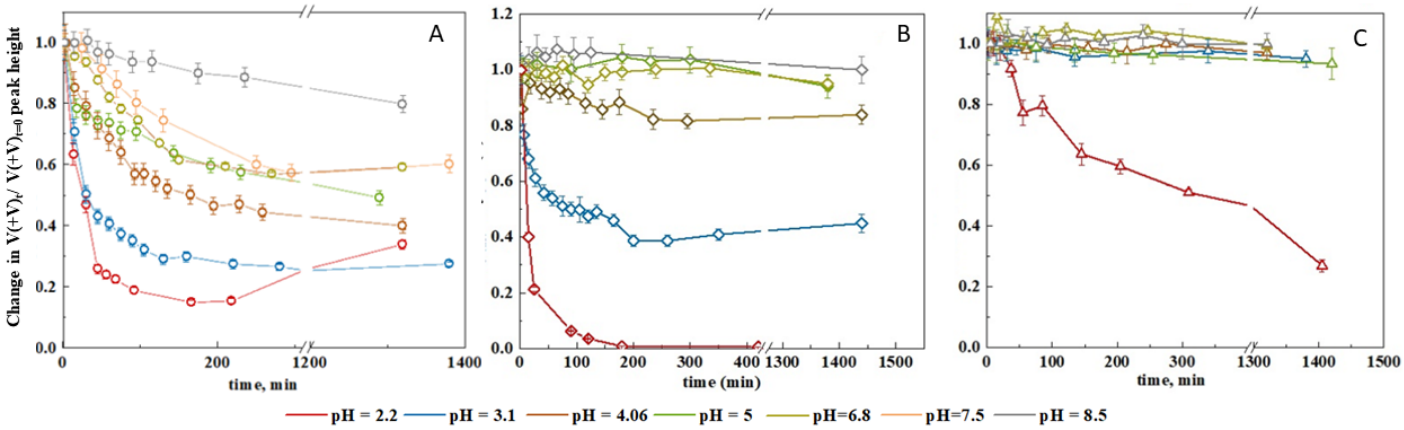

Figure 14. Time-dependent chromatographic measurement of V(+V)-EDTA in the sample solution containing $5x10^{-5}$ mol L^{-1} V(+V),$1x10^{-4}$ mol L^{-1} , thiol and $1x10^{-2}$ mol L^{-1} EDTA in the time span of 24 hours at each pH: A. L-cysteine B. Thioacetic acid. C. Ethanethiol.

5.3.2. Spectrophotometric measurements of V(+V) reduction in the presence of investigated thiols.

The chromatographic data on V(+V) interaction with thiols are accompanied by spectral curves recorded at equilibrium time at each pH (Figure 15). Sample solutions were recorded over time span of 24 hours and the full spectral events recorded over time at each pH value can be found in the Appendix IV. The spectral changes with time available in the Appendix IV. confirm previously postulated reaction mechanism in the research paper III, especially in case of L-cysteine. Based on the performed chromatographic and spectral analysis we can assume the formation of protonated V(+IV) complexes from pH=2 to 5, coordinated through both an amino and carboxyl group (Legrum, 1986; Costa Pessoa and Vilas Boas, 1990). In near-neutral and neutral pH region coordination of the thiol, amino and carboxylic groups in *cis*- geometry is suggested, dominantly present in ML_2H and ML_2 stoichiometry, respectively (Costa Pessoa and Vilas Boas, 1990). In the indicated pH range, the observed intense band attributed to the cis-trans V(+IV)-thiol complex shifts to the lower energy region of the spectrum, with $\lambda_{max} \sim 560$. The band positions could potentially mark the formation of a bis-chelate V(+IV) complex in which coordination occurs through deprotonated carboxyl and thiol groups, which is consistent with the literature data for this

type of coordination (Micera and Dessi, 1988; Williams, Barrio and Etcheverry, 1999; J. Costa Pessoa, I. Tomaz, T. Kiss, 2001; Monga V., Thompson. K.H., Yuen V.G., Sharma V., Patrick B.O., McNeill J.H., 2005). Also, a broad band appears at pH=6.8 and 7.5 in the near infrared (IR) region which is previously linked with intervalence transfer (IT) transition, possibly indicating the formation of a compound with mixed V(+V/IV) valence states (Dutta *et al.*, 1997; Dinda *et al.*, 2006). Following deprotonation of the amino group in a slightly alkaline medium, very discrete absorption bands were observed, and a low concentration of V(+IV) was observed in the chromatographic analysis (as observed on Figure 14). This suggests that the loss of the available proton catalyst from the amino group would inhibit the reduction of V(+V). The band characteristic of the formation of V(+V) - thioester intermediate species was evident in all cases where V(+IV) was detected, proving that this step is essential for the reaction to proceed to the formation of V(+IV) compounds. However, the slower reduction observed with increasing pH may allow sufficient time for V(+V)-thiol complexation (Paul and Tracey, 1997). Complexes of V(+V) with thiols are especially characteristic for neutral or slightly alkaline media (Bhattacharyya S., Batchelor R.J., Einstein F., no date; Nxumalo F and Tracey, 1998; Crans D.C., Zhang B., Gaidamauskas E., Keramidas A.D., Willsky G.R., 2010). Certainly, this possibility cannot be excluded due to the broad band near IR region at neutral pH values.

Spectral curves recorded in the solutions containing TAA and ethanethiol at pH=2.2 (Figure 15, B and C, red curve) depict formation of vanadyl cation with characteristic λ_{max} at 800 nm (Choi, Kwon and Kim, 2013). The spectral results support the proposed hypothesis of the occurrence of precipitation at acidic pH, since the mentioned process usually indicates a lack of complex formation (Crans and Tracey, 1998). In case of TAA, we can follow formation of the V(+V)-thioester band at pH=3 and 4, which decomposes into a compound exhibiting absorption properties at around $\lambda_{max} \sim 600$ nm. In addition, the solution turned green during the measurement, characteristic for V(+IV)-thiol complex (Williams and Baran, 2006; Crans D.C., Zhang B., Gaidamauskas E., Keramidas A.D., Willsky G.R., 2010). Thus, we can attribute the absorption properties of the reaction solution to the d-d transitions of V(+IV) complexes, possibly involving O,S-coordination (Williams, Barrio and Etcheverry, 1999; Maurya M.R., Khurana S., Zhang W., 2002). Above pH=5, no reduction was detected in the data obtained by chromatographic measurements. However, the spectral band shows an increase in the wavelengths associated with the d-d transitions of V(+IV) complexes (Micera and Dessi, 1988; Williams, Barrio and

Etcheverry, 1999). We can exclude the possibility that the spectral changes originate from V(+V) complexes since they do not exhibit d-d transitions (Maurya M.R., Khurana S., Zhang W., 2002). However, due to the dilution of the reaction solution, the V(+IV) species could be below the detection limit of the chromatographic analytical system. Regardless of this, it can be stated that upon pH increase above 5, the reduction of V(+V) is inhibited by TAA.

In case of ethanethiol, change in the spectrum wasn't observed at pH=3 and only V(+V) was determined in these solutions by means of IC. In this pH range, the aqueous speciation of V(+V) is dominated by hydrolyzed decavanadates, which might be stable towards interaction with ethanethiol. The discrete band at about 600 nm would then be possibly assigned to the VOL_2 species, which are under chromatographic limit of detection, where coordination takes place through two pairs of S-donors (Williams and Baran, 2006; Crans D.C., Zhang B., Gaidamauskas E., Keramidas A.D., Willsky G.R., 2010). Absorbance spectra of the reaction solutions prepared on a suitable pH (10.5) showed that deprotonation of ethanethiol doesn't induce any spectral changes assigned to the formation of V(+IV) species (data not shown). The poor reduction of thioalcohols has been observed in previous studies with DTT and is attributed to the formation of stable V(+V)-thiol complexes (Nxumalo F and Tracey, 1998). The data obtained in this study do not exclude proposed hypothesis.

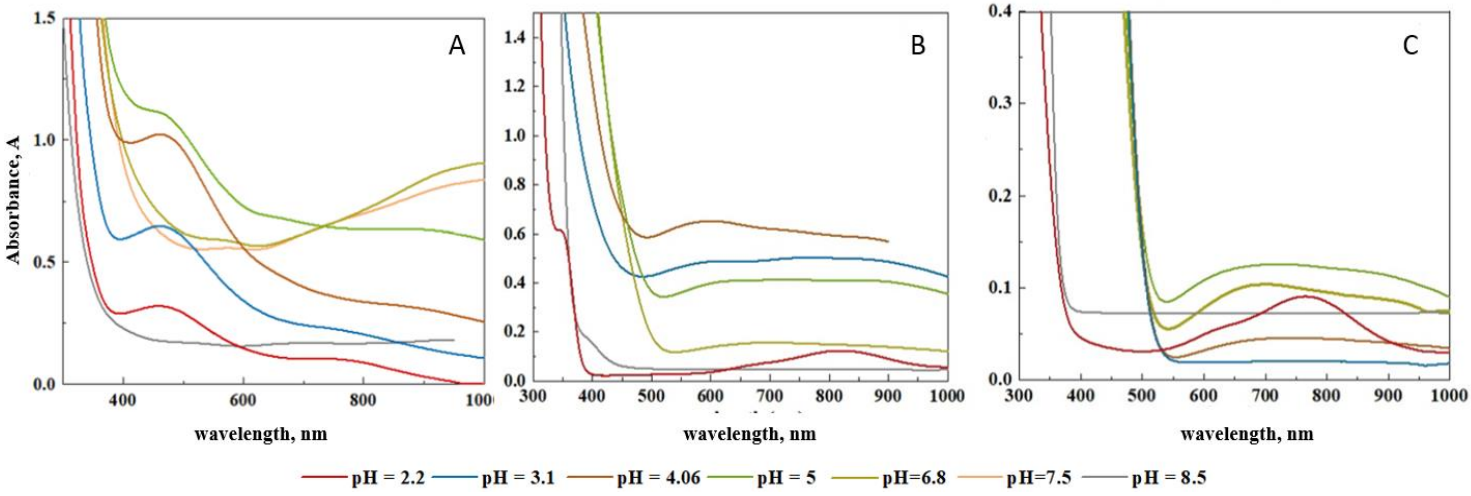

Figure 15. Absorption spectra of reaction solution containing $5x10^{-3}$ mol L^{-1} V(+V), $1x10^{-2}$ mol L^{-1} thiol at each pH recorded in t_{eq}. A. L-cysteine B. thioacetic acid C. ethanethiol.

5.3.3. Kinetics of V(+V) reduction with L-cysteine.

The determined decrease in V(+V) measured chromatographically followed a second order reaction in which the values of the net rate reaction coefficients correlated negatively with an increase in pH. Numerical values of net rate reaction coefficients are given in Appendix IV (Table A11). Plotting the log k values of each pH value investigated against the corresponding pH values shows a clear non-linear reduction rate of V(+V) in the sample solution (Figure 16). This is probably result of several processes simultaneously occurring in the reaction solution. Firstly, as mentioned earlier, the reduction reaction is proton catalyzed, so the deprotonation of each functional group present in L-cysteine has a major effect on the V(+V) reduction. In the nonlinear pH range, the catalysts of the reaction process is thought to be predominantly the amino group (pK_a ~ 8.16), while at lower pH values the reaction could be additionally catalyzed by the carboxyl group and an acidic medium (Nxumalo F and Tracey, 1998). Secondly, V(+V) shows a rich hydrolytic chemistry with the formation of oligomerized species above pH=3, which show very different chemical behaviour compared to the monomeric V(+V) species (Aureliano M., 2009; Ramos *et al.*, 2009). Especially, non-linear kinetics are observed in the pH range from 5 to 7. The predominant species from pH=3 to 7 are highly stable oxovanadates, mainly decavanadates (Figure 6). In studies by Aureliano *et.al.* (2009) it was observed that decavanadate species respond differently to biological reactions compared to monomeric V(+V) species (Aureliano M., 2009). Independent of their solution stability, Ramos *et al*. (2009) have shown that decavanadate species promote cysteine oxidation and faster production of V(+IV) in the medium (Ramos *et al.*, 2009). Above pH=6, the degree of oligomerization of V species changes and moves from the predominant V_{10} species to lower degree oligomerized species (V_4 and V_2). However, decavanadates are known to exist at neutral pH due to their slow decomposition and oligomerization of oxovanadates (Aureliano M., 2009; Ramos *et al.*, 2009).

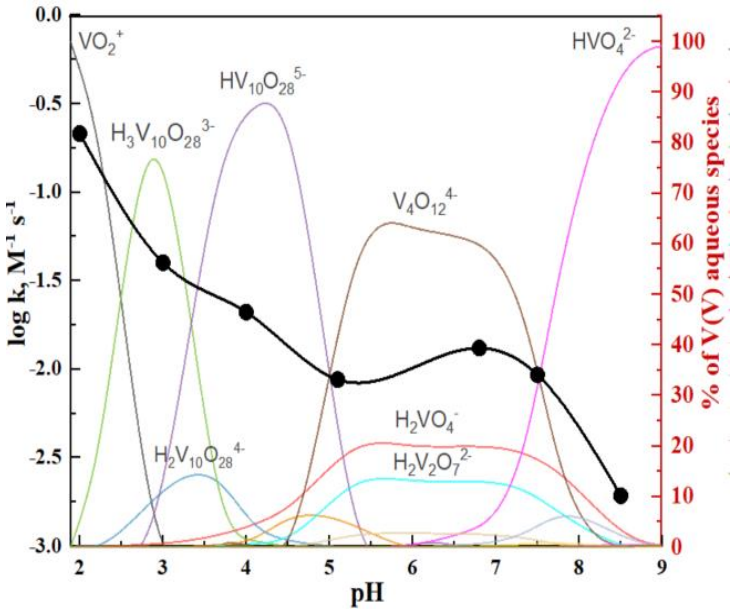

Figure 16. Dependence of determined net rate coefficients for V(+V)-L-cysteine reaction at examined pH values. Graph is plotted along V(+V) aqueous speciation over examined pH range. V(+V) speciation was modelled using VISUAL MINTEQ for fixed V(+V) concentration (0.005 mol L^{-1}) and ionic strength (0.1 mol L^{-1}, borate buffer).

Study in the research paper III., on V(+V) reduction with 3-mercaptopropionic acid showed a similar reduction mechanism of V(+V) compared to L-cysteine (Bura-Nakić, Knežević and Mandić, 2022). However, in the presence of L-cysteine, the reduction was more favourable up to a pH of 8.5 compared to 3-mercaptopropinoic acid (pKa ~ 4.57), suggesting that in this pH range the presence of an additional ligand amino group in L-cysteine promotes reduction and complexation of V(+IV) on higher pH values compared to 3-meracptopropionic acid (Nxumalo F and Tracey, 1998). Moreover, each proton donor affects the structural properties of the formed compounds in solution, as shown by the spectral data obtained. Thus, the coordination properties are also a pH-dependent phenomenon determined by the pK_a values of the species in the reaction system (Bhattacharyya S., Martinsson A., Batchelor R.J., Einstein F.W.B., 2001).

The additional study performed to accompany research paper III., on the interaction of V(+V) with L-cysteine, TAA and ethanethiol helps to better understand the ability of the R-SH group to reduce V(+V) based on different structural properties of thiols. In addition, presented findings are of great biological and ecological importance. The ionization properties of certain thiol compounds containing additional functional groups capable of V-bonding influence pH-dependent reduction mechanism of V(+V). At the same time, carbon-bonded thiol groups (R-SH) solely, are unlikely to

reduce V(+V) fast enough in certain media in the absence of an additional functional group. Thus, this study proves that the presence of additional functional groups in a thiol-compound has the potential to favour the reduction of V(+V) over the complexation of V(+V). In addition, it seems highly likely that in proteins based on the cysteine residues (ATPase, vanadate phosphatase ect.) and sulphur donor ligands, V(+V) reduction is a strong mechanism to prevent enzymatic inhibition by phosphate like-V(+V). At the same time, this suggests stability of V(+V) in its most toxic state in the presence of structurally simple thiols.

6. CONCLUSIONS

In this work, determination of redox species V(+IV) and V(+V) was performed in the water column and bioavailable fraction of surface sediments of the Krka River estuary based on chromatographic methods which were adapted and successfully applied. Introduced methodological improvements enabled V redox speciation in the samples of complex matrix, both sediment extracts and water samples of varying salinity. Furthermore, stability of V species with simple organic ligands and V(+V)/V(+IV) redox conversion in the presence of relevant thiol compounds was investigated to understand how factors such as pH and ligand structure influence redox distribution under modelled conditions. Stability of V species with succinic acid was performed using capillary zone electrophoresis, while stability of V(V) in the presence of thiols was performed using new approach based on simultaneous use of ion chromatography and spectrophotometry. A combined approach enabled accurate measurement of V(V) reduction kinetics, as well as observation of spectral changes occurring in the solution.

Obtained results may be summarized as follows:

- Through the salinity gradient of the surface layer of Krka River estuary V species show conservative behaviour. Redox speciation of all samples showed dominant presence of V(V) species. However, up to 26% of V(IV) species was found in the samples taken from the head of the estuary, characterized with oxygen depletion. In addition, V(IV) species were determined in the fully oxygen saturated samples, but under presumed anthropogenic burdening. Obtained results suggest V(+V) reduction with natural ligands present in the water column. Formed V(+IV) is later stabilized, possibly due to the complexation with natural organic and/or inorganic ligands, leading to exceptional redox distribution of V in oxic medium.
- Surface sediments of the Krka River estuary did not show recorded V pollution and mobility of V is generally considered low. Observed slight enrichment of V_{TOT} with respect to the geological background at anthropogenically burdened sampling sites, is almost in all cases accompanied with V binding to the reducible and residual solid phases. Acid-extractibility of V was high in samples characterised with high clay content, samples under direct terrigenous input and samples which are dominated by carbonates. On the upper part of the estuary, mobility trend seems to be affected by the irregular input and type of material. However, even in the case of determined high mobility, it is presumed that toxic effects of

V to surrounding biota is not a concern since speciation studies showed predominance of V(+IV) species.

- Based on the study on the complexation of V(+IV) and V(+V) species with simple organic ligand (succinic acid) formation of protonated complexes was established. Formation of V(+IV)-succinic acid complexes with appropriate stability constants of $\log\beta°_{111}=8.3 \pm 0.2$ and $\log\beta°_{122}=15.6 \pm 0.5$ were determined. Also, formation of V(+V) -succinic acid complex with stability constant of $\log\beta°_{111}=7.9 \pm 0.1$ was calculated as well. Values were extrapolated to zero ionic strength using Davies equation. Obtained results suggest greater stability of V(+IV) complexes with simple organic ligands, which could support redox speciation of V obtained in the water column of the Krka River estuary.
- Reduction of V(+V) in the presence of thiol compounds is proton catalysed reaction, meaning that the presence of additional functional group supports V(+V) reduction in the aqueous solution. In case of 3-mercaptopropionic acid, L-cysteine and thioacetic acid, V(+V) reduction is pH dependant process, where deprotonation characteristics of each ligand inhibit V(+V) reduction. Only, in case of ethanethiol reaction doesn't seem to show pH dependence. In case of monodentate ligands, obtained experimental results don't exclude favoured V(+V) complexation, instead of reduction. Reduction of V(+V) in the presence of L-cysteine was observed on the psychological pH, indicating that reduction reaction could be potential powerful biological mechanism against V(+V) toxicity.
- Ion chromatography proved as a valuable analytical tool in investigation of V redox speciation in environmental samples. Facile connection with spectrometric instrumentation enabled accurate, simple, and robust measurements in natural water samples of variable salinity. In addition, chromatographic technique was used as a novel approach in the studying of V(+V) reduction in the presence of thiol compounds. Simplicity, cost effective, and easy connection to spectrometric instrumentation makes this analytical technique to stand out as suitable analytical choice in the study of V speciation.

Overall, this work offers novel contributions to the existing knowledge on V(+IV) and V(+V) biogeochemical pathways. Oxic conditions govern predominance of V(+V) species in the water column of the Krka River estuary, while stabilisation of reduced species is noticed at sites under hypoxic conditions and anthropogenic input. Predominance of V(+IV) species in acid-extrcatable fraction of the Krka River estuarine surface sediments is linked with electron transfer reactions

between organic/inorganic ligands in the sediment phase with respect to V species. Furthermore, studies performed under modelled conditions prove that V(+IV) has higher affinity to organic ligands compared to V(+V), which supports stabilisation of V(+IV) determined in oxic estuarine water samples. Lastly, V(+V) reduction in the presence of sulphurised organic ligands could be additional mechanism impacting V redox distribution in natural systems as well as biological mediums. Based on obtained results it can be presumed that bio-reduction of V(+V) is highly dependent on ionisation properties of sulphur bio-ligands. Specifically, polydentate thiols will cause V(V) reduction more readily, compared to monodentate thiols. Lastly, this study presents pioneer research on V redox speciation in the surface sediment and water column of highly stratified Krka River estuary showing importance of trace metals speciation studies to accurately interpret their mobility, toxicity and chemical reactivity in natural aquatic system.

7. APPENDIX

7.1.Appendix I.

Table A1. Review of developed extraction methods for redox speciation of V in natural water samples.

Analytical method	Determined V species	Sample pretreatment	Principle of V redox species separation	Type of natural sample	LOD	Reference
SPE-GFAAS („on-line“ configuration)	V(+V), V(+IV)	Filtration and acidification of the sample (pH=4.5, acetate buffer)	Retention of V(IV) and V(V) redox species on CHELEX-100 ion-exchange resin at pH=4.5 and their elution with 0.1 M NH_4OH/0.2 N $HClO_4$	Estuary	30 ng L^{-1}	(Wang and Sañudo-Wilhelmy, 2008)
SPE-ETAAS („off-line“ configuration)	V(+IV), V(+V)	Filtration	Complexation of V(IV) and V(V) species with organic ligands and their elution with ascorbic acid on an anion exchange stationary phase	Seawater	20 ng L^{-1}	(Nukatsuka, Shimizu and Ohzeki, 2002)
SPE-AAS („off-line“ configuration)	V(+IV), V(+V)	Filtration	Solid-phase extraction of V(V) and V(IV) on ion-exchange resin, and eluation with NaOH/ malonic acid.	Natural aqueous samples	-	(Banerjee *et al.*, 2003)
LLE ICP-OES („off-line“ configuration)	V(+V)	Filtration and acidification	Extraction of V(V) from dibenzo-18-crown-6 using dichloromethane as a solvent which forms a colorless complex with V(V) (λ_{max}=285 nm)	Seawater	$2x10^2 – 5x10^3$ ng L^{-1}	(Agrawal, Menon and Jain, 2003)
SPE-UV/Vis („off-line“ configuration)	V(+V)	Filtration and acidification (pH=1, HNO_3)	Preconcentration and determination of V(V)-PAR complex on XAD resin. V(IV) species were masked by complexation with CDTA.	Seawater	$1.6x10^3$ ng L^{-1}	(Filik *et al.*, 2004)

LPME-ICP-OES („on-line“ configuration)	V(+V)	Filtration and acidification (pH=5)	Extraction of the formed V(V)-APDC complex in CCl_4 at pH=5. V(IV) was masked with the CDTA.	Seawater, lake water	71 g L^{-1}	(Li and Hu, 2007)
SPME-ETAAS („on-line“configuration)	V(+V)	Filtration	Adsorption of V(V) on the active material of the stationary phase and elution with HNO_3	Seawater	8 ng L^{-1}	(Naeemullah, Tuzen and Kazi, 2018)
SPE-ETAAS („off-line“ configuration)	V(+V), V(+IV)	Filtration and storage at +4°C	Retention of V(IV) and V(V) chemical species on CHELEX-100 ion-exchange resin and their elution with HNO_3/NH_3	Seawater	$1.5x10^2$ ng L^{-1}	(Alcalde-Isorna, Barciela-Alonso and Bermejo-Barrera, 2011)
SPE- ICP-OES („on-line“configuration)	V(+V)	Filtration	Retention of V(V) on the stationary phase (CTAB-modified alkyl SiO_2 microcolumn) at pH=2-7 and elution with HNO_3	Seawater, freshwater	30 ng L^{-1}	(Xiong, Qin and Hu, 2010)

Table A2. Review of developed electrochemical methods for redox speciation of V in natural water samples.

Analytical method	Determined V species	Sample pretreatment	Principle of V redox species separation	Type of natural sample	LOD	Reference
CAdSV	V(+V)	-	Accumulation of V(V)-CAA complex on HMDE catalyzed by the addition of BrO_3^-	Natural water samples	0.05 ng L^{-1}	(Bobrowski, Nowak and Zarebski, 2005)
DP CSV	V(+V)	Filtration	Adsorption and desorption of V(V)-catechol complex on HMDE at pH=6.9 (PIPES buffer)	Seawater	15 ng L^{-1}	(van den Berg and Huang, 1984)
SWAdSV	V(+V)	Filtration	Adsorption of V(V)-cuperferon complex on HDME	Seawater	8 ng L^{-1}	(Greenway and Wolfbauer, 1995)
AdCSV	V(+V)	Filtration	Accumulation of the V(V)-COD complex on the HMDE electrode.	Rivers, estuaries, coastal and open sea	50 ng L^{-1}	(Schneider *et al.*, 2015)
AdCSV	V(+V)	Filtration	Accumulation of V(V)-chloranilic acid on the HMDE electrode at pH=4.6 (acetate buffer)	Seawater	1000 ng L^{-1}	(Sander and Henze, 1996)

Table A3. Review of developed spectrophotometric methods for redox speciation of V in natural water samples.

Analytical method	Determined V species	Sample pretreatment	Principle of V redox species separation	Type of natural sample	LOD	Reference
Spectrophotometry	V(+V)	Filtration	Determination of V(V) based on the oxidation of AB (Azure B) in the presence of KI at acidic pH	Natural water samples	7.5×10^5 ng L^{-1}	(Narayana and Sunil, 2009)
Catalytic spectrophotometry	V(+V)	Filtration	Catalytic effect of V(V) oxidation of THAPPH with H2O2 (pH=2.8)	Natural water samples	2×10^4 ng L^{-1}	(Chalapathi *et al.*, 2014)
Catalytic spectrophotometry	V(+IV), V(+V)	Filtration	Catalytic effect of V(V) oxidation of DPH (N,N-diphenylhydrazine) with CTA	Seawater	10 ng L^{-1}	(Nakano *et al.*, 2009)
Catalytic spectrophotometry	V(+V)	-	Catalytic effect of V(V) oxidation of VBB (Victoria Blue B) with $KBrO_3$	Natural water samples	4.2×10^2 ng L^{-1}	(Keyvanfard, 2009)
Catalytic spectrophotometry	V(+V)	Filtration	Catalytic effect of V(V) oxidation of GB+ (Gallamine blue) with $KBrO_3$ (pH=2.0)	Natural water samples	3.1×10^2 ng L^{-1}	(Gürkan, Tamay and Ulusoy, 2017)
Spectrophotometry	V(+IV)	Filtration , sample storage at +4°C	Formation of V(IV)-HBMATC complex in organic solvent (dimethyl formamide)	Natural water samples		(Prem Kumar *et al.*, 2012)
LLME- spectrophotometry	V(+V)	-	Extraction of the V(V)-PAR complex in an organic solvent in the presence of CTAB.	Natural water samples	60 ng L^{-1}	(Uslu *et al.*, 2013)
Spectrophotometry	V(+V)	Filtration	Complexation of V(V) with 2,4-DNPH in an acidic medium (H_2SO_4)	Natural water samples	1.2×10^4 ng L^{-1}	(Al-Tayar *et al.*, 2012)
Spectrophotometry	V(+V)	Filtration	Determination of V(V) based on the oxidation of AB (Azure B) in the presence of KI at acidic pH	Natural water samples	7.5×10^5 ng L^{-1}	(Narayana and Sunil, 2009)

Table A4. Review of developed chromatographic and related methods for redox speciation of V in natural water samples.

Analytical method	Determined V species	Sample pretreatment	Principle of V redox species separation	Type of natural samples	LOD	Reference
RPLC-ICP-MS	V(+V), V(+IV)	-	Formation and separation of V(IV)-EDTA and V(V)-EDTA complexes on C18 stationary phase and mobile phase (3 mM EDTA, 0.5 mM tetrabutylammonium phosphate, 12% v/v methanol, pH=6.5)	Seawater	V(IV)- 25 ng L^{-1} V(V) – 41 ng L^{-1}	(Wann and Jiang, 1997)
RPLC-ICP-QMS	V(+IV), V(+V)	Filtration	Formation and separation of V(IV)-EDTA and V(V)-EDTA complexes on a C18 column	Estuary	V(IV)- 7 ng L^{-1} V(V) – 13 ng L^{-1}	(Liu and Jiang, 2002)
RP-HPLC	V(+V)	Filtration	Complexation of V(V) on pre-column HQ and separation on C18 stationary phase	Seawater	$1x10^3$ ng L^{-1}	(Ohashi, Uehara and Shijo, 1991)
CE	V(+IV), V(+V)	Filtration	On-column complexation of V(IV) and V(V) with EDTA	Groundwater	V(IV) – 3 µmol L^{-1} V(V) – 1 µmol L^{-1}	(Chen and Naidu, 2002)
CE	V(+IV), V(+V)	Filtration	Pre-capillary complexation with HEDTA	Groundwater	V(IV) – 3.4 µg L^{-1} V(V) – 0.4 µg L^{-1}	(Chen, Owens and Naidu, 2007)

Table A5. Review of developed X-ray spectroscopic related methods for redox speciation of V in the solid aquatic sediment samples.

Analytical method	Determined V species	Sample pretreatment	Principle of V redox species determination	Type of natural samples	Reference
XANES	V(+III), V(+IV)	Dried and frozen -20°C	X-ray absorption spectra recorded at the V K-edge (5463.76 eV). By linear combination fitting (LFC) sample standards were compared to reference standards	Coastal marine sediment	(William W. Bennett, Enzo Lombi, Edward D. Burton, Scott G. Johnston and Daryl L. Howard, 2018)
XANES	V(+IV), V(+V)	Nitrogen purged and stored at 4°C	Analysis of collected data on the basis of V standards: $V_2(III)O_{3(s)}$, $V(IV)O_{2(s)}$, $V_2(VI)O_{5(s)}$	Surface lake sediments	(Nedrich *et al.*, 2018)
XANES	V(+III), V(+IV), V(+V)	Dried and stored at room temperature	Spectra were collected at the V K-edge energy (5465 eV). Spectra were compared to reference samples: V_2O_5, VO_2, V_2O_3, VS_2, V_2S_3 by linear combination fitting	Alum Shale of the Scandinavian region	(Bian *et al.*, 2022)

7.2.Appendix II.

I. Supplement file to the research paper I.

Redox speciation of vanadium in estuarine waters using improved methodology based on anion exchange Ion Chromatography coupled to HR ICP-MS system

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Analytical validation of the IC-HR ICP-MS data. For the accurate V(V) concentration determination due to the high influence of sample matrix, 4-point standard addition method was employed. On Figure S1a. is shown example of chromatograms obtained using the stated method on one of the estuarine samples (SB, Jan.2020.) Samples were spiked using V(V) standard solution, with the exact spike concentration being 20 nmol L-1. Concentration range studied was up to 80 nmol L-1. Peak heights were used in the process of data treatment for obtaining quantitative information on V(V) concentration. Additionally, on Figure S1b. is presented dependence of peak height and peak areas showing high linearity between peak characteristics evidencing that the choice of usage of peak heights did not affected accuracy of obtained data.

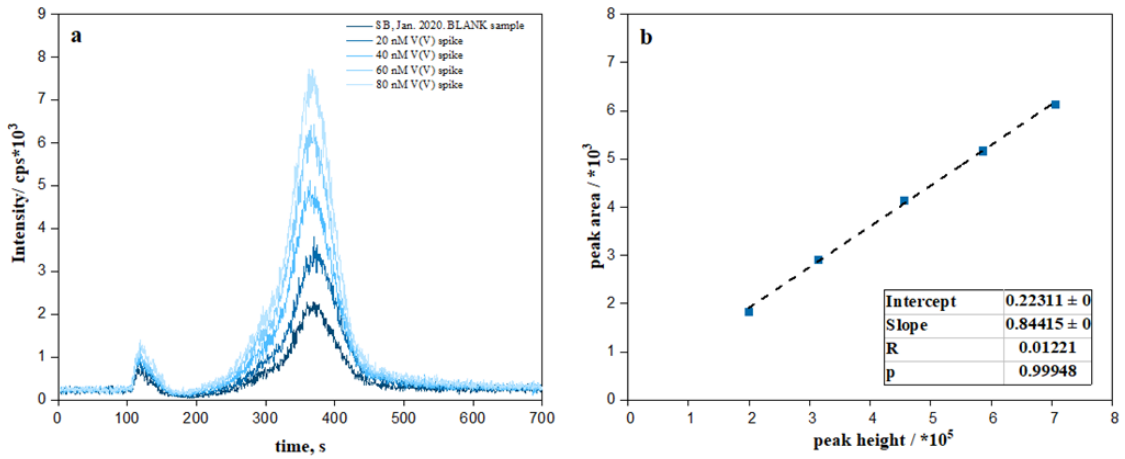

Figure S1. a) Example of obtained chromatograms using standard addition method for V(V) concentration determination on the estuarine sample SB. Jan.2020. (S=35). b) linear dependence of peak heights and peak

areas for data treatmenet of obtained chromatograms using standard addition method on estuarine sample SB.Jan,2020. (S=35).

The detection limits were determined using modified blank determination method. They were evaluated to be matrix dependant as well, with the exact values being 0.6 nmol L-1 in the freshwater and 2 nmol L-1 in the seawater. Limits of quantification were determined as 3 times of detection limits: 1.8 nmol L-1 in the freshwater and 6 nmol L-1 in the seawater. The reproducibility of the method was tested by triplicate analysis of seawater sample (S=35). The confidence interval was 0.8 % while relative standard deviation amounted to 1.1 % for V(V) species showing high accuracy. Repeatability accuracy of peak height for V(V) species was > 95 % (n=5). Recovery of V(V) species was evaluated by spiking estuarine sample with known amount of V(V) standard (20 nmol L-1 and 40 nmol L-1). Results showed high recovery of V(V) species, with values around 97% for 20 nmol L-1 and 101 % for 40 nmol L-1 spike of V(V) standard. Performed stability tests of V(V) species obtained by spiking a seawater sample with known amount of V(V) standard (40 nmol L-1) showed satisfactory results. In the Figure S2 are presented chromatograms supporting Figure 2d. in the main manuscript.

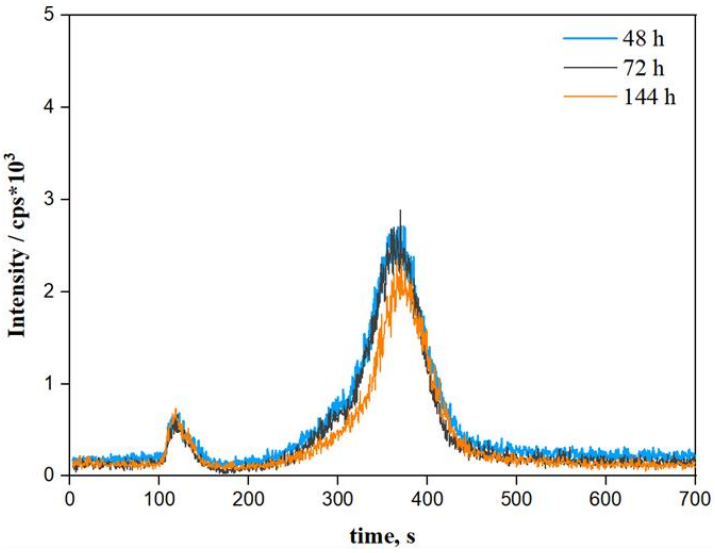

Figure S2. Chromatograms showing stability of V(V) in the seawater sample spiked with 40 nmol L^{-1} V(V) standard measured repeatively in the period of 144 hours.

All of the analytical figures of merit as well as additional information on method are shown in Table S1 and compared with the work done by Li et a. as it served as a basis for modifications made in presented method. Also, comparison with the work done by Wann et al. (1997) is also presented due to the similarity in matrix of the processed samples.

Table S1. Comparison of the method information and analytical figures of merit of the presented work and work done by Li et al. [1] and Wann et al. [2].

Method information and analytical merit	Li et al. 2018.	Wann et al. 1997.	p.w.
Method of V(V) concentration determination	Calibration curves	Calibration curves	Standard addition method
Instrumentation	IC-ICP MS	RP LC-ICP MS	IC-ICP MS
Mobile phase composition	2 mmol L^{-1} EDTA, 80 mmol L^{-1} ammonium bicarbonate, 3% acetonitrile	3 mmol L^{-1} EDTA, 0.55 mmol L^{-1} tetrabutylammonium phosphate, 12% (v/v) methanol solution	8 mmol L^{-1} EDTA, 40 mmol L^{-1} ammonium bicarbonate, 40 mmol L^{-1} ammonium sulphate, 3% acetonitril
Samples matrix	Coke pore water samples, bacterial cultures	Seawater reference material	Estuarine samples (S =4-35)
Retention time V(V)-EDTA, min	4.9	3.221	5.0
Recovery	97 %	93-104 %	97-101 %
LOD	V(V) 19.63 nmol L^{-1}	V(V) 0.078 nmol L^{-1}	V(V) 0.6 nmol L^{-1} (in the freshwater) 2 nmol L^{-1} (in the seawater)
LOQ	-	-	V(V) 1.8 nmol L^{-1} (in the freshwater) 6 nmol L^{-1} (in the seawater)
RSD, %	0.92	-	1.1
Repeatability of peak height, %	-	5.6	4.6

Table S2. Numerical values of total dissolved V determined dissolved V(V) concentration and calculated percentage of V(IV) within the Krka River estuary samples.

sample	total dissolved V determined by ICP-MS / nmol L^{-1}	st. deviation / nmol L^{-1}	dissolved V(V) determined by IC-ICP-MS / nmol L^{-1}	Replicate	st. deviation / nmol L^{-1}	% V(IV)
SM – 0.5 m depth (January 2020)	18.4	1.2	18.2	1	3.6	1.08
SM – 0.9 m depth(January 2020)	25.3	0.4	24.2	2	0.5	4.35
SM –5 m depth(January 2020)	30.7	1.2	26.4	1	1.9	14.00
SM – 6.1 m depth(January 2020)	30.2	1.2	29.8	2	1.79	1.32
M – 0. 5 m depth (January 2020)	21.6	1.0	21.0	2	4.51	2.70
M – 2.2 m depth (January 2020)	20.7	0.6	19.0	1	4.6	8.21
LV – 1.8 m depth (January 2020)	24.5	1.4	21.8	2	0.1	11.00
LV – 2.8 m depth (January 2020)	25.9	1.2	23.1	2	1.66	10.81
LV – 7 m depth (January 2020)	33.8	0.4	26.3	2	1.22	22.18
LV – 10 m depth (January 2020)	37.0	0.8	31.1	1	3.34	15.94
SM – 0.4 m depth(November 2019)	12.3	0	12.1	1	1.98	1.63
SM – 1.5 m depth(November 2019)	20.1	0	19.4	1	3.62	3.48
SM – 3.5 m depth(November 2019)	29.94	0	26.3	1	0.37	12.16
SM – 5.0 m depth(November 2019)	24.06	0	17.8	1	1.27	26.01
SM – 6.0 m depth(November 2019)	21.65	0	17.1	1	0.14	21.01

Table S3. Operating conditions of High Resolution Inductively Coupled Plasma Mass Spectrometry (HR ICP-MS).

Instrument	Element 2 (Thermo, Germany)
RF power	1200 W
Cool (plasma) gas	15.0 L min^{-1}
Auxiliary gas	0.92 L min^{-1}
Sample gas	1.00-1.02 L min^{-1}
Nebulizer	Sea-spray, 0.4 mL min^{-1}
Spray chamber	Twister, 50 mL, Cyclonic
Sample cone	Nickel
Skimmer cone	Nickel, „H“ model

II. Vanadium(+IV) and (+V) complexation by succinic acid studied by affinity capillary electrophoresis

Chemicals and solutions. 1 mol L^{-1} perchloric acid solution was from Fisher Scientific. Sodium perchlorate monohydrate (≥ 99 %) was supplied by Merck. Ammonium vanadate solution (≥ 99 %), vanadyl sulfate hydrate (≥ 99 %), succinic acid (≥ 99 %) and dimethyl sulfoxide (99.9%) were supplied by Sigma Aldrich. All solutions were prepared with fresh, high-purity deionized water (R = 18.2 MΩ cm) produced by a Millipore direct Q cartridge system.

Apparatus, procedure and data treatment. Affinity cappilary electrophoresis system (P/ACE system MDQ, Beckman Coulter, France) operated based on a 0−30 kV high-voltage built in power supply equipped with a UV-vis spectrophotometric diode array detector (200 nm). A capillary (50 µm inner diameter, 363 µm outside diameter) made from fused silica was obtained from Beckman Instruments. It had a total length (L_t) of 31.2 cm and an effective separation length (L_d) of 21 cm. The capillary was housed in an interchangeable cartridge with circulating liquid coolant (temperature 25°C). Every measurement was repeated at least three times. Data acquisition and processing are carried out with Karat 32 software (Beckman Coulter, France). The capillary was conditioned prior to use by successive washes with 0.1 M sodium hydroxide, deionised water and the buffer solution used in the study. It was rinsed for 2 minutes (at a pressure of 103.4 kPa) with the buffer between two runs and kept filled with deionised water overnight. The values of ionic strength were fixed with $NaClO_4$ as BGE at 0.1 mol L^{-1}.

Combined ion specific electrode (ISE) “Fischerband” for perchlorate from Fischer Scientific was used to check perchlorate concentrations of stock solutions and to control ionic strength. The RSD was less, than 2%. The pH values of BGE solution was kept at ≤ 2.5 by using pecrhloric acid addition. The pH was controlled with a pH-meter. pH-meter GLP-21 (Crison, France) and a combination electrode was used for pH measurements after calibration against NIST standards (4.01 and 7.00). The pH values of the solutions were in an interval of ±0.05 units from desired values. Acidic pH conditions were used to avoid hydrolysis and/or polymerisation of the V(+IV) and V(+V) species, that could potentially lead to the formation of additional species and/or modification of V species mobility values (V. Sladkov *et al.*, 2018). At such pH values and V(IV) and V(V) concentrations of 5×10^{-4} and $1x10^{-3}$ M respectively, the contribution of hydrolysed and polymeric species is not noticeable (Fig. A1).

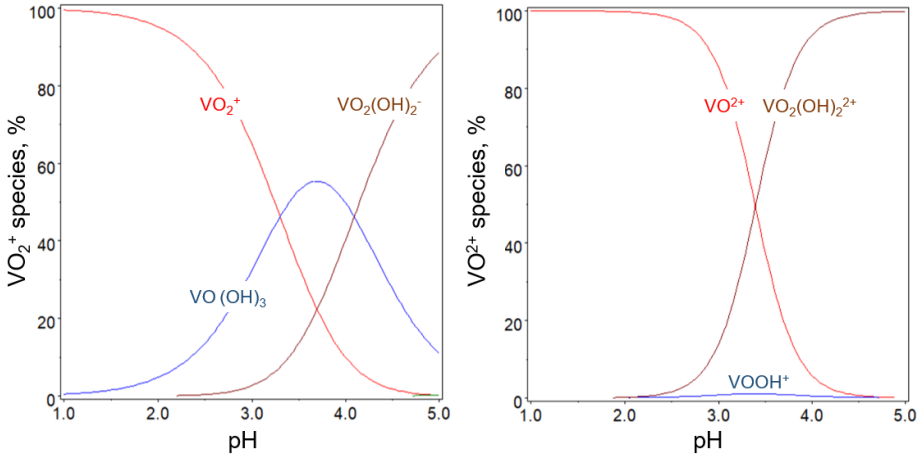

Figure A1. Distribution diagrams of the hydrolysis species of V(V) (A) and V(IV) (B) for fixed initial concentrations of V(V) ($1x10^{-3}$ M) and V(IV) ($5x10^{-4}$ M) from pH=1 to pH=5 at T = 25 °C and I = 0.1 M. Hydrolysis constants used for the speciation diagram are taken from the Baes and Mesmer.

The normal polarity mode was applied (injection was performed at the positive capillary end). The separation voltage applied was 5 kV and the injection time (by a pressure of 3.45 kPa) was 4 s. The value of applied voltage was chosen to respect the Ohm’s law. The current was about 45 µA and the input power was about 0.2 W. In these conditions we can estimate that the effect of Joule heating is insignificant in the thermostated part of the capillary and the deviation from the desired temperature 25°C is at most 1°C. The separation was performed at constant forward pressure of 1.4 kPa. The sample, containing the analyte (or analytes) and the neutral marker, was injected in the capillary. Dimethyl sulfoxide (DMSO, 0.05%) was used as a neutral marker for electroosmotic flow mobility determination. The capillary contained BGE with fixed concentrations of succinic acid. Sets of runs were then made for different concentrations of

succinic acid (from 0 to 0.4 M) in BGE. The separation was performed at constant forward pressure of 1.4 kPa. The electrophoretic mobility μ (m^2 s^{-1} V^{-1}) of V species is calculated using the following expression:

$$\mu = L_t L_d (1/t - 1/t_{eof})/U \quad (1)$$

where L_t (m) is the total capillary length, L_d (m) is the length between capillary inlet and the detection window, U (V) is applied voltage, t (s) is the migration time of the studied species, and t_{eof} (s) is the migration time of neutral marker (V. Sladkov *et al.*, 2018). All concentration and constant calculations were performed with the EXCEL® and ORIGIN® software programs. The experimental points were fitted by a least squares curve methods.

Electrophoretic mobilities

Table A6. Average values of the electrophoretic mobilities measured each in triplicate for V(IV) and V(V) species introduced in the capillary in mixed sample solution at $5x10^{-4}$ and $1x10^{-3}$ M respectively, as a function of various succinic acid (H_2Succ) concentrations in background electrolyte at fixed ionic strength values (I = 0.1 M, $NaClO_4$ – $HClO_4$) [a].

	V(IV)		V(V)		
$C[H_2Succ]_{tot}$ (M)	$\mu_{obs} \times 10^8$ ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	$\mu_{obs} \times 10^8$ ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	pH
0	3.78 ± 0.16	1.73	2.36 ± 0.12	0.19	2.40
5.0×10^{-6}	3.72 ± 0.10	1.03	2.22 ± 0.04	0.64	2.40
1.0×10^{-5}	3.83 ± 0.09	0.97	2.25 ± 0.14	2.27	2.39
5.0×10^{-5}	3.71 ± 0.01	0.11	2.11 ± 0.07	1.33	2.39
1.0×10^{-4}	3.71 ± 0.01	0.04	2.09 ± 0.08	1.63	2.40
1.0×10^{-3}	3.75 ± 0.18	1.98	2.13 ± 0.15	2.88	2.42
2.0×10^{-3}	3.74 ± 0.14	1.54	2.13 ± 0.06	1.04	2.48
5.0×10^{-3}	3.65 ± 0.01	0.11	2.04 ± 0.11	2.12	2.46
8.0×10^{-3}	3.61 ± 0.01	0.06	2.05 ± 0.06	1.16	2.39
1.0×10^{-2}	3.61 ± 0.05	0.56	2.02 ± 0.12	2.30	2.41
2.5×10^{-2}	3.49 ± 0.03	0.32	1.90 ± 0.08	1.71	2.40
5.0×10^{-2}	3.28 ± 0.21	2.51	1.78 ± 0.12	2.63	2.38
8.0×10^{-2}	2.98 ± 0.11	1.50	1.64 ± 0.09	2.15	2.42
1.0×10^{-1}	2.86 ± 0.13	1.76	1.56 ± 0.15	3.78	2.41

[a] T = 25 °C, t_{inj} = 4 s, ΔP_{inj} = 3.45 kPa, U = 5 kV; capillary: L_t = 31.2 cm, L_d = 21 cm, $\varnothing_{int}$ = 50 µm; UV detection: λ = 200 nm. Errors correspond to the estimated uncertainty of the average of three replicates at a probability of 95% ($u = t\sigma/\sqrt{3}$) according to the student t distribution.

Table A7. Average values of the electrophoretic mobilities measured each in triplicate for V(IV) and V(V) species introduced in the capillary in mixed sample solution at at $5x10^{-4}$ and $1x10^{-3}$ M respectively, as a function of various succinic acid (H_2Succ) concentrations in background electrolyte at fixed ionic strength values (I = 0.1 M, $NaClO_4$ – $HClO_4$) [a].

	V(IV)		V(V)		
$C[H_2Succ]_{tot}$ (M)	$\mu_{obs} \times 10^8$ ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	$\mu_{obs} \times 10^8$ ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	pH
0	3.73 ± 0.02	0.19	2.34 ± 0.01	0.21	2.00
1.0×10^{-3}	3.70 ± 0.02	0.23	2.31 ± 0.01	0.21	1.98
2.0×10^{-3}	3.73 ± 0.06	0.65	2.32 ± 0.05	0.80	1.95
6.0×10^{-3}	3.70 ± 0.02	0.16	2.30 ± 0.01	0.18	1.95
8.0×10^{-3}	3.69 ± 0.02	0.21	2.30 ± 0.01	0.22	1.94
1.0×10^{-2}	3.69 ± 0.02	0.20	2.29 ± 0.02	0.39	1.95
2.0×10^{-2}	3.66 ± 0.03	0.30	2.28 ± 0.02	0.30	1.94
4.0×10^{-2}	3.57 ± 0.03	0.34	2.20 ± 0.03	0.45	1.94
6.0×10^{-2}	3.52 ± 0.03	0.36	2.16 ± 0.04	0.77	1.96
8.0×10^{-2}	3.49 ± 0.02	0.27	2.14 ± 0.02	0.38	1.98
1.0×10^{-1}	3.45 ± 0.06	0.66	2.12 ± 0.05	0.99	1.97
2.0×10^{-1}	3.19 ± 0.04	0.55	1.91 ± 0.01	0.29	1.97
3.0×10^{-1}	3.02 ± 0.18	2.37	1.77 ± 0.13	2.96	1.95
4.0×10^{-1}	2.83 ± 0.04	0.62	1.63 ± 0.02	0.50	1.96

[a] T = 25 °C, t_{inj} = 4 s, ΔP_{inj} = 3.45 kPa, U = 5 kV; capillary: L_t = 31.2 cm, L_d = 21 cm, $\varnothing_{int}$ = 50 µm; UV detection: λ = 200 nm. Errors correspond to the estimated uncertainty of the average of three replicates at a probability of 95% ($u = t\sigma/\sqrt{3}$) according to the student t distribution.

Table A8. Average values of the electrophoretic mobilities measured each in triplicate for (IV) and V(V) species introduced in capillary in mixed sample solution at $5x10^{-4}$ and $1x10^{-3}$ M respectively, as a function of various succinic acid (H_2Succ) concentrations in background electrolyte at fixed ionic strength values (I = 0.1 M, $NaClO_4$ – $HClO_4$)[a].

	V(IV)		V(V)		
$C[H_2Succ]_{tot}$ (M)	$\mu_{obs} \times 10^8$ ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	$\mu_{obs} \times 10^8$ ($m^2\ V^{-1}\ s^{-1}$)	RSD (%)	pH
0	3.72 ± 0.01	0.14	2.37 ± 0.01	0.09	1.48
1.0×10^{-3}	3.72 ± 0.01	0.10	2.37 ± 0.01	0.05	1.45
2.0×10^{-3}	3.72 ± 0.01	0.08	2.37 ± 0.01	0.08	1.48
6.0×10^{-3}	3.71 ± 0.01	0.14	2.36 ± 0.01	0.19	1.45
8.0×10^{-3}	3.72 ± 0.01	0.08	2.37 ± 0.01	0.06	1.45
1.0×10^{-2}	3.71 ± 0.01	0.12	2.36 ± 0.01	0.08	1.46
2.0×10^{-2}	3.69 ± 0.02	0.22	2.35 ± 0.01	0.13	1.45
4.0×10^{-2}	3.66 ± 0.01	0.10	2.33 ± 0.01	0.08	1.45
6.0×10^{-2}	3.63 ± 0.01	0.09	2.30 ± 0.01	0.14	1.45
8.0×10^{-2}	3.60 ± 0.01	0.06	2.28 ± 0.001	0.02	1.50
1.0×10^{-1}	3.56 ± 0.01	0.10	2.25 ± 0.001	0.01	1.48
2.0×10^{-1}	3.43 ± 0.01	0.06	2.15 ± 0.01	0.12	1.45
3.0×10^{-1}	3.30 ± 0.01	0.13	2.06 ± 0.01	0.16	1.46
4.0×10^{-1}	3.19 ± 0.01	0.07	1.97 ± 0.01	0.11	1.45

[a]T = 25 °C, t_{inj} = 4 s, ΔP_{inj} = 3.45 kPa, U = 5 kV; capillary: L_t = 31.2 cm, L_d = 21 cm, $\varnothing_{int}$ = 50 µm; UV detection: λ = 200 nm. Errors correspond to the estimated uncertainty of the average of three replicates at a probability of 95% (u = tσ/√3) according to the student t distribution.

Complexation of metal ions by succinic acid. Tables A9 summarizes values of the stability constants log β_{111} for succinic acid metal complexation (reactions *4* and *5*), and log K_{MOH} of the corresponding first metal hydrolysis constant (*6*). Values were retrieved from the available literature and used to construct the linear free-energy plot (Figure 8). Only data acquired at ionic strength of 0.1 M have been selected.

$$M^{z+} + H^+ + L^{2-} \rightleftarrows [MHL]^{z+1-2} \qquad (4)$$

$$\beta_{111} = \frac{[MLH^{z+1-2}]}{[M^{z+}][H^+][L^{2-}]} \qquad (5)$$

$$M^{z+} + OH^- \xrightleftharpoons{K_{MOH}} [M(OH)]^{(z-1)+} \qquad (6)$$

Table A9. Literature data of the stability constants log β_{111} for succinate complexes and log K_{MOH} for different metal ions. I=0.1 M. T=25 °C.

M^{z+}	log K_{MOH}	Ref	Logβ_{111}	Medium	Ref
Al^{3+}	8.45	(Ekberg and Brown, 2016)	6.78	-	(Woolard, 1994)
Cd^{2+}	3.73	(Ekberg and Brown, 2016)	6.49	-	(Woolard, 1994)
Ca^{2+}	2.50	(Ekberg and Brown, 2016)	5.78	-	(Woolard, 1994)
Co^{2+}	3.96	(Ekberg and Brown, 2016)	6.23	-	(Woolard, 1994)
Cu^{2+}	5.95	(Ekberg and Brown, 2016)	7.03	$NaClO_4$	(Morphy *et al.*, 1990)
Mg^{2+}	2.02	(Baes and Mesmer, 1976)	5.82	-	(Woolard, 1994)
Mn^{2+}	2.97	(Ekberg and Brown, 2016)	6.05	-	(Woolard, 1994)
Ni^{2+}	4.03	(Ekberg and Brown, 2016)	6.15	-	(Woolard, 1994)
Pb^{2+}	6.87	(Ekberg and Brown, 2016)	7.32	-	(Woolard, 1994)
VO^{2+}	8.95	(Ekberg and Brown, 2016)	7.4±0.2	$NaClO_4$ – $HClO_4$	this work
VO_2^+	10.70	(Baes and Mesmer, 1976)	7.3±0.1	$NaClO_4$ – $HClO_4$	this work
UO_2^+	8.40	(Ekberg and Brown, 2016)	7.54±0.1	$NaClO_4$ – $HClO_4$	(V. Sladkov *et al.*, 2018)

7.3.Appendix III.

Variability of bioavailable V(IV) in surface sediments of Krka River estuary

Table A10 a. Determined concentrations of bioavailable V in surface sediments samples of Krka River estuary at stations K2-K21. Concentrations of V_{TOT} and Al_{TOT} are taken from the Cukrov *et al.* (2021) (Cukrov, 2021).

Sample	$C[V]_{BIOAVAILABLE}$, μg g^{-1}	$C[V]_{TOT}$, μg g^{-1}	$C[Al]_{TOT}$, μg g^{-1}
K5	27.39	50.60	21666
K6	19.42	50.00	21025
K7	16.98	65.60	27130
K8	14.36	43.90	18939
K9	16.48	56.70	27948
K10	14.08	67.30	33003
K11	16.50	80.30	40700
K12	33.56	80.08	36247
K13	15.59	108.00	49234
K14	13.40	76.00	43109
K15	14.18	35.10	16948
K16	13.90	77.20	41421
K17	10.67	87.40	49204
K18	10.57	70.60	33211
K19	13.91	80.10	38883
K20	12.01	77.70	44543
K21	18.33	92.40	52861
K22	15.93	89.20	38615
K23	11.89	73.10	40501
K24	13.90	73.00	39524

Table A10 b. Determined concentrations of bioavailable V in surface sediments samples of Krka River estuary at stations K22-K36. Concentrations of V_{TOT} and Al_{TOT} are taken from Cukrov et al. (2021) (Cukrov, 2021).

Sample	$C[V]_{BIOAVAILABLE}$, µg g^{-1}	$C[V]_{TOT}$, µg g^{-1}	$C[Al]_{TOT}$, µg g^{-1}
K25	11.83	76.30	39000
K26	9.09	31.60	8432
K27	10.10	74.00	34064
K28	10.09	89.60	32450
K29	10.59	94.20	38117
K30	10.79	48.40	19992
K31	10.36	85.70	41191
K32	10.85	83.20	35222
K33	11.38	71.20	33175
K34	13.84	78.30	35469
K35	13.80	76.10	37143
K36	11.08	51.80	22999
K37	8.47	39.50	17264
K38	10.41	19.70	5453
K39	10.87	25.30	9778

7.4.Appendix IV.

I. Supplementary file to research paper III.

Supplementary material

Chromatographic and spectrophometric studies of vanadate (+V) reduction by 3–mercaptopropionic acid

Elvira Bura-Nakić[a*], Lucija Knežević[a], Jelena Mandić[b]

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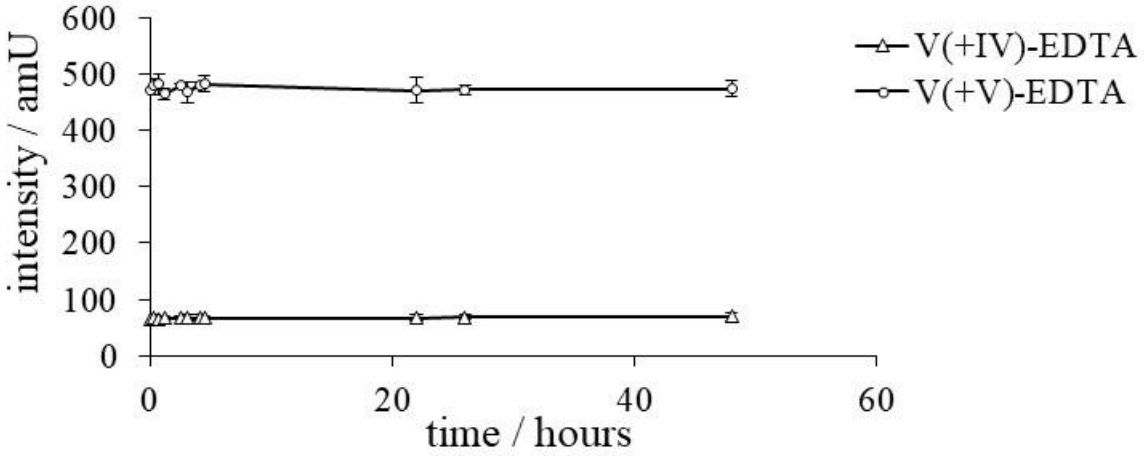


Figure S1. Time-resolved chromatographic determination of the V oxidation states (+V and + IV) in subsamples of the reaction solution. The reaction solution contained 0.01 mol dm-3 EDTA while the concentration of 3-MPA was 10-fold lower (0.001 mol dm-3). The concentration of VV and VIV added was 0.0006 mol dm-3 and the solution pH was 4.7.

Ordinary Differential Equation (ODEs) code developed in Mathematica for the numerical solution of the system

```
(*Define  known
values:*)pH = 4.5;
V0 = 0.009;
S0 = 0.02;
Clear[kf,
kb];H = 10^
-pH;

(*Read in absorbance vs time data and rescale absorbance to concentration of
V^V:*)expdata  = Import[StringJoin[NotebookDirectory[], ToString[pH], ".txt"],
"Table"]; expdata = {60, V0 / Quiet[Interpolation[expdata, 0]]} # & /@ expdata;

(*Set the maximum of time to the timestamp of the last measured value:*)
tmax = Max[expdata[[All, 1]]];

(*Define the differential rate
equations:*)vf = kf V5[t]^2 SH[t]^2 H^2;
vb = kb V4[t]^2 SS[t];

(*Define the model in the form of a set of ODEs for the
concentrations:*)model = ParametricNDSolveValue[
   {
    D[V5[t], t] ·  -2 vf + 2 vb,
    D[V4[t], t] ·  2 vf - 2 vb,
    D[SH[t], t] ·  2 vf - 2 vb,
    D[SS[t], t] ·  vf + -vb,
    V5[0] ·  V0,
    V4[0] ·  0,
    SH[0] ·  S0,
    SS[0] ·  0
   },
   V5, {t, 0, tmax}, {kf, kb}];

(*Optimize the parameter values kf and kb so that the modelled
 curve fits the measured data, calculate K as K=kf/kb, print the
 results:*)
fitpars = FindFit· expdata, {model[kf, kb][t]}, · · kf, 10^10· , {kb, 1}· , t· ;
kf = kf /. fitpars;
kb = kb /. fitpars;
K = kf / kb;
Print["kf=", kf, ";       kb =", kb, ";       K =", K]

(*Plot together the measured data and the fitted model:*)
dataplot = ListPlot[expdata, PlotLabel ·  StringJoin["pH=", ToString[pH]],
   AxesLabel ·  · {"t / s", "c_V^(V) / (mol dm^-3)"}· , ImageSize ·  {600, 400}, PlotRange ·  {0, V0}· ;

solplot = Plot[model[kf, kb][t], {t, 0, tmax}, PlotRange ·  All];
Show[dataplot, solplot]
```

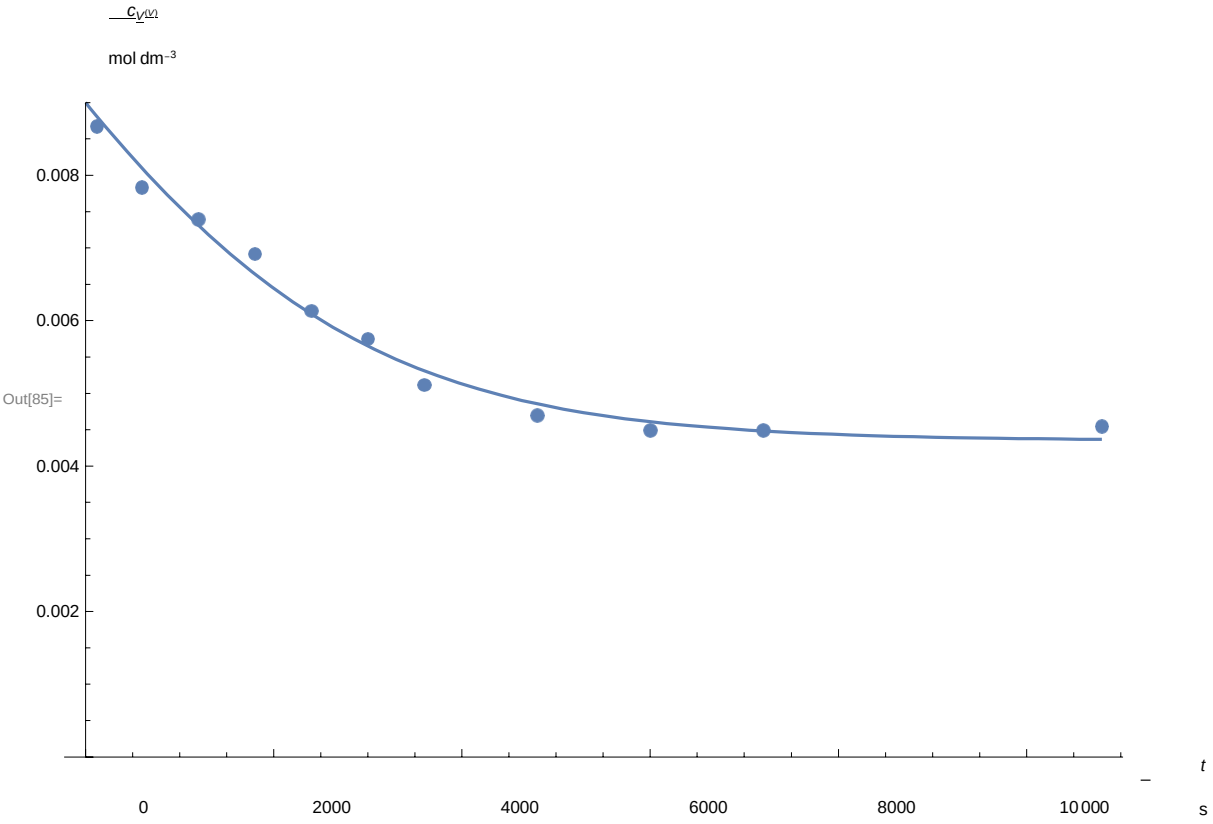
```
(*Export the results:*)
Export[StringJoin[NotebookDirectory[], ToString[pH], ".cr
```

```
Table[{N[t / 60], model[kf, kb][t]}, {t, 0, tmax, 200}], "Table"];expdata = {1 / 60,
1} # & /@ expdata;
        Export[StringJoin[NotebookDirectory[], ToString[pH], ".out"], expdata, "Table"];
```

kf=2.46832× 10^{10}; kb =5.6533; K =4.36616× 10^{9}

pH=4.5

Out[85]=



II. Investigation of relevant thiol compounds (L-cysteine, thioacetic acid and ethanethiol) with V(+V) and V(+IV) using combined spectroscopy and chromatography

pH-dependant spectral measurements

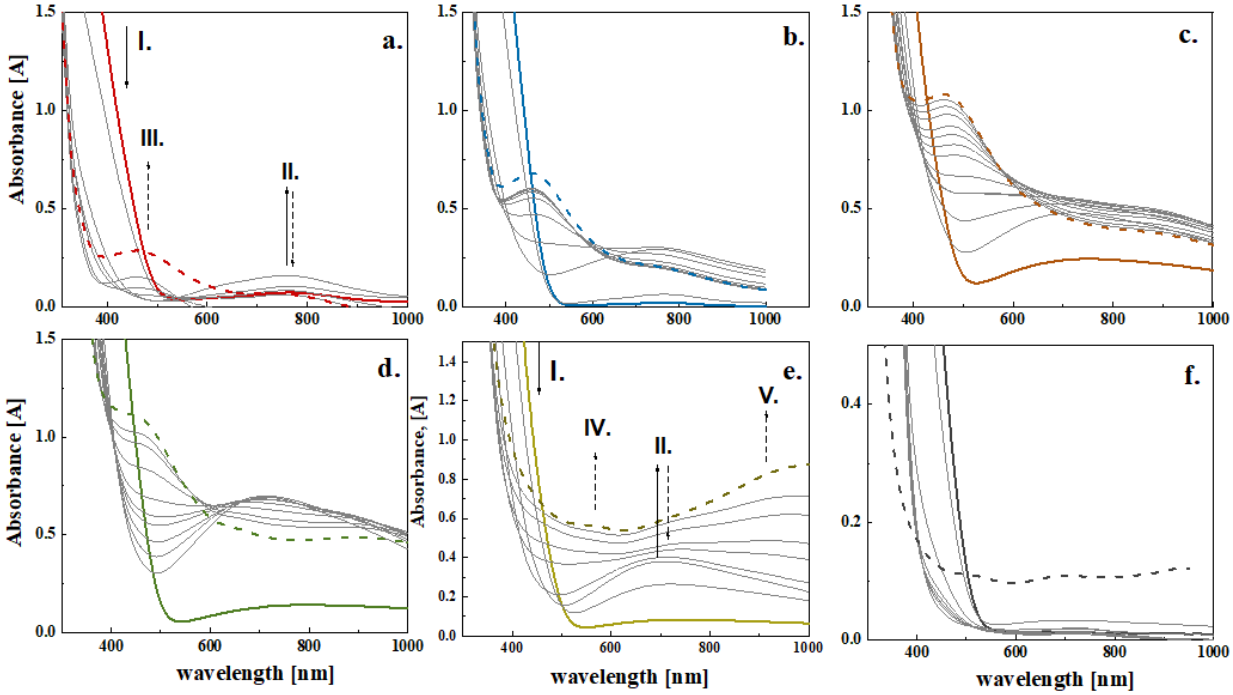


Figure A2. Absorbance change of the solutions containing 0.005 mol L^{-1} V(V) and 0.01 mol L^{-1} L-cysteine recorded in 1 cm cuvette at various pH: a.) pH=2.2 b.) pH=3.1 c) pH=4.06 d.) pH=5.0 e.) pH=6.8 f.) pH=8.5. Full line marks measured solution in t =2 min and dashed line marks solution measured in t_{eq}.

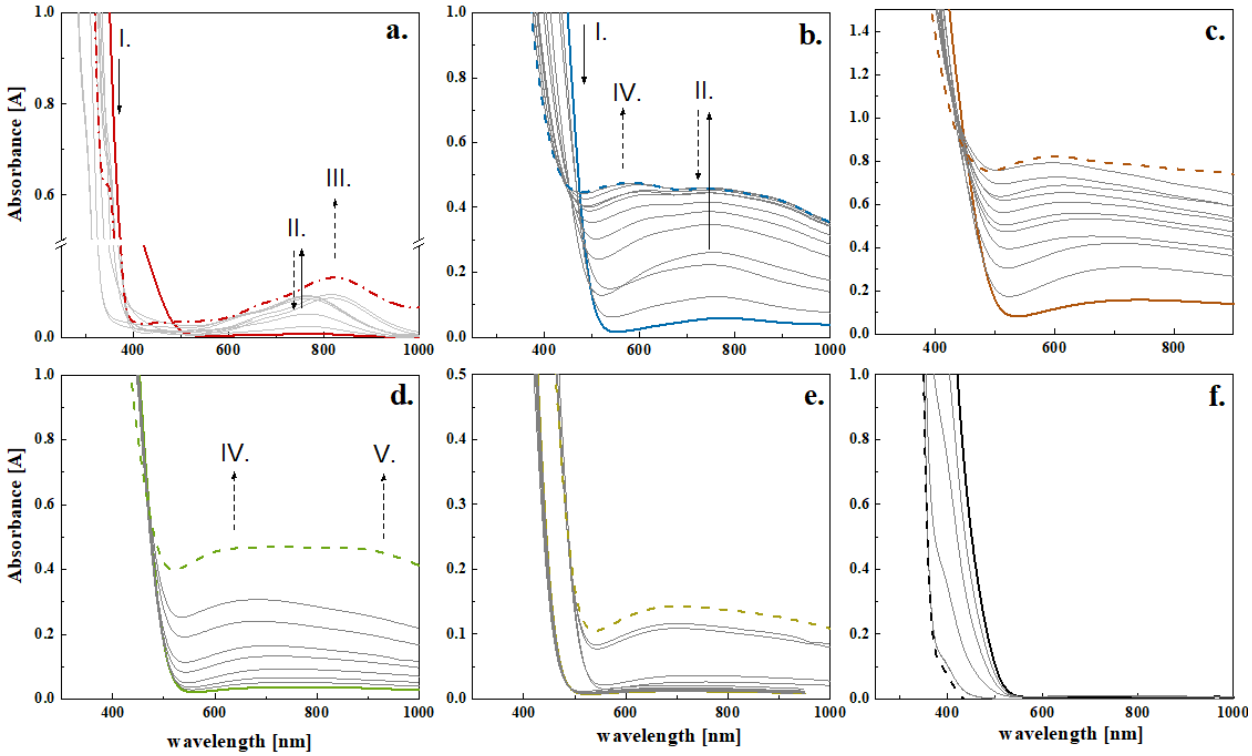


Figure A3. Absorbance change of the solutions containing 0.005 mol L^{-1} V(V) and 0.01 mol L^{-1} Thiolacetic acid recorded in 1 cm cuvette at various pH : a.) pH=2.1 b.) pH=3.0 c) pH=4.0 d.) pH=5.1 e.) pH=6.8 f.) pH=8.5. Full line marks measured solution in t =2 min and dashed line marks solution measured in t_{eq}.

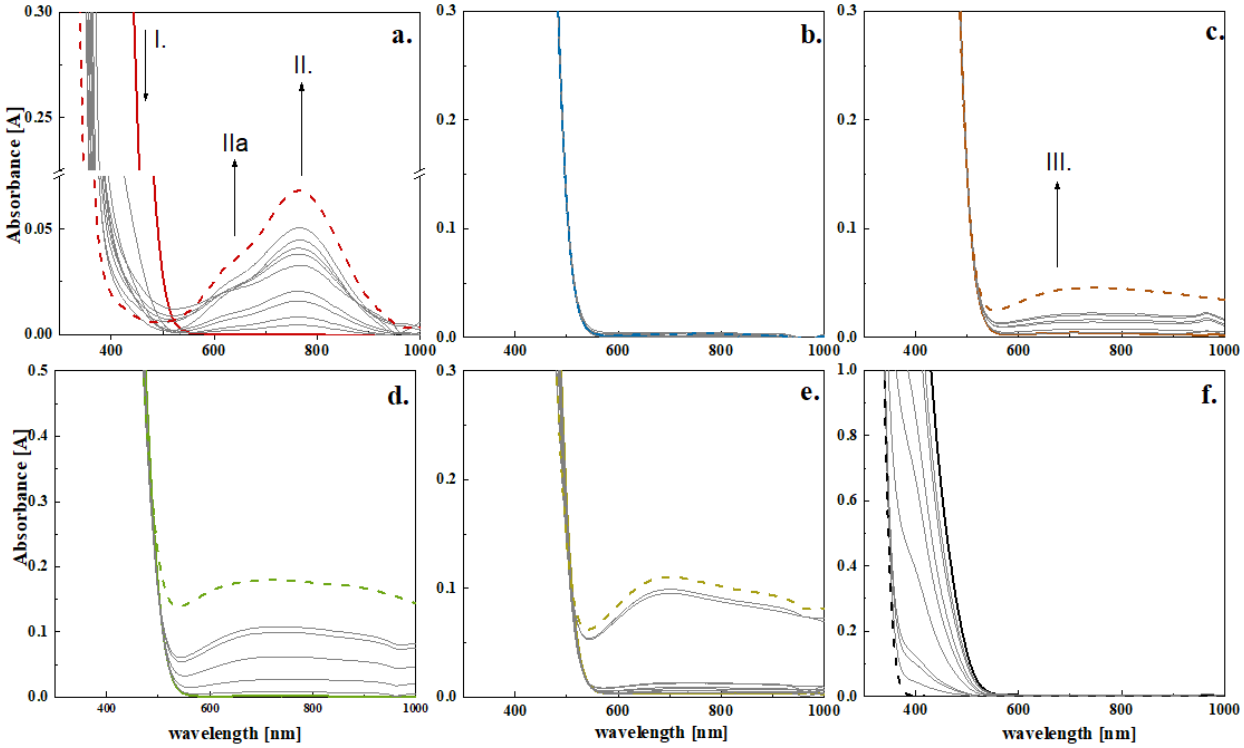


Figure A4. Absorbance change of the solutions containing 0.005 mol L^{-1} V(V) and 0.01 mol L^{-1} Ethanethiol recorded in 1 cm cuvette at various pH: a.) pH=2.2 b.) pH=3.1 c) pH=4.0 d.) pH=5.1 e.)pH=6.8 f.) pH=8.6. Full line marks measured solution in t =2 min and dashed line marks solution measured in t_{eq}.

V(+V)-L-cysteine reaction kinetics

The pH-dependant reaction of V(V) and L-cysteine followed a second-order reaction as mentioned in the main manuscript. The data were calculated based on the equation:

$$\frac{1}{C} = \frac{1}{C_0} + k * t$$

Where C is the time dependant V(V) concentration, C_0 is inital V(V) concentration and k is net rate reaction coefficient. Concentration of V(V) was expressed by extrapolating V(V) intensities measured in time to the one measured at t = 0 by ion chromatography. Net rate reaction coefficient and R values were calculated using SOLVER option in Excel by means of maximum non-linear regression. In Table S1 measured net rate reaction coefficients and R^2 values are presented. Non-linear V(V) reduction is visible and explained in more details in the main document. Accuracy of measurements expressed by R^2 seem to be lower on acidic values which could be linked to the more dynamic redox medium on such conditions.

Table A11. Dependance of net rate coefficient (k) and accuracy (R^2) on pH of the solution containing 0.005 mol L^{-1} V(V) and 0.01 mol L^{-1} L-cysteine.

pH	k, M^{-1} s^{-1}	R^2
2.2	2.2×10^{-1}	0.83
3.1	3.9×10^{-2}	0.84
4.1	2.1×10^{-2}	0.97
5.0	8.7×10^{-3}	0.89
6.8	1.3×10^{-2}	0.99
7.5	9.2×10^{-3}	0.99
8.5	1.9×10^{-3}	0.99

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