Platinum speciation in the presence of natural organic matter in a simplified freshwater medium investigated using HPSEC-ICP-MS

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AUTHOR CONTRIBUTION STATEMENT

Océane Hourtané: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. **D. Scott Smith:** Resources, Supervision, Visualization, Writing – review & editing. **Claude Fortin:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

ENVIRONMENTAL CONTEXT

Platinum is a metal of emerging concern in ecotoxicology, mainly due to its dissemination in the environment through the abrasion of car catalysts. However, its aqueous speciation in natural waters remains poorly known and its binding to organic matter may modify its effects on organisms. Experimental measurements revealed the complex chemistry of Pt, low complexation and very slow kinetics. The use of Pt^{II} would be recommended for chronic exposure experiments.

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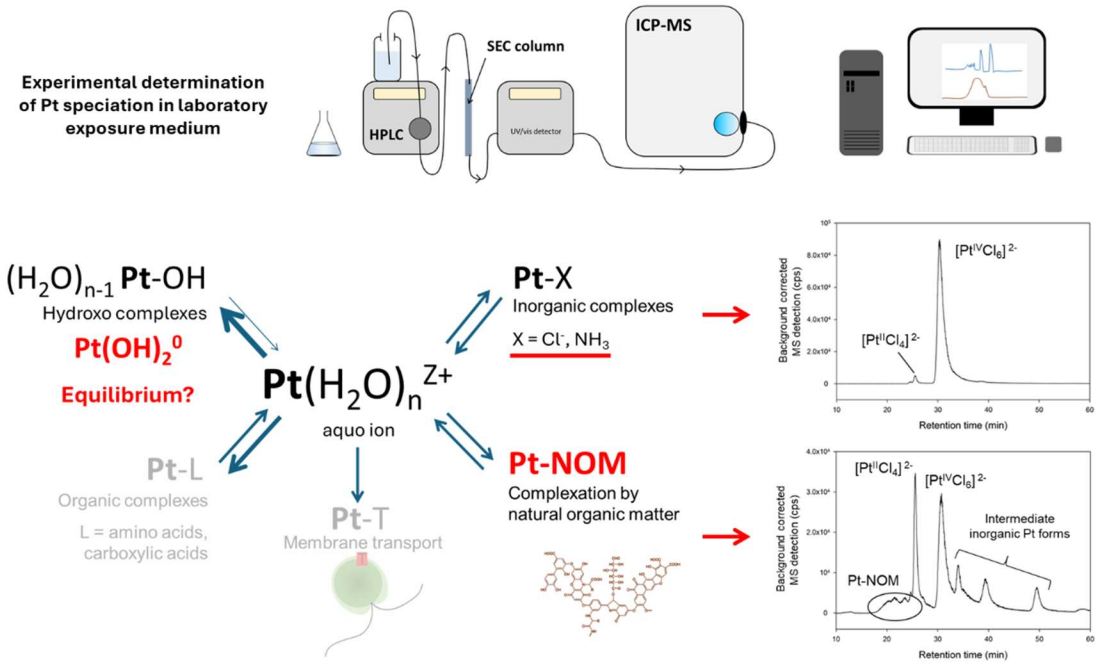
ABSTRACT

Rationale. It is a common consensus that the free ion concentration of a metal in an aqueous medium can be a good indicator of its toxicity. In this context, the ability to determine metal speciation is of paramount importance to evaluate possible impacts on aquatic ecosystems. While speciation can be predicted for well-studied metals, the task is difficult when little information on thermodynamic constants is available, as it is the case for platinum. It is then necessary to turn to experimental methods. **Methodology.** For this purpose, a high-performance size exclusion liquid chromatography method coupled with inductively coupled plasma mass spectrometry for online metal detection was used. In a synthetic freshwater medium, we first explored the inorganic speciation of platinum (added as Pt^{IV}) at pH 5 and 6, and using three equilibration periods (4 min, 48 hours and 1 week). We then tested two different conditions with freshwater natural organic matter (NOM) from four different origins, both expected to provide different levels of complexation: Low (pH = 5; [NOM] = 3 mg C L^{-1}) and high (pH = 6; [NOM] = 10 mg C L^{-1}). **Results.** Several inorganic forms of Pt (II and IV) were identified, with good separation and repeatability. In the absence of NOM, dissociation of $[Pt^{IV}Cl_6]^{2-}$ complexes was clearly more important at pH 5 than at pH 6. Also, $[Pt^{IV}Cl_6]^{2-}$persisted over time, even after a week even though this redox form is believed to be unstable in these conditions, suggesting that thermodynamic equilibrium was not reached. **Discussion.** Weak complexation by NOM was observed and the initial form of $[Pt^{IV}Cl_6]^{2-}$ persisted. However, the presence of NOM resulted in the formation of additional Pt inorganic species. These unidentified peaks, which were relatively more abundant at high NOM levels, were interpreted as intermediate species between $[Pt^{IV}Cl_6]^{2-}$ and Pt-MON complexes.

Keywords: complexation, data-poor metal, dissociation, equilibrium, hyphenated technique, liquid size exclusion chromatography, natural organic matter, platinum, reduction, speciation.

GRAPHICAL ABSTRACT



INTRODUCTION

Any natural aquatic environment is both complex and unique. The same goes for natural organic matter (NOM), which may give water its brownish color. NOM is a heterogeneous mixture of functionalized organic macromolecules, derived from living organisms. It is also omnipresent in natural waters with 90% dissolved organic carbon concentrations measured in the aquatic continuum ranging from 0.2 to 34.3 mg C L^{-1} according to the extensive literature review by Massicotte *et al.* (2017). NOM from a terrestrial origin, described as allochthonous, mainly comes from soil run-offs from watershed while autochthonous NOM originates mostly from primary production in the water column (Thurman 1985, Wetzel 2001, Thurman 2012). Both their origin and composition can also vary depending on other factors associated with seasonal variations and the extent of organic matter degradation (Beckett and Ranville 2006, Ma *et al.* 2021). Therefore, the

molecules composing NOM can have a wide range of characteristics. This complex nature of NOM has led to extensive research about its composition, in the 70's and in the 80's. From early on, NOM was operationally separated into different fractions, the main ones being humic acids (HA) and fulvic acids (FA). The former tends to be composed of larger aromatic molecules, mainly from a terrestrial origin compared to the latter fraction, which tends to contain smaller more functionalized molecules. However, whether in terms of molecular weight or chemical composition, there is no strict difference between HA and FA (Thurman *et al.* 1982, Thurman 1985, Findlay and Parr 2017, Schellekens *et al.* 2017). Therefore, such compartmentalization of NOM has since lost some of its appeal. It is more accurate to consider the NOM molecular weights as a continuum with no threshold value between HA and FA. Determination of optical properties are often determined, as they can offer a good insight into NOM composition (Al-Reasi *et al.* 2013, Vogt *et al.* 2023, Zilber *et al.* 2024).

Size exclusion chromatography (SEC) can be used for the determination of humic substances molecular weight (MW) (Zhou *et al.* 2000, McAdams *et al.* 2018). The mobile phase drives the sample through a column containing a porous stationary phase, generally silica or polymer gels (Lubomirsky *et al.* 2021). Low MW molecules can pass through small pores that are inaccessible to their high MW counterparts. They cover a greater distance and therefore exit the column later. On the contrary, high MW molecules are excluded from small pores, reaching the end of the column faster (Linsay 1987). Hence the name: size exclusion chromatography. An important factor to take into consideration is the choice of calibration standards. When studying humic substances, sodium polystyrene sulfonate standards (PSS) are recommended as they have a comparable size to mass ratio, allowing good comparison of their retention times with MW (Chin *et al.* 1994, Schimpf and Petteys 1997). Both PSS standards and NOM molecules can be detected at 254 nm when exiting the column using an UV/visible spectrometer (Zhou *et al.* 2000, Bolea *et al.* 2006). While this detection method has been criticized in the past because NOM molecules are unequally optically active, it has been found to yield acceptable results and is commonly used for SEC (Her *et al.* 2002). Furthermore, the choice of elution parameters is also of paramount importance. To avoid humic substances conformation modifications (such as coiling and uncoiling) during elution, which would lead to poor resolution for the

chromatograms, mobile phase and sample matrix should have similar characteristics: pH and ionic strength (Chin and Gschwend 1991). Size exclusion chromatography allows to determine the range of MW for NOM, as well as to calculate its average and median MW. Studies for which this method was used determined the average dissolved NOM MW to be mostly around 0.5 to 6 kDa (Chin *et al.* 1994, Zhou *et al.* 2000, O'Loughlin and Chin 2001, Her *et al.* 2002, Perminova *et al.* 2003, McAdams *et al.* 2018). In addition, the MW determined for the same NOM standard from Suwannee River Fulvic Acid (SRFA), were comparable between the different studies: 1.7 to 2.3 kDa for the weight average MW and 0.9 to 1.6 kDa for the number average MW.

Since SEC allows a separation of NOM molecules based on their MW, a hyphenated technique of high-performance size exclusion chromatography (HPSEC) and inductively coupled plasma mass spectrometry (ICP-MS) can be used to determine metal complexation by NOM, which is of critical importance in both the geochemical and ecotoxicological context. Indeed, the presence of NOM has been shown to modify Pt mobility through environmental compartments (Kubrakova *et al.* 2017), bioavailability to aquatic organisms (Dubiella-Jackowska *et al.* 2009) as well as its biological impact (Diehl and Gagnon 2007). Through the MW based separation, HPSEC-ICP-MS also allows to determine a metal's susceptibility to complexation by different MW fractions NOM range (Wu *et al.* 2004, Zhao *et al.* 2024). Indeed, functional groups on NOM molecules allows the binding of cations, among which, trace metals. Hard ligands such as oxygen-containing functional groups (carboxylic, phenolic, carbonyl) have a higher affinity for hard metals, while soft ligands nitrogen- or sulfur-containing functional groups (amines, thiols) will preferentially bind soft metals such as Pt (Pearson 1993, Smith *et al.* 2002). Furthermore, HPSEC-ICP-MS has been used to determine Pt complexation by different organic macromolecules in the past: NOM (Menzel *et al.* 2001) but also proteins to study intracellular fate and potential sequestration of this metal (Klueppel *et al.* 1998, Lesniewska *et al.* 2004). Therefore, HPSEC-ICP-MS coupling is a valuable tool to assess metal speciation both in intra- and extracellular media. In addition to the estimation of complexation by organic molecules, this technique can allow the separation of some inorganic Pt species (Menzel *et al.* 2001) which could then be identified. It thus has the potential to provide a general picture of speciation.

This hyphenated method shows promise for successfully determining Pt speciation in the presence of NOM: both organic and inorganic. Indeed, the increase in the discharge of Pt in aquatic environments due to its use in automobile catalysts as well as cancer treatment, has led to an increased interest for its possible environmental mobility and toxicity (Artelt *et al.* 2000, Lenz *et al.* 2005). However, the lack of thermodynamic data, as well as the controversy surrounding the few available ones (Azaroual *et al.* 2001, 2003, Byrne 2003) and the suspected instability of Pt^{IV} has led to difficulties in predicting its speciation. The high affinity of Pt^{2+} for NH_3 (Feifan *et al.* 2017) as well as the estimation of binding constants using Linear Free-Energy Relationships (Tipping *et al.* 2011, Le Faucheur *et al.* 2019) suggests a high affinity for NOM. Yet, experimental data indicate a lower affinity of Pt for NOM than anticipated (Menzel *et al.* 2001, Hourtané *et al.* 2022, Hourtané *et al.* 2024).

Insufficient data from the literature calls for thorough examination of Pt^{IV} speciation in the presence of NOM using an experimental technique. For this purpose, HPSEC-ICP-MS was used in this work to (1) assess the adaptability of this method to a freshwater context (low ionic strength of both samples and mobile phase to keep humic substances uncoiled) to study Pt(II, IV) speciation in exposure media; (2) obtain a good resolution and separation of inorganic Pt forms and identify the main ones; (3) evaluate complexation of Pt by NOM in two different conditions: low and high complexation.

MATERIAL AND METHODS

Labware and solutions

Before use, labware was washed with deionized water, soaked in a 10% nitric acid solution for 24-h and then rinsed with deionized water three times and with ultrapure (MilliQ) water (≥18 MΩ·cm) five times. All solutions (stock solutions, samples, mobile phase as well as calibration standards) were all prepared using ultrapure water. pH was measured with electrode Fisherbrand Accumet 13 620 299A and adjusted using NaOH (ACS grade; Fisher) and HNO_3 (Trace Metal grade; Fisher) solutions. Unless specified otherwise, all solutions were stored at 4°C.

The aqueous speciation of Pt was determined in the presence of NOM from different origins. Two of them were Suwannee River standards: humic acid (SRHA; catalogue number 3S101H) and fulvic acid (SRFA; catalogue number 2S101F) and were purchased as a dry powder from the International Humic Substances Society (IHSS). The powder was dissolved in a 10^{-2} M NaOH (Fisher) solution and agitated for 24 h in the dark before filtration through a polyethersulfone (PES) membrane (0.45 µm porosity; Pall Corporation; previously stored in ultrapure water for 24 h) to obtain stock solutions. The other two NOMs: Bannister Lake (BL-NOM) and Luther Marsh (LM-NOM) were obtained by reverse osmosis and treated with a batch of preconditioned cation exchange resin, following the protocol described in Al-Reasi *et al.* (2012). Some of their characteristics are further described in the work of Al-Reasi *et al.* (2013) and Zilber *et al.* (2024). All NOM stock solutions were stored at 4°C in the dark and their dissolved organic carbon concentrations were determined analytically (Shimadzu VCPH).

To optimize elution quality and obtain suitable chromatograms, it is generally recommended in the literature that the mobile phase and samples have similar ionic strength and pH (Chin and Gschwend 1991). In addition, the conformation of NOM molecules is also sensitive to these parameters: coiling at low pH and/or high ionic strength and uncoiling at high pH and/or low ionic strength (Ghosh and Schnitzer 1980). To minimize such conformational changes, the sample matrix was chosen as the mobile phase for the experiment carried out in this work. Both were a simplified freshwater algal growth medium, the Modified High Salt Medium – Simplified (MHSM-S; $[K^+] = 4.00 \cdot 10^{-3}$ M, $[Na^+] = 1.02 \cdot 10^{-4}$ M, $[NH_4^+] = 9.37 \cdot 10^{-4}$ M, $[Ca^{2+}] = 6.82 \cdot 10^{-4}$ M, $[Mg^{2+}] = 8.11 \cdot 10^{-5}$ M, $[NO_3^-] = 6.30 \cdot 10^{-3}$ M, $[SO_4^{2-}] = 8.11 \cdot 10^{-5}$ M; hardness of 0.76 mM). Samples and mobile phase were adjusted to either pH = 5.00±0.05 or pH = 6.00±0.05 depending on the experiment carried out. This medium was to mimic the experimental conditions of Hourtané *et al.* (2024) who showed that the presence of NOM could cause a significant increase of Pt uptake by a green alga.A fresh mobile phase was prepared for each experiment at least 48 h before in 1 or 2 L Teflon bottles and the pH adjusted using NaOH and HNO_3 solutions. Once the preparation was complete, the solution was filtered through a 0.2 µm PES membrane (Pall corporation) that was previously soaked in ultrapure water for 24 h. Since changes in pH can lead to important modifications in speciation (Stumm

and Morgan 2012), and since no pH buffers were used, pH of the mobile phase was verified and adjusted again if needed before use. For each experimental condition, a 10 mL sample was prepared in a 50 mL polypropylene (PP) tube (Sarstedt) using MHSM-S medium as the electrolyte matrix. Pt was added under different forms as detailed in following section.

Investigation of inorganic speciation

In the absence of NOM, different platinum compounds were added to the sample matrix (360 nmol L^{-1}) and injected in order to identify peaks and relate them to species. Commercially available complexes $[Pt^{II}(NH_3)_4]^{2+}$, $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{IV}Cl_6]^{2-}$ were respectively added from dissolved $(NO_3)_2Pt^{II}(NH_3)_4$ (4.260% (w/w) as a stock solution; Umicore), $K_2Pt^{II}Cl_4$ (powder; Heraeus chemicals), and $(NH_4)_2Pt^{IV}Cl_6$ (1000 µg/mL stock solution in 10% (v/v) HCl; Plasma CAL, SCP Science). For the first two Pt forms, pH was adjusted to 6.00, and speciation was determined by HPSEC-ICP-MS in less than 3 h. Because of the suspected instability of $[Pt^{IV}Cl_6]^{2-}$, especially when exposed to light (Michaud-Valcourt *et al.* 2024), the Pt^{IV} solution was prepared in the dark immediately prior to its injection without prior pH adjustments (pH = 4.09 ± 0.43 (n = 5) determined in subsequent solutions prepared in the same conditions).

Complexation determination

To study speciation in the presence of NOM, a mixture of critical metals Ga, La and Pt (360 nmol L^{-1}; prepared from Plasma CAL, SCP Science standard solutions) was used, however, only Pt speciation is presented in this work. Measured Pt concentrations were comprised between 276 and 352 nmol Pt L^{-1}, this metal having been added in the initial form of $[Pt^{IV}Cl_6]^{2-}$. The presence of the other metals is not expected to have affected neither Pt inorganic speciation nor its complexation by NOM considering the low metal to ligand ratio (see Supplementary material, *Repeatability of speciation results* section). In order to reproduce the experimental conditions of Hourtané *et al.* (2024), an equilibration period of 48 h at 20°C in the dark was respected. The air-exchange was rendered possible by a light unscrewing of the tube lids. Samples were opened and swirled to perform pH adjustments: immediately after preparation and then every 24 h. Bacterial growth was not tested but is believed to have been nonsignificant. Different NOM conditions (No NOM, SRHA, SRFA, BL-NOM, LM-NOM) were compared for two different conditions that were expected to

provide different levels of complexation: Low (pH = 5.00 and [NOM] = 3 mg C L^{-1}) and high (pH = 6.00 and [NOM] = 10 mg C L^{-1}). For each experiment, samples were adjusted to the same pH as the mobile phase before the equilibration period. Given the importance of pH for speciation determination, pH was adjusted in the samples every 12 to 24-hours during the equilibration period and again before filtration (0.45 µm PES syringe filters, VWR) and injection.

Speciation measurements with HPSEC-ICP-MS

Speciation was experimentally determined using the online coupling of high-performance liquid chromatography (HPLC, Spectra, Thermo Fisher Scientific) with a SEC column (Superdex^{TM} 75 Increase 10/300 GL; Cytiva) with real time metal quantification of the eluate with an ICP-MS (Thermo scientific iCAP RQ) without using a collision cell. The method allows semi-quantitative determination of which size fraction a metal is present. The stationary phase in the column is composed of highly crosslinked agarose with covalently bound dextran chains. As described in the previous sections, the mobile phase had the same composition as the sample matrix. For all tests and experiments, an injection volume of 100 µL and a flow rate of 0.6 mL·min^{-1} were used.

As recommended in the literature when working with NOM, calibration of the column was performed using poly(styrenesulfonic acid sodium salt) (PSS) molecular mass analytical standards (Chin *et al.* 1994, Zhou *et al.* 2000, Bolea *et al.* 2006). Indeed, the standards used must have structures and behavior in solution similar to the studied macromolecules. In this context, the use of PSS standards is thus considered more appropriate than globular proteins which would then lead to an overestimation of molecular weight. PSS standards with certified MW of 208, 4230, 14900 and 80100 Da (Sigma Aldrich) were solubilized in the mobile phase/sample matrix (MHSM-S), at 250 g/L. Cysteine and EDTA (ethylenediaminetetraacetic acid) (10^{-2} mol L^{-1}) were also used as standards. Detection of standards as well as NOM was performed by UV/vis absorbance measurement at 254 nm at the end of the column. For metal detection, ^{194}Pt intensity (number of counts) was recorded. Both parameters were plotted as a function of retention time in order to determine metal speciation, particularly its complexation with NOM molecules of a wide MW range. To evaluate Pt recovery, samples were injected and collected at the end of the column for

metal quantification. This operation was performed twice: with and without the column, in order to determine the proportion of Pt losses that occur in the HPLC system and in the column. To maintain pressure in the absence of a column, a polyether ether ketone (PEEK) tube of 0.127 mm internal diameter was used instead. Total Pt concentrations were also measured in the samples, offline, using the same ICP-MS. Subsamples were collected prior to HPSEC-ICP-MS analysis and acidified at HCl 5% (V/V) using Trace Metal HCl (Fisher) to be quantified. The calibration curve was prepared in HCl 5% (v/v) using a 1000 µg Pt mL^{-1} stock solution ($(NH_4)_2Pt^{IV}Cl_6$; 10% HCl (V/V) Plasma CAL, SCP Science). Another solution: S409, 10 µg Pt mL^{-1} (C00-215 061-409, PlasmaCAL) was used to prepare quality controls with a tolerance threshold of 5% for curve validation and a Re/Rh internal standard was also co-injected with the samples to correct any possible drift during analysis. In-between samples, a solution of HCl 5% (v/v) was automatically pumped to rinse the system. No memory effect was observed.

As recommended by the supplier, the column was stored in 20% ethanol (v/v) (prepared from 95% denatured ethanol, HistoPrep grade, Fisher). Before use, the column was conditioned with MilliQ water, then with the mobile phase using twice the column volume (50 mL) for each. Every 5 samples, a sample containing EDTA and cysteine (10^{-2} mol L^{-1}) was injected to rinse the column. After each experiment, the column was flipped upside down to be rinsed with 50 mL of NaOH (0.5 M) to clean it from potential remaining NOM, then stored in 20% ethanol (v/v). A spring top device was added on the column to maintain pressure during storage and avoid any shrinking of stationary phase that could alter its properties.

Determination of NOM MW

For each NOM, the MW was experimentally determined based on chromatograms, using the method described in Zhou *et al.* (2000) from Yau *et al.* (1979). As humic substances can have a wide range of MW, the weight average MW (M_w), and the number average MW (M_n) (see Equations 1 and 2 below) tend to be different. Indeed, there is a greater contribution of the large molecules to M_w despite their smaller number. Therefore, polydispersity, which can be determined as $p = M_w/M_n$ is above 1. Equations 1 and 2

respectively show how M_w and M_n were calculated, h_i being the chromatogram height and M_i is the molecular weight corresponding to the eluted volume.

$$M_w = \sum_{i=1}^{n}(h_i \cdot M_i) / \sum_{i=1}^{n} h_i$$

Equation 1: weight average MW

$$M_n = \sum_{i=1}^{n} h_i / \sum_{i=1}^{n}(h_i / M_i)$$

Equation 2: number average MW

Data analysis

Column calibration and determination of NOM MW was performed with the HPLC and the absorbance data was automatically converted in mV before being processed with Chromeleon software (ver. 7.2SR5, see https://www.thermofisher.com/order/catalog/product/CHROMELEON7). For real time detection of Pt, HPLC and ICP-MS were used online with the software Qtegra (ver. 2.10.4345.236, see https://www.thermofisher.com/order/catalog/product/IQLAAGGACBFAOVMBJD), able to communicate with Chromeleon 7 through ChromControl. Data for real time ^{194}Pt detection and for total Pt quantification were acquired by Qtegra, the former were later exported to Chromeleon 7 for peak integration. Chromatogram data were exported, background corrected and presented using SigmaPlot (ver. 10.0, see https://grafiti.com/sigmaplot-detail).

The relative proportions of different Pt species were determined as the area of its specific peak divided by the total area of the chromatogram. Note that they do not systematically add up to 100%. Moreover, the obtained values are purely indicative, as HPSEC-ICP-MS is a semi-quantitative method.

RESULTS AND DISCUSSION

Choice of sample and mobile phase matrix

For the determination of the MW of humic substances by HPSEC, the choice of column, mobile phase, and composition of calibration materials is crucial. It is typically

recommended in the literature to use a high ionic strength when performing SEC. One of the benefits of increasing ionic strength is that it can effectively reduce non-specific interactions including those between the M-NOM complexes and the stationary phase as showed by Zhao *et al.* (2024) with Ag. However, a large increase of ionic strength in the mobile phase compared to the sample could modify speciation before and/or during elution by reducing metal complexation by NOM due to the presence of competing ions in solution. So, there is a balance to be found between increasing recovery while maintaining the initial speciation. Nevertheless, the mobile phase and sample matrix used in this work both have a low ionic strength of $6 \cdot 10^{-3}$ eq L^{-1}. Indeed, sample matrix and mobile phase should have similar characteristics, and the purpose of this study was to determine Pt speciation in solutions with the same composition as the ones used for exposure of freshwater organisms (Hourtané *et al.* 2024). Furthermore, Superdex^{TM} columns are designed to reduce non-specific interactions with their agarose-based stationary phase and results from the work of Menzel *et al.* (2001) in a similar research context demonstrate that this type of column can be used at low ionic strength. Indeed, the authors showed that Superdex^{TM} 200 HR 10/30 allowed a good separation of NOM and platinum inorganic species in complex samples, even using MilliQ water as an eluent. In addition, increasing ionic strength would create another bias in MW determination as it would impact NOM molecules conformation compared to the desired experimental conditions (low ionic strength). Specifically, it would cause a coiling that could result in an underestimation of the molecular weight, since humic substances are likely to be found in uncoiled structures under typical freshwater conditions: neutral pH and low ionic strength (Ghosh and Schnitzer 1980).

In light of this information, special attention was given to the quality of the elution. As presented in the chromatograms from the following sections, it was satisfactory at low ionic strength with well-defined peak for PSS analytical standards and inorganic forms of Pt from the samples, good resolution of the column in the range of MW of interest and repeatable retention times. Recovery of Pt added as $[Pt^{IV}Cl_6]^{2-}$ was 57 ± 6% (n = 5) and similar in the absence and the presence of NOM. It was considered satisfactory, especially compared to the 32% obtained by Menzel *et al.* (2001) using Superdex^{TM} 200. Moreover, the potential dissociation of M-NOM complexes during elution could have negatively

impacted resolution. But, despite the important dilution throughout the elution process, this seems unlikely for Pt. This metal is known to have very slow reaction kinetics as suggested by Pt^{2+} low k_{-w} (water exchange rate constant within the coordination sphere) of $10^{-3}/10^{-4}$ s^{-1} (Richens 1997). Indeed, this implies a low reactivity of a metal in aqueous media, while elution was completed within 60 min. Therefore, the experimental conditions chosen were deemed suited to determine Pt speciation with this method.

HPSEC - Standard separation and evaluation of NOM MW

The Superdex^{TM} 75 Increase 10/300 GL column was calibrated using a mix of four PSS analytical standards prepared in MHSM-S (250 mg L^{-1} each). The retention times were respectively 12.6, 13.6, 15.7 and 28.1 min for the 80.1, 14.9, 4.2 and 0.2 kDa standards. The chromatogram obtained (Figure 1) shows symmetrical peaks and a good resolution of the column, especially for the MWs of interest with wide gap between the 4.2 and 0.2 kDa standards. There was also a good repeatability for the standards retention times, whatever the standard conservation method 4°C or -20°C, and retention times were similar at both pH conditions used in this work: pH = 5 and pH = 6 (see the 'SEC column calibration' section of the Supplementary material).

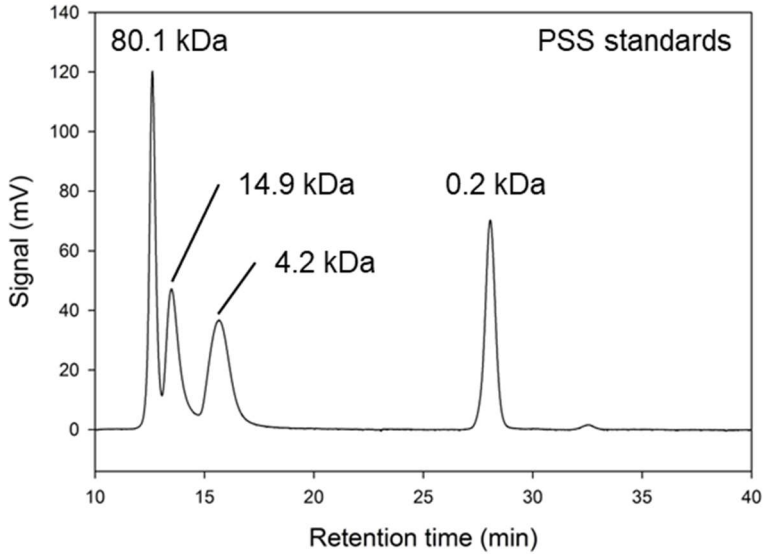


Figure 1: Chromatogram of the UV/vis detection after elution of the sodium polystyrene sulfonate standards standards mix in MHSM-S freshwater medium (pH = 6), column SuperdexTM 75 Increase 10/300 GL, mobile phase: MHSM-S, flow rate: 0.6 mL·min-1, injection volume: 100 µL

The retention times obtained for all four NOMs were similar between pH 5 and 6. As expected, the NOM solution contain a mixture of macromolecules of a wide range of MW, resulting in large peaks (Figure 2). In addition, NOM seem to be composed of 3 to 4 main classes of molecules as suggested by the apparent presence of several unseparated peaks. Based on the absorbance signal at 254 nm, MW appeared to be globally comprised between 0.1 and 15.5 kDa. Some differences in the respective average MW among the four NOMS were noted (Table 1). They can be sorted from the highest to the lowest MW with the following order: LM≥SRHA≈SRFA≥BL, which is in accordance with optical indices determined by Zilber *et al.* (2024).

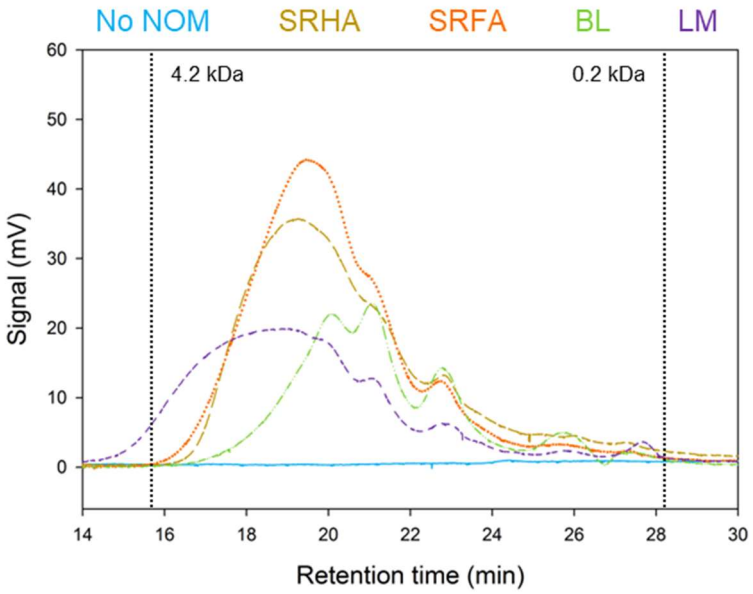


Figure 2: Chromatogram of the UV/vis detection after elution of NOM solutions in MHSM-S [NOM] = 30 mg C/L on the column (pH = 6); No NOM: solid line, blue; SRHA (Suwannee River Humic Acid): long dash, gold; SRFA (Suwannee River Fulvic Acid): dotted line, orange; BL (Bannister Lake): dash-dot-dot, green; LM (Luther Marsh): short dash, purple, column Superdex^{TM} 75 Increase 10/300 GL, mobile phase: MHSM-S, flow rate: 0.6 mL·min^{-1}, injection volume: 100 µL

The measured MW values presented in Table 1 are in good agreement with previous work on NOM, MW being mostly comprised between 0.5 to 6 kDa. (Chin *et al.* 1994, Zhou *et al.* 2000, O'Loughlin and Chin 2001, Her *et al.* 2002, Perminova *et al.* 2003, McAdams *et*

al. 2018). For SRHA and SRFA, which have been studied in a similar context in the past, average MW reported in the literature are slightly lower, especially in the case of SRHA (see Table 1). However, the opposite might have been expected due to various reasons related to the low ionic strength elution conditions of this work. The potential smaller apparent size of the pores due to repulsion effects from the stationary phase might have led to an overestimation of NOM MW. Moreover, NOM molecules could actually have been larger than previously reported because of their uncoiled form in the experimental conditions. Besides, PSS standards are also expected to have been in an uncoiled conformation, likely more linear than the NOM molecules, which could have induced a bias on column calibration and also lead to an overestimation of NOM MW. As for the increase of non-specific interactions in the column, it could influence the observed MW in different ways: either an underestimation due to increased adsorption, attraction or H bonding delaying elution or an overestimation due to repulsion of anionic NOM molecules and an earlier exit of the column (Pelekani *et al.* 1999). While a lot of factors could have impacted MW estimation at low ionic strength, results suggest that the use of PSS standards was still adequate for calibration of the column used in this work. Indeed, the obtained MW are satisfyingly close to values from the literature. It should also be noted that improvements in stationary phase and calibration standards characteristics since the 90's can also lead to lower observed average MW values due to a greater contribution of low MW molecules, as documented by McAdams *et al.* (2018).

Table 1: Weight average MW (M_w), number average MW (M_n) and polydispersity (p) of the four NOMs tested as experimentally determined by HPSEC based on prior calibration with sodium polystyrene sulfonate standards. SRHA = Suwannee River Humic Acid; SRFA = Suwannee River Fulvic Acid; BL = Bannister Lake; LM = Luther Marsh (n=1). Comparable values from the literature are also listed: SRHA and SRFA (O'Loughlin and Chin 2001, Her et al. 2002) ; SRFA only (Chin and Gschwend 1991, Chin et al. 1994, Zhou et al. 2000, McAdams et al. 2018)

Determined in this study (HPSEC)

NOM	M_w (Da)	M_n (Da)	p
SRHA	942	517	1.8
SRFA	1020	640	1.6
BL	762	513	1.5
LM	1277	628	2.0

From the literature (HPSEC, similar context and calibration)			
NOM	**M_w (Da)**	**M_n (Da)**	**p**
SRHA	3305, 3703	1807, 1934	1.7-2.8
SRFA	1310-1700	909-1614	1.2-2.3

Pt inorganic speciation – identification of complexes

Chromatograms obtained for all Pt inorganic forms tested are shown in Figure 3. Results for $[Pt^{II}(NH_3)_4]^{2+}$ (Figure 3.a, pH adjusted to 6, t<3 h) were inconclusive. No change in speciation were suspected to be responsible for this result since this form has been shown to be stable over 96 h under comparable experimental conditions (Michaud-Valcourt *et al.* 2024). The low count as well as delayed and wide peaks both point to adsorption, which can be caused by non-specific interactions such as attraction of opposite charges. Since $[Pt^{II}(NH_3)_4]^{2+}$ is the only cationic complex tested, it is likely that its positive charge and subsequent attraction to electronegative O-donor sites of the polymer materials from the HPLC tubes (polyether ether ketone) and the stationary phase (crosslinked agarose and dextran) might have led to this result. This suggests that this column and/or setup is not adequate for observing positively charged inorganic Pt species. However, the satisfying recovery obtained for the other samples presented in the following sections suggests that negatively charged complexes are detected. It also allows for some degree of interpretation on Pt inorganic speciation. Results obtained for the other inorganic forms: $[Pt^{II}Cl_4]^{2-}$ (Figure 3.b) and to $[Pt^{IV}Cl_6]^{2-}$ (Figure 3.c), allowed for an identification of the peaks. One main peak was obtained at 26 min for $[Pt^{II}Cl_4]^{2-}$ and at 31 min for $[Pt^{IV}Cl_6]^{2-}$. In the solution containing $[Pt^{II}Cl_4]^{2-}$, another peak which could be a dissociation and/or hydrolysis product of $[Pt^{II}Cl_4]^{2-}$ (likely an intermediate species between $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{II}OH_2]^{0}$) was observed at 39 min. For $[Pt^{IV}Cl_6]^{2-}$, two other small peaks were observed at 25 and 26 min. The second one seems to correspond to $[Pt^{II}Cl_4]^{2-}$ and the first one could not be identified but might be an intermediate form between $[Pt^{IV}Cl_6]^{2-}$ and $[Pt^{II}Cl_4]^{2-}$. Candidate complexes include $[Pt^{IV}Cl_4(OH)(H_2O)]^{-}$, $[Pt^{III}Cl_5]^{2-}$, $[Pt^{III}Cl_6]^{3-}$, $[Pt^{III}Cl_4(OH)(H_2O)]^{2-}$ (Pozdnyakov *et al.* 2015).

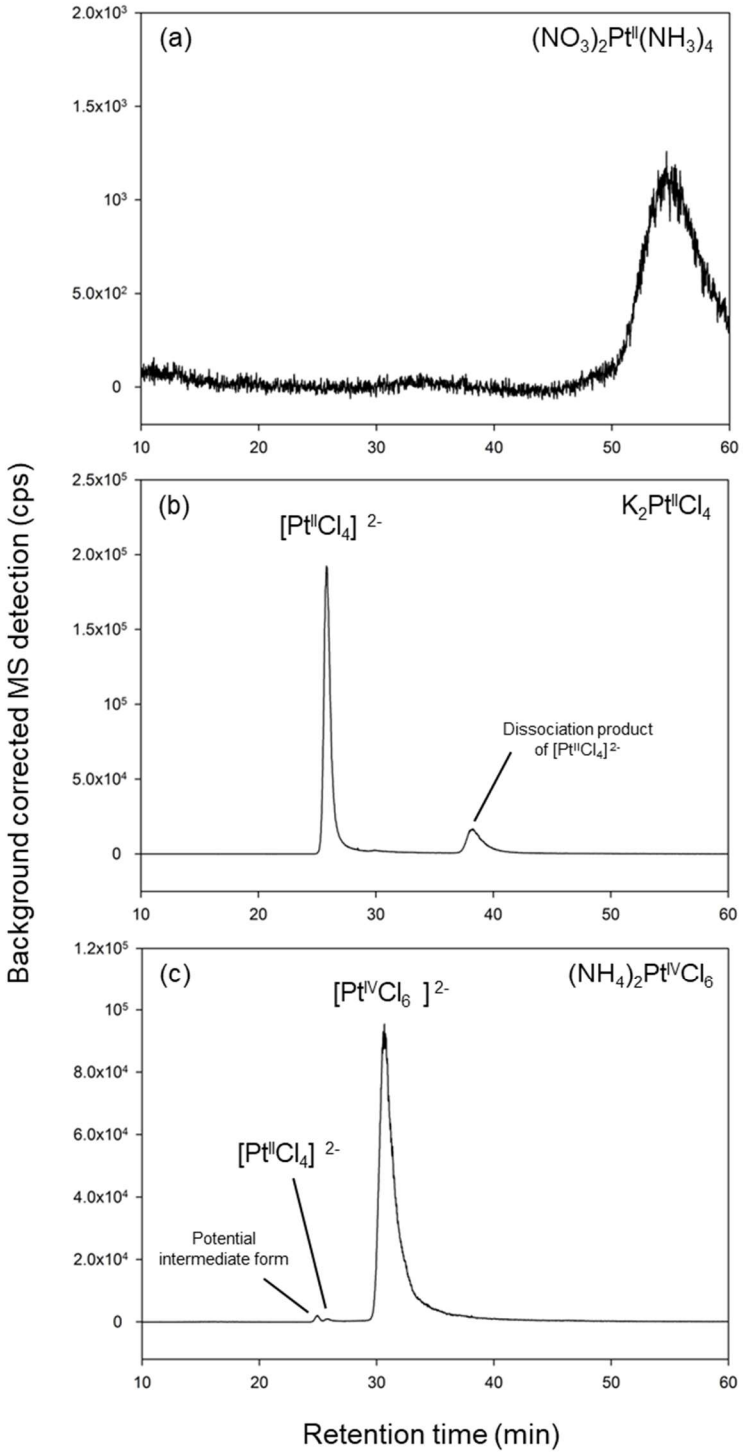


Figure 3: Chromatograms of Pt using different initial forms. $[Pt^{II}(NH_3)_4]^{2+}$ (a, pH = 6.00, preparation time <3 h, [Pt] = 480 nM); $[Pt^{II}Cl_4]^{2-}$ (b, pH = 6.00, preparation time <3 h), [Pt] = 168 nM); and $[Pt^{IV}Cl_6]^{2-}$ (c, pH non-adjusted, preparation time = 4 min, [Pt] = 374 nM), detection of ^{194}Pt, column Superdex™ 75 Increase 10/300 GL, mobile phase: MHSM-S, flow rate: 0.6 mL·min^{-1}, injection volume: 100 µL

The shorter retention time of $[Pt^{II}Cl_4]^{2-}$ compared to $[Pt^{IV}Cl_6]^{2-}$ was somewhat unexpected. However, while the MW distribution observed for NOM molecules is consistent with other studies, the elution of the inorganic Pt species seems to be largely governed by non-specific interactions with little regard for their actual MW over such a small size scale. Nevertheless, the obtained peaks are well defined and have repeatable retention times. Thus, the interpretations of Pt inorganic speciation in the following sections were based on these retention times.

Platinum inorganic speciation – Influence of pH

Platinum speciation was examined for the 48 h equilibrated solutions of $(NH_4)_2PtCl_6$ at the two working pH values in the absence of NOM (Figure 4). While retention times of the inorganic forms were similar at pH 5 and 6, the peak areas were different.

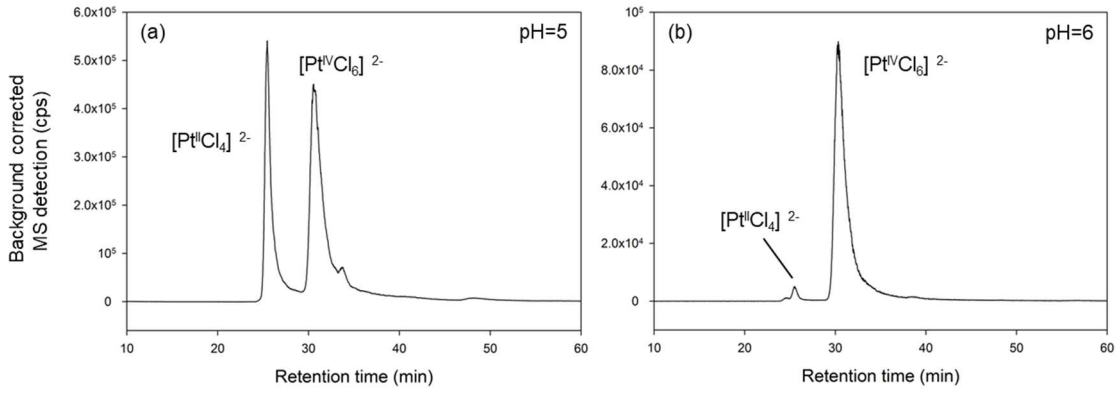


Figure 4: Chromatograms of 48 h equilibrated solutions of $(NH_4)_2PtCl_6$ in the absence of NOM at pH = 5.00 (a), [Pt] = 276 nM and pH = 6.00 (b), [Pt] = 312 nM, detection of ^{194}Pt, column Superdex™ 75 Increase 10/300 GL, mobile phase: MHSM-S, flow rate: 0.6 mL·min^{-1}, injection volume: 100 µL

Thermodynamically, Pt^{IV} in our conditions should be reduced to Pt^{II} and be present at equilibrium as $[Pt^{II}(OH)_2]^0$ (Colombo *et al.* 2008). The fact that distinct peaks are observed when Pt^{IV} and Pt^{II} are injected suggests that the equilibrium is not reached despite the 48-h equilibration period. An aging experiment of similar samples presented in the supplementary material showed that $[Pt^{IV}Cl_6]^{2-}$ actually remains the main species in solution at pH = 6 for over a week, even when exposed to light. In our conditions, Pt^{IV}

dissociation and reduction are slower than what others have found (Nachtigall *et al.* 1997, Michaud-Valcourt *et al.* 2024). However, they both used UV-vis absorbance for Pt complexes detection which is less sensitive than real time ICP-MS detection (Nischwitz *et al.* 2003) and therefore required the use of higher concentrations of Pt complexes. Also, as stated above, Pt^{II} is expected to have slow kinetics in aqueous media (Richens 1997). At pH = 6 (Figure 4.b), two other minor inorganic forms were also obtained. With retention times of 25 and 26 min, they seem to respectively correspond to the possible intermediate form and $[Pt^{II}Cl_4]^{2-}$ that were identified for samples prepared only 3 h before injection (see Figure 3.c). In fact, both chromatograms (Figure 4.b and Figure 3.c) are very similar. This suggests that speciation remains relatively stable at this pH and in this time span, with only a slightly greater proportion of $[Pt^{II}Cl_4]^{2-}$ at 48 h (2.3% of detected Pt) compared to 4 min (0.2%), suggesting a very slow dissociation.

On a different note, after equilibration at pH = 5 (Figure 4.a) speciation is quite different compared to pH = 6 (Figure 4.b) suggesting a faster dissociation and reduction. At pH = 5, the peak areas corresponding to $[Pt^{II}Cl_4]^{2-}$ (26 min) and $[Pt^{IV}Cl_6]^{2-}$ (31 min) were of the same order of magnitude (35 and 47% of detected Pt respectively). A peak corresponding to another unidentified inorganic Pt form was also observed at 34 min, but is co-eluted with $[Pt^{IV}Cl_6]^{2-}$. Such a large difference in dissociation kinetics over such a small pH difference is surprising. It is possible that the dissociation/reduction mechanisms occurring at pH = 5 and 6 might be different, and/or that a limiting step could be favored at pH 5.

Pt speciation and complexation in the presence of NOM

Speciation of Pt was determined in two conditions, low (pH = 5 and [NOM] = 3 mg C L^{-1}) and high (pH = 6 and [NOM] = 10 mg C L^{-1}) complexation, and the chromatograms obtained are presented in Figure 5. For both conditions, some complexation by NOM is observed as a low intensity large cluster of peaks corresponding to the NOM peak that was previously determined in Figure 2, the range of retention time corresponding to Pt complexation (16 to 24 min) is highlighted in all chromatograms of Figure 5. The low extent of Pt binding by NOM is coherent with previously published results in similar conditions (Hourtané *et al.* 2022, Hourtané *et al.* 2024). It is also consistent with its low affinity for carboxylic groups. As a soft metal, Pt^{2+} would preferentially be bound to N or

S containing groups, high affinity group which are present in lower proportions (Pearson 1993, Smith *et al.* 2002). This might result in a saturation of NOM complexation sites for Pt. Since dissociation of Pt-NOM complexes during elution is unlikely due to the slow kinetics of Pt^{2+} in aqueous solutions and because the recovery of Pt was similar in the presence and absence of NOM (57 ± 6%; n = 5), it follows that Pt losses (43 ± 6% including 32 ± 3% in the HPLC system and 11 ± 4% in the column; n = 5) might be mainly from inorganic forms. It is possible that positively charged species might have been retained through adsorption as hypothesized to explain the $[Pt^{II}(NH_3)_4]^{2+}$ delayed wide peak. Table 2 shows the proportion of the different Pt species obtained by peak integration detected in the chromatograms. These values must be interpreted with caution since HPSEC-ICP-MS is a semi-quantitative method. Moreover, it should also be noted that the retention time for $[Pt^{II}Cl_4]^{2-}$ being included in the range for Pt-NOM complexes made integration of both peaks, and thus the estimation the relative proportion of the corresponding species difficult.

As expected, complexation seems to be greater at pH = 6 and [NOM] = 10 mg C L^{-1} (Figure 5.e to h and Table 2) compared to pH = 5 and [NOM] = 3 mg C L^{-1} (Figure 5.a to d and Table 2). Moreover, in terms of NOM, there seems to be a distinction between two groups: there was a systematically lower complexation of Pt with SRHA and LM compared to SRFA and BL. The former have a higher molecular weight (Table 1) and are also believed to be more aromatic (Zilber *et al.* 2024). The latter might be more functionalized, which could explain the complexation results.

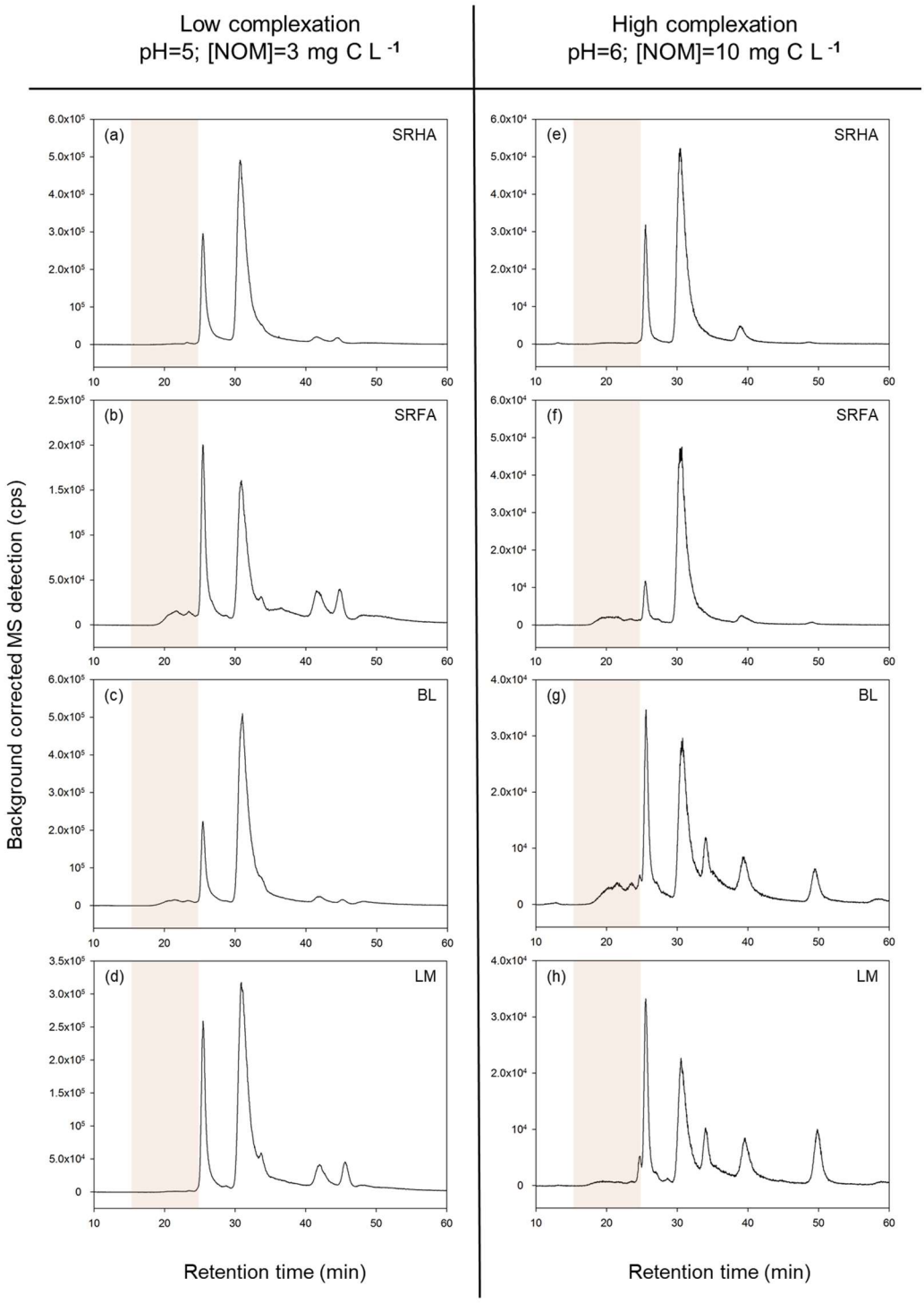


Figure 5: Chromatograms of 48 h equilibrated solutions of $(NH_4)_2PtCl_6$ in the presence of four different NOMs; pH = 5 and [NOM] = 3 mg C L^{-1} (graphs a, b, c, d); pH = 6 and [NOM] = 10 mg C L^{-1} (graphs e,f,g,h); [Pt] = 324 ± 11 nM; SRHA = Suwannee River Humic Acid; SRFA = Suwannee River Fulvic Acid; BL = Bannister Lake; LM = Luther Marsh, detection of ^{194}Pt, column Superdex™ 75 Increase 10/300 GL, mobile phase: MHSM-S, flow rate: 0.6 mL·min^{-1}, injection volume: 100 µL. For purposes of illustration, the retention time interval for which Pt complexation by NOM was observed (16 to 24 min) is represented in light brown

Table 1: Proportions of the total peak areas attributed to different Pt species for each of the experimental conditions, HPSEC-ICP-MS being a semi-quantitative method.

Species / elution time	% of total peak area									
	pH = 5; [NOM] = 3 mg C L^{-1}					pH = 6; [NOM] = 10 mg C L^{-1}				
	No NOM	SRHA	SRFA	BL	LM	No NOM	SRHA	SRFA	BL	LM
Pt-NOM 16-24 min	/	0.8	7.3	4.3	0.5	/	1.3	9.9	11.1	3.2
$Pt^{II}Cl_4^{2-}$ 26 min	34.7	21.7	22.6	15.6	20.8	2.3	17.4	10.1	18.5	20.3
$Pt^{IV}Cl_6^{2-}$ 31 min	46.8	62.0	35.1	70.9	61.3	92.3	70.0	71.0	39.3	38.1
Pt unknown 34 min	0.9	0.1	0.6	0.4	0.8	/	/	/	3.9	5.3
Pt unknown 39 min	/	/	/	/	/	0.3	4.3	2.5	5.2	8.2
Pt unknown 42 min	/	1.6	6.0	1.8	4.7	/	/	/	/	/
Pt unknown 45 min	/	1.2	4.5	0.7	3.5	/	/	/	/	/
Pt unknown 48 min	0.9	0.4	3.1	1.5	0.4	/	0.3	0.5	4.7	9.3
Total of other Pt species < 1%	0.1	0.0	0.7	0.1	0.1	0.5	0.4	0.3	1.4	3.3

Aside form complexation, the chromatograms of Figure 5 show notable changes in Pt inorganic speciation in the presence of NOM. Inorganic species $[Pt^{IV}Cl_6]^{2-}$ and $[Pt^{II}Cl_4]^{2-}$ seem to be present in comparable proportions for the four NOMs and the two complexation conditions tested. This is consistent with the corresponding chromatogram in the absence of NOM at pH = 5, but less so for the pH = 6 condition (Figure 4). For the latter pH, dissociation and reduction of $[Pt^{IV}Cl_6]^{2-}$ seem more important in the presence of NOM, humic substances being known for their role in metal reduction (Szilágyi 1971, Matthiessen 1998, Andre and Choppin 2000, Palmer *et al.* 2006). Thus, equilibrium could possibly be reached faster in the presence of NOM, especially at pH = 6, although further experiments would be required to formally conclude on this point. In addition, a certain number of Pt inorganic forms were observed, up to 16 unidentified species in the presence of NOM. This is consistent with the work of Nachtigall *et al.* (1997). The authors observed a variety of chloro mixed aquo/chloro complexes obtained by hydrolysis of $[Pt^{IV}Cl_6]^{2-}$ and $[Pt^{II}Cl_4]^{2-}$. It is also consistent with the assumption of faster kinetics in the presence of NOM as the unknown peaks observed in Figure 5 are most likely intermediate forms between $[Pt^{IV}Cl_6]^{2-}$ and the Pt-NOM complexes. Indeed, inorganic Pt complexes must first dissociate, in order for the metal to be complexed by NOM. The presence of ligands thermodynamically favors the dissociation of the original forms.

Furthermore, the same peaks are consistently observed in the presence of all the different NOMs, but with different patterns between pH 5 and 6. At pH = 5 (left panels), two late peaks were observed at 42 and 45 min. Both species were probably slightly retained on the column, which suggests they might have been positively charged inorganic Pt species. Despite the suspected non-specific interactions, the peaks were well defined. Their formation also seems to be slightly more important in the presence of SRFA and LM NOMs. At pH = 6 (Figure 5.e to 5.h), two additional peaks were observed in all NOM conditions. The one at 39 min has previously been hypothesized to be a product of $[Pt^{II}Cl_4]^{2-}$ dissociation. The other unknown inorganic form is retained for a longer time in the column (49 min), suggesting again a positively charged species. For the two local NOMs (BL and LM), these two peaks are of greater proportions as displayed in Figure 5 and Table 2. In addition, a peak at 34 min is clearly observed and represents over 5% of the total peak area at pH 6 for BL and LM. It seems to be the same Pt form that was obtained at pH = 5, both in the absence and presence of NOM. Since this peak at 34 min comes out immediately after $[Pt^{IV}Cl_6]^{2-}$ at 31 min, it is possible the presence of the former in low proportions might have been concealed by the peak of the latter in the other samples in which a small shoulder can be seen at that elution time. It should be noted that the chromatograms obtained at pH 6 in the presence of 10 mg C L^{-1} of either BL or LM indicate the presence of much more of, presumably, intermediate species. The two NOMs in question were obtained by reverse osmosis, this method being less aggressive than extraction processes and resulting in a better recovery of NOM. It follows that their composition would be more complex and more representative of environmental conditions than that of the extracted NOMs: SRHA and SRFA (Sun *et al.* 1995, Kleber and Lehmann 2019, IHSS 2024). Therefore, this result illustrates the importance of the sampling method, with an apparent impact on Pt dissociation kinetics and inorganic speciation at pH = 6. Furthermore, the observation of different intermediate forms at pH = 5 and pH = 6 highlights the critical role of pH. As suggested in the previous section (*Pt inorganic speciation – Influence of pH*) the dissociation and reduction mechanisms could potentially be different between pH = 5 and pH = 6.

At chemical equilibrium, over 99% of inorganic Pt would be expected to be present as $[Pt^{II}(OH)_2]^0$ (Colombo *et al.* 2008). Although this species could not be identified in the

present work, it could be one of the minor unidentified species. Because of its neutral charge, it may have a retention time greater than $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{IV}Cl_6]^{2-}$. However, in the absence of NOM and when Pt was added as $(NH_4)_2PtCl_6$, identified inorganic Pt species: $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{IV}Cl_6]^{2-}$, represented 89 ± 7% (n = 3) of detected Pt after the 48-h equilibration period. Since, total Pt recovery was 57 ± 6% (n = 5), it follows that ~51% of the Pt present in the sample has been identified. Therefore, $[Pt^{II}(OH)_2]^0$ could not have represented more than ~49% of all species. Indeed, equilibrium was clearly not reached, despite the presence of a higher number of species suggesting apparent faster kinetics in the presence of NOM. These results provide insights on the environmental conditions that facilitates the dissociation of $[Pt^{IV}Cl_6]^{2-}$ and the formation of intermediate species. However, further identification of the multiple inorganic Pt forms obtained would require the use of other types of columns, such as, for example ion-exchange based retention columns that could provide more information over the different species charges (Nischwitz *et al.* 2003, Hann *et al.* 2005, Vitkova *et al.* 2014, Yao *et al.* 2022).

From an ecotoxicological perspective, our understanding of the relationships between Pt speciation, bioavailability and toxicity remains largely incomplete. However, previous work has shown that the presence of NOM in similar experimental conditions (pH = 6 and [NOM] = 10 mg C L^{-1}) led to an increased toxicity and bioavailability of Pt for unicellular green algae (Hourtané *et al.* 2022, Hourtané *et al.* 2024). While it might be hypothesized that the presence of one of the intermediary species observed in the present work could be responsible for this observation, it remains unlikely. Indeed, the present results showed similar modification of Pt inorganic speciation with NOMs sampled using the same method, and no such trend could be observed regarding Pt bioavailability (Hourtané *et al.* 2024).

To determine the effect of platinum on aquatic biota, the choice between Pt^{IV} or Pt^{II} is not without consequences. Because Pt^{IV} persists over a time frame that may exceed most standard toxicity test, a different biological response may result from both redox forms. In other words, it should not be assumed that Pt^{IV} is readily reduced to Pt^{II} and quickly reach equilibrium like most transition elements. In fact, a recent study showed that Pt^{IV} is more toxic to algae than Pt^{II} using $[Pt^{IV}Cl_6]^{2-}$, $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{II}(NH_3)_4]^{2+}$ (Michaud-Valcourt *et*

al. 2024). For a chronic exposure, the use of Pt^{II} would be recommended in order to facilitate the achievement of chemical equilibrium.

CONCLUSIONS

In this work, aqueous Pt speciation was studied in solutions representative of surface fresh water which contains heterogeneous and polyfunctional NOM. For this purpose, we used an HPSEC-ICP-MS technique that provided a satisfactory Pt recovery of 57 ± 6% (n=5), as well as good repeatability and resolution. The Superdex^{TM} column seemed to be a good choice when working at low ionic strength. Result show that average MW of the NOMs studied is around 1 kDa, which was consistent with previous work, despite the uncoiling of humic substances expected in our experimental conditions. Retention times for PSS standards, NOM molecules and Pt inorganic species were similar between the two pH of interest: 5 and 6, and the main inorganic forms of Pt in solutions were identified as $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{IV}Cl_6]^{2-}$. However, the elution of the inorganic Pt species seems to be largely governed by non-specific interactions, as suggested by the counter intuitive exit order of $[Pt^{II}Cl_4]^{2-}$ and $[Pt^{IV}Cl_6]^{2-}$. Not to mention the importantly delayed elution of $[Pt^{II}(NH_3)_4]^{2+}$, which, along with the characteristics of the column and tube materials suggests a poor recovery of positively charged Pt species.

Pt complexation by NOM was weak (0.5 to 11.1 %), even at the relatively high NOM concentration of 10 mg C L^{-1}, but might have been more important if an equilibrium had been reached. Indeed, the initial form of $[Pt^{IV}Cl_6]^{2-}$ was still the main species in solution in all tested conditions, despite being expected to be thermodynamically unfavored. Equilibrium seemed far from being attained after the 48-h equilibration period respected in this work, which is consistent with Pt known slow kinetics. On top of the appearance of Pt-NOM complexes, the presence of NOM also modified the inorganic speciation of Pt, especially at pH = 6. Since the equilibrium was not reached, this can be explained by the increased reaction rates in the presence of NOM: dissociation and reduction seemed favored, along with the detection of numerous potential intermediate Pt inorganic forms between $[Pt^{IV}Cl_6]^{2-}$ and Pt-NOM, one of them being $[Pt^{II}Cl_4]^{2-}$. The other intermediate forms remain to be identified, however the mechanisms at play seemed to be different at pH = 5 and pH = 6. This highlights the complex chemistry of Pt and suggests that our

understanding of the hydrolysis of chloroplatinate complexes in aqueous media remains incomplete.

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DATA AVAILABILITY STATEMENT

Data presented in this work are available at https://doi.org/10.5683/SP3/2N5HZF.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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