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Identifying Ni-complexed natural organic matters in a relatively stagnant river water

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ABSTRACT

Pretreatment protocols, using preparative liquid chromatography (prep-LC), and high resolution mass spectrometer methods, were developed to identify potential Ni-natural organic matter (NOM) complexation, for a relatively stagnant river water sample. The former and the latter were performed, using a large separation column, packed with C18 and size exclusion resin media, and ion trap-time of flight (IT-TOF) mass spectrometer, respectively. The NOM samples were effectively fractionated into four different peaks, with helps of both RI and UV detections, and further subjected to mass analyses using the IT-TOF, which provided distinct *m/z* peaks pairs in mass spectra, with *m/z* peaks difference of ca. 58, as evidence of Ni-complexed NOM.

Keywords: Ni-NOM complexation; Preparative LC; Ion trap-time of flight (IT-TOF)

1. Introduction

The organic carbon partition coefficient (K_{oc}) of nickel (Ni), with atomic mass of ca. 58, has not been known yet [1]. Research of Ni complexation with organic matters has been very rare compared to other metals, such as iron, to our knowledge; some studies revealed the Ni-organic complexation, with model organic compounds, including flovoprotein, ethylendiamine, and glycinate, using electron spin resonance peak with free radicals and complexation (stability) constant [2,3]. Nickel has been known to contribute, through complexation/incorporation with proteins, to metabolism in cell and tissue, and molecular genotoxicity through Ni-DNA-protein cross-link, systematic toxicity (e.g., respiratory, immunotoxicity, neurotoxicity, reproduction), and also interact with other metals, such as iron, for a low-affinity cell transport pathway [1,4,5].

Nickel in surface water has been recognized resulted from soil, mining piles, and atmospheric deposition, but, it can also be originated from anthropogenic contribution, such as industrial and municipal wastewater, and landfill leachate [5,6]. Nickel is known to exist mostly in the form of green hexaquonickel $[Ni(H_2O)_6]^{2+}$ in aquatic solution environments, like surface water, as other forms, including hydroxides, sub-sulfide, sulfides, carbonates, phosphate, elemental form, and bimetallic compounds, are insoluble in water. Most of nickel compounds may become relatively soluble at pH levels below 6.5 [1].

With natural bulk water samples, identification of Ni-complexation with natural organic matters (NOM) is believed not easy, due to interferences of non-bound

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organic matters and other metals, and also relatively low levels of nickel in surface waters, even when sensitive quantification tools are applied; to our knowledge, there is no research to be published to have suggested characterizing tools and methods to be able identify complexations between Ni and NOM. Thus, a new preparative method is believed necessary to be able to provide tools for fractionating NOM into various portions having different characteristics, including hydrophobic and molecular weight, and also potential with further analyses for the fractionated NOM samples. Existing classical NOM fractionation and preparation methods, such as XAD-8/4 resins, have been used to fractionate NOM samples into hydrophobic versus hydrophilic portions, but they require relatively long isolation time and provide high salts levels with the fractionated samples, which may be obstacle to further analysis, such as mass spectrometer measurement.

2. Methods and materials

A surface water sample from the Muan site of the Yeongsan River, South Korea, was selected for the research as the site is located at the downstream of the river, with relatively bad water qualities, and relatively stagnant due to constructed estuary dam. General water qualities of this site, with respects to total organic carbon (TOC), biochemical oxygen demand, total nitrogen (TN), and most of heavy metals, as compared to other upstream sites of the river, have been found bad, according to our previous studies (not shown in this paper). TOC and TN were measured by a catalytic combustion type of organic analyzer (TOC Analyzer, Shimadzu, Japan), equipped with auto-sampler. Sampling was conducted on June, 2010, with pH of 8.5, a water temperature of 26.0°C, conductivity of 744.0 μS cm^{-1}. The fractionation of NOM samples was performed using recycling preparative HPLC unit (LC-9201, JAI, Japan) with UV (UV detector 3702, JAI) and RI (RI-50s, JAI) detections. The unit allows relatively large volume injection up to 10 ml (in this study, 5 ml was injected) as it is equipped with a preparation specialized column (Jaigel-GS-310, 20ϕ × 500 mm, JAI), thus, samples fractionation is possible for further analyses, without any further concentration or purification step. According to the manufacturer, separation by the column is made based on combination of hydrophobic interaction (C18) and size exclusion. Once the sample was fractionated using the pretreatment, that is, prep-LC system, it was subjected to further analysis by ion trap-time of flight (IT-TOF) to obtain mass spectrum results (Fig. 1). Mass spectra of NOM fractions were obtained using IT-TOF LC/MS system (Shimadzu, Tokyo, Japan) equipped with an electron spray

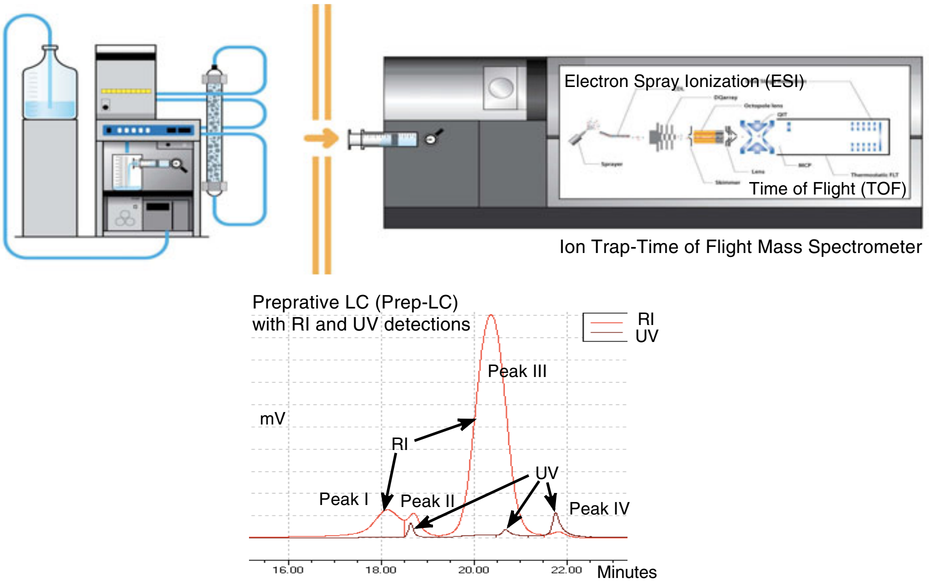


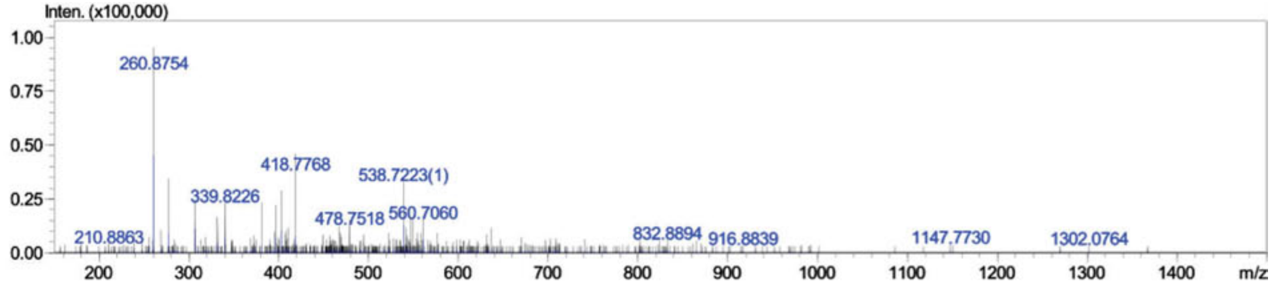
Fig. 1. Schematic of the preparation liquid chromatography (prep-LC) system, equipped with a large volume separating column based on C18 and size exclusion (left), and ion trap-time of flight (IT-TOF) mass spectrometry (right). Four different peaks were separated from the prep-LC system with the separation column; based on the column properties for separation, peaks I and II samples were believed to exhibit less hydrophobic with higher molecular weights than peaks III and IV samples.

ionization (ESI) source operating in both positive and negative modes. Depending on sample, each ionization mode can give very different mass spectra. For example,a sample with easily ionizable and/or numerous acidic groups, such as carboxylic acids, readily loses a proton thus is negatively ionized. In contrast, a sample with many basic groups, such as amines, easily picks up a proton thus can be positively ionized. As positive ion spectra for the sample investigated exhibited less intensity and more complicated spectra with more spurious ions than negative ion spectra, which were attributed to sodium adducts. Thus, the mass spectrometer was operated in negative ion mode, along with another supposition that most NOMs dissolved in water contain many acidic groups which are necessary for complexation with metals. A mobile phase was delivered at a flow rate of 0.2 ml min^{-1} consisting of 5 mM ammonium acetate in water and methanol. The optimized mass conditions were as follows: negative ion mode electro-spray voltage of −4.5 kV, CDL temperature of 200°C, block heater temperature of 200°C, nebulizer gas (N_2) flow of 1.5 l min^{-1}, drying gas (N_2) pressure of 200 kPa, pressure of ion trap of 1.8×10^{-2} Pa, pressure of TOF region of 2.1×10^{-4} Pa, ion accumulated time of 30 ms. Mass spectra were acquired in the range of m/z 200–2000 for MS^1. Sample injection (1 μl) was performed in a HPLC system (Shimadzu, Japan), consisting of an LC-20AB binary pump and a SIL-20AC auto-sampler, without a chromatographic separation. In automatic mode, all ions were firstly accumulated in octopole and then rapidly pulsed into IT for MS^n analyses, according to criteria settings. All ions produced were finally introduced into the TOF instrument for accurate mass measurements. The ionized molecules fractured by the ESI step exhibit certain molecular masses (denoted as m) and valences (z), and the molecular ions are separated by the TOF, using different times of flight of the ions. The separating results are represented with peak intensities and corresponding m/z values. Ion accumulation time was set at 30 msec. Prior to data acquisition, the instrument was calibrated with trifluoroacetic acid sodium solution which was used as standard solution for calibrating the instrument, against entire mass range (m/z 50–5,000). Data recorded were processed by the LC solution 3.5 software (Shimadzu, Japan), including a formula predictor to predict potential chemical formula. Nickel and other heavy metals were measured using ICP-MS (Agilent, 7500ce, USA), equipped with an octopole reaction system, and ultra pure hydrogen and helium gases. For accuracy and precision verification, internal and standard samples with known concentrations were also measured using standard reference materials (SRM 1640, NIST) and those used for the calibration. All samples were measured in triplicate and then averaged.

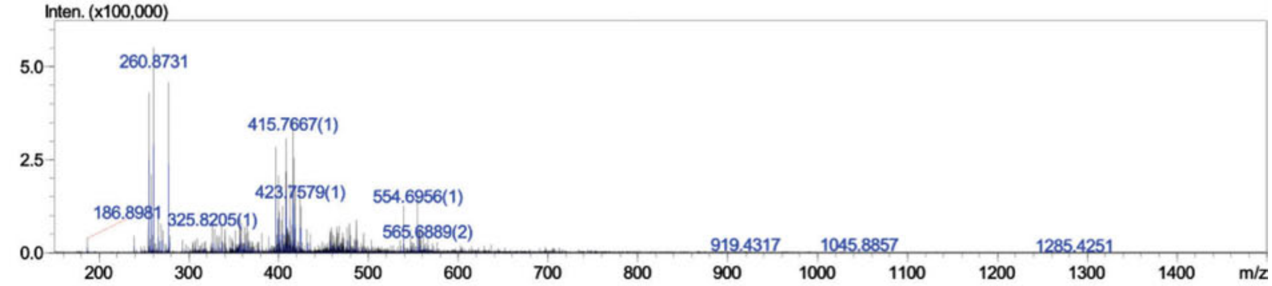
3. Results and discussion

Four peaks were separated, as shown in Fig. 1, then, fractionated samples were manually collected into vials which were conveyed to both the IT-TOF mass spectrometer to obtain corresponding mass spectra and the ICP-mass to measure levels of heavy metals, including nickel. The peaks I and IV were detected preferentially by RI and UV detections, respectively; RI and UV detections are generally sensitive to saccharides/amino sugars and aromatic compounds, respectively. Even though the fractionated samples were not further analyzed, with respect to molecular weight and hydrophobicity measurements, the peaks I and II samples were believed to exhibit less hydrophobic with higher molecular weights than the peaks III and IV samples, based on the properties of the prep-LC column. With this preposition, m/z difference with value of ca. 58 was investigated for IT-TOF mass peaks pair of the fractionated four samples to identify potential Ni-NOM complexation, which were found for the peaks III and IV samples, not for peaks I and II samples, as shown in Fig. 2. With the peaks III and IV samples, there are 3 or 4 m/z mass peak-pairs of which m/z differences are ca. 58, which tells there are at least 3 or 4 functionality sites on the NOM which are available for complexation with Ni. The fact was also supported by the measurements results of heavy metals per unit organic carbon with the raw and prep-LC fractionated NOM samples. Levels of nickel per unit organic carbon for the peaks III and IV samples are much higher than the peaks I and II samples, and unfractionated raw samples, as summarized in Table 1. Nickel also exhibited much higher levels per unit organic carbon than the other heavy metals, for all the tested samples.

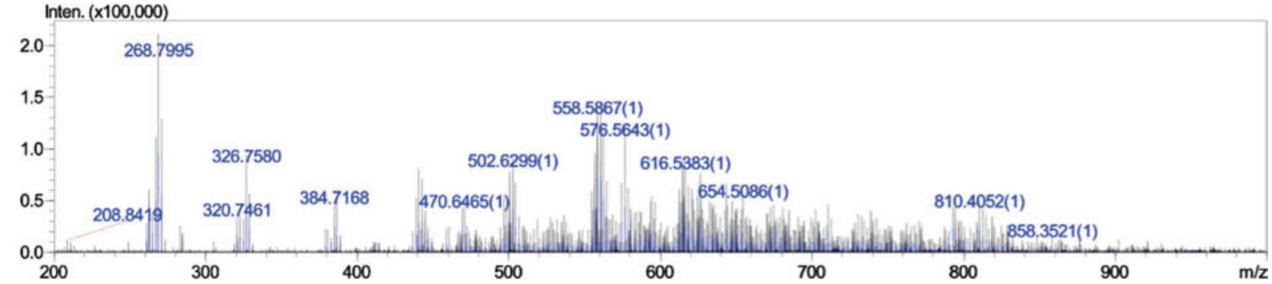
More detailed characteristics for the prep-LC fractionated NOM samples were not analyzed in this study, thus, had better be obtained for better understanding to explain (or confirm) Ni-NOM complexation. Further characterizations may include analyses using higher levels of mass spectra, such as MS^2 and MS^3, with helps of natural isotope abundance information, analyses with more purified/separated NOM samples with another column, such as C18, prior to the IT-TOF, and analyses to identify the fractionated NOM samples, with respect to comprising bio-polymers which can be obtained using pyrolysis GC-Mass (being performed), and others.



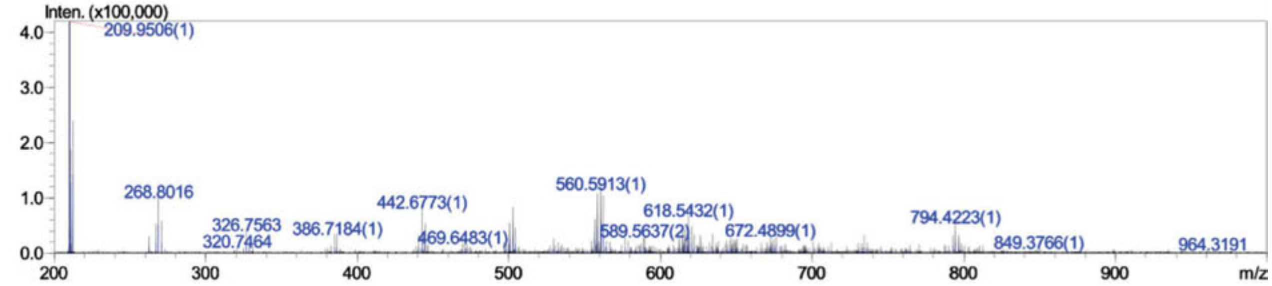
Peak I sample



Peak II sample



Peak III sample (Δm/z=ca. 58, between 268.7995-326.7580-384.7168, and 502.6299-558.5869-616.5383)



Peak IV sample (Δm/z=ca. 58, between 268.8016-326.7563, and 502.6328-560.5913-618.5432)

Fig. 2. Mass spectra measured by the IT-TOF, for the NOM samples fractionated using the prep-LC; the first two spectra (peaks I and II samples) do not contain mass peaks pairs of which *m/z* differences are ca. 58, while, the last two (peaks III and IV samples) do, which is believed evidence of complexations between Ni and available functional sites of NOM.

Table 1
Water characteristics of the raw and prep-LC fractionated samples

Raw water sample			Prep-LC fractionated samples heavy metal per unit organic carbon [μg (mg C)$^{-1}$]						
TOC (mg C l^{-1})	TN (mg N l^{-1})	SUVA[a] (l (m-mgC)$^{-1}$)	Sample	Ni/C	Fe/C	Mn/C	Cu/C	Zn/C	Co/C
4.7	2.7	2.4	Peak I + Peak II	2.45	0.00	0.08	0.22	0.04	0.01
			Peak III	17.96	0.00	0.80	0.11	0.81	0.04
			Peak IV	7.61	0.00	0.56	0.27	0.05	0.01
			U.F.[b]	2.02	0.00	0.00	0.34	0.02	0.02

[a] SUVA: specific UV absorbance is UV absorbance at 254 nm divided by TOC, which is relatively aromaticity index.

[b] U.F.: unfractionated sample.

4. Conclusions

Even the analyses and corresponding findings, regarding the Ni-NOM complexation, are in initial stages, and need more demonstrations for better proving, results of mass spectra with helps of NOM preparation steps, using the prep-LC, were proved effective to identify potential functional sites on NOM, from a relatively stagnant river water sample, for complexation with Ni over other heavy metals. Considering researches of complexation between Ni and NOM are very rare, both pretreatment protocols, and analyses tools and methodologies, are believed to be able to contribute to both further intensive studies of Ni complexation and other related water quality management issues, in connection with NOM and heavy metals.

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