Vol. 11, No. 4, 2020
ISSN 2233-4203/ e-ISSN 2093-8950
ARTICLE
www.msletters.org | Mass Spectrometry Letters

Characterization of Natural Organic Matter in Spring Water

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Received November 25, 2020; Revised December 14, 2020; Accepted December 15, 2020
First published on the web December 31, 2020; DOI: 10.5478/MSL.2020.11.4.90

Abstract : Interest in aspects of industrialization relating to human health has increased. Accordingly, the use of labels such as 'natural foods' and 'organic ingredients' has become more widespread, and greater emphasis is being placed on improving quality of life. Water is an essential element for human life, and water quality has a significant impact on human health. However, technology that can precisely determine the substances present in water is still lacking. This study was conducted to establish a complete mass spectrometry process, from pretreatment to analysis, to measure and characterize natural organic matter (NOM) in Korean spring water samples. Salts and other matrices were removed from the samples using solid-phase extraction (SPE) with two different columns (PPL and C18). After establishing an accurate analysis method, the experimental results were evaluated based on Van Krevelen diagrams and analysis of molar O/C and H/C ratios. The method for characterizing NOM introduced herein should facilitate evaluation of water quality.

Keywords : natural organic matter, solid-phase extraction, pretreatment, mass spectrometry, water quality

1. Introduction

Natural organic matter (NOM) accounts for a large proportion of all carbon (C)-based compounds found in natural, engineered, terrestrial, glacial and aquatic environments. NOM is a heterogeneous mixture of plant, animal and microbial residues present above and below ground, and showing varying degrees of degradation. It is a major reservoir for C and plays an important role in biogeochemical cycles in soil-based ecosystems. NOM is composed of C from various sources, in the form of monosaccharides, organic acids and amino acids, as well as more complex polymers such as cellulose, lignin, and lignocellulose. The structural properties of C from different sources are heterogenous, for example in terms of the degree of polymerization and aromaticity.[1-4] The properties of NOM are considered an important aspect of the nutrient cycle.[5,6] Elucidating the chemical composition of NOM is very important for bio-environmental studies. Characterization of NOM in natural environments can be achieved using a range of analysis techniques, such as excitation emission matrix (EEM) fluorescence spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, ultraviolet visible light (UV-vis) spectroscopy and high-performance liquid chromato-graphy/mass spectroscopy (HPLC/MS).[7-9] These analytical methods are useful for investigating the chemical properties of NOM in various environments, but are not sufficient to reliably identify the presence of individual compounds within NOM. Fourier transform- ion cyclotron resonance mass spectrometry (FT-ICR MS) with electrospray ionization (ESI) and atmospheric pressure ionization is currently the only analytical technique capable of obtaining detailed information on specific compounds in complexes such as natural dissolved organic matter (DOM).[10]

Here, a method is reported for characterizing dissolved NOM. As a pretreatment, high-resolution mass spectrometry with two different solid-phase extraction (SPE) columns (Agilent PPL and Phenomenex C18) is used for molecular and chemical cha-racterization of compounds in DOM. Established pretreatment techniques and high-resolution analyses allow specific components of various natural organic substances dissolved in surface water to be characterized at the molecular scale. There is a demand for techniques that can easily and precisely analyze the properties of organic materials.

Materials and Methods

A consideration regarding the accuracy of FT-ICR-MS is proper sample preparation. Several methods for

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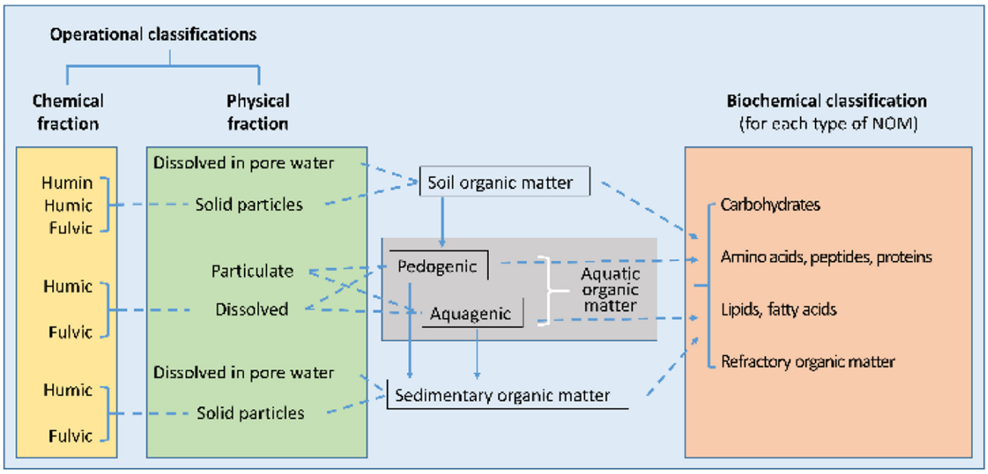

Figure 1. Properties of natural organic matter (NOM).

characterizing under-water organic matter have been reported in the literatures.[11-19] There are many extraction methods have been used to concentrate DOM from water sources including ultrafiltration, SPE, electro-dialysis and combined reverse osmosis electro-dialysis. However, explanations on how to analyze specific components of NOM in surface water are very scarce. Also, in the previous study, the SPE column of C18 or PPL was used for comparison and analysis, except that it was applied to the analysis of natural organic matter in spring water. In addition, the drying and re-extraction steps described here are in accordance with previously reported methods. Therefore, the NOM analysis method developed in this study differs from existing methods.

Samples

HPLC-grade formic acid and ammonium hydroxide reagents were purchased from Sigma-Aldrich. Hydrochloric acid (HCl) was purchased from Samchun and methanol was purchased from JT Baker. Ultrapure water was obtained using the Milli-Q Integral system (Merck-Millipore). To develop the NOM characterization method, a standard NOM sample (Suwanee River fulvic acid; SRFA) was analyzed. Samples were first subjected to SPE pretreatment to remove salt and other substrates, and to concentrate small amounts of NOM.

Sample preparation

Before the FT-ICR MS analyses, we used Bond-Elut PPL (500 mg/6 mL) cartridges and Phenomenex C18 cartridges (Agilent, USA) for SPE.

The sample was adjusted to a pH value of 2~4 using 1 M HCl and then extracted using a Bond-Elut PPL cartridge. Each cartridge was conditioned with 2 mL methanol and equilibrated with 2 mL 0.1% formic acid in ultrapure water. One liter of acidified sample was loaded into the cartridge at a flow rate of 12 mL/min, washed with 2 mL ultrapure water and then eluted in 2 mL if 2% ammonium hydroxide in methanol. The PPL-eluted samples were dried using a speed vacuum for 2 hours and stored at -80°C in a freezer immediately after preparation. Using this method, NOM present in very small amounts in a 1 L water sample was extracted, and then analyzed using a 15T FT-ICR mass spectrometer equipped with an electrospray ionization (ESI) source (solariX™ system, Bruker Daltonics, Billerica, MA).

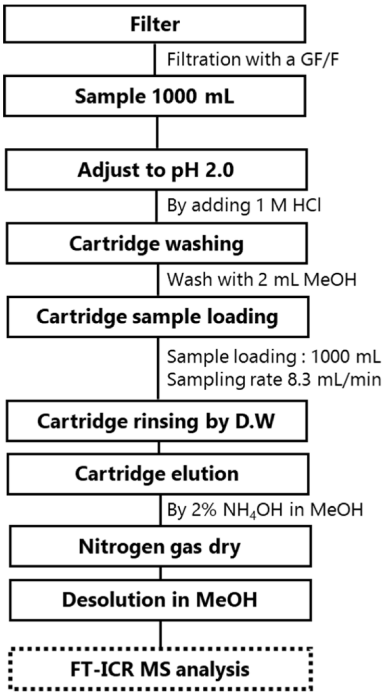

Figure 2. Schematic overview of the NOM analysis method.

15T-FT-ICR MS analyses

SPE-eluted samples were dissolved in 1 mL methanol, centrifuged at 13,000 rpm for 10 minutes, diluted 50 times in 1 mL of methanol, and centrifuged at 13,000 rpm for 10 minutes to obtain 100 µL of supernatant. Ultra-high resolution MS analysis was performed using the 15T FT-ICR MS with the Apollo II ESI source. The method was performed in negative ion mode and the instrument was operated in broadband mode, between 150 and 1,200 m/z. The samples were injected using a 250-µL Hamilton syringe with a flow rate of 2 µL/min. The spray current was set to –3.0 kV, and the drying gas temperature was 180°C with a 4.0 L/min flow rate. The skimmer voltage was set at –45 V. A total of 100 temporary scans, which were collected with 4MWord time domain, were co-added to one mass spectrum.

The results were categorized into five component series (CHO, CHON, CHOS, CHONS and other) based on the molar hydrogen/carbon (H/C) and oxygen/carbon (O/C) ratios. As shown in Table 1, there were eight categories of molecular compounds: aromatic formula, condensed aromatic structure (CAS), lignin/CRAM, protein, carbohydrate, lipid, unsaturated hydrocarbon, and tannin; these were also represented on Van Krevelen diagrams.[20-22]

Van Krevelen diagrams

The raw data from 15T FT-ICR MS were processed using the Bruker Daltonics Data Analysis 4.2 and Sierra Analytics Composer (Sierra Analytics, Modesto, CA) software. After 15T FT-ICR MS measurements, the raw data were imported into the mentioned software for peak detection and recalibration. Composer, a formula calculator, was used to assign elemental compositions.[23] In short, the empirical molecular formulas were calculated for the masses of singly charged ions in the range of *m/z* 150–1,100 by combinations of up to 100 ^{12}C, 200 ^{1}H and 60 ^{16}O atoms, followed by additional calculations of molecular formula including up to 2 ^{14}N and 1 ^{32}S atoms.[24] And then, the molecular formulas with assignment errors of 0.5 ppm or more were excluded from further processing.

Table 1. Compound classes used for the FT-ICR MS analysis.

Compound class	Abbreviation/ Name	Criterion
Aromatic formula	AF	AImod = 0.5-0.67
Condensed aromatic structure	CAS	$0.2 \le H/C \le 0.7$ $0 \le O/C \le 0.67$
Lignin/CRAM	Lignin	$0.7 \le H/C \le 1.5$ $0.1 \le O/C \le 0.67$
Protein	Protein	$1.5 \le H/C \le 2.2$ $0.3 \le O/C \le 0.67$
Carbohydrate	Carbohydrate	$1.5 \le H/C \le 2.4$ $0.67 \le O/C \le 1.2$
Lipid	Lipid	$1.5 \le H/C \le 2.0$ $0 \le O/C \le 0.3$
Unsaturated hydrocarbon	UnsatHydroC	$0.7 \le H/C \le 1.5$ $0 \le O/C \le 0.1$
Tannin	Tannin	$0.5 \le H/C \le 1.5$ $0.65 \le O/C \le 1$

Van Krevelen diagrams have been used as optimally graphical methods for elemental analysis in conjunction with FT-ICR MS in previous studies.[11-19] In a Van Krevelen diagram, the molar H/C ratio is plotted vertically and the molar O/C ratio is plotted horizontally. Each peak in the soil organic matter spectrum is indicated by a point on the diagram. The relative abundance of a given compound can be of circular size, and other official classes are color coded. In general, the major classes of biogeochemical compounds (i.e., lipids, proteins, carbohydrates, lignin, tannins, unsaturated hydrocarbons, and condensed aromatics) have characteristic H/C and/or O/C molar ratios, so occupy a specific area of the Van Krevelen diagram. These diagrams are particularly effective for comparing compounds with different numbers of heteroatoms.

Results and Discussion

In order to select an appropriate SPE cartridge before proceeding with mass spectrometry, an experiment was conducted comparing two types of cartridges using groundwater as a prerequisite. Using the C18 cartridge, CHO-based compounds were most abundant in the NOM (25.53%). In comparison, for the Agilent PPL cartridge, CHON-based compounds were most abundant (44.16%),

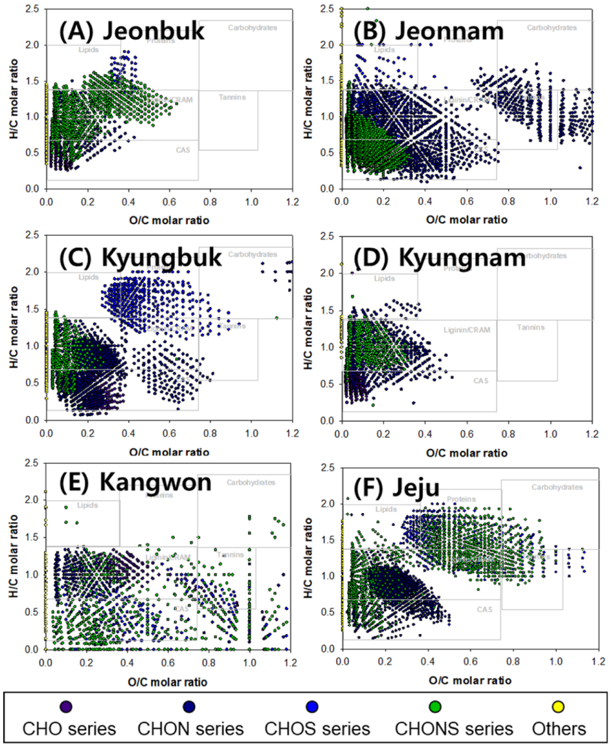

Figure 3. Van Krevelen diagrams for mineral spring water samples obtained from various sites in Korea.

Table 2. Molecular constituents of mineral spring water samples obtained from various sites in Korea.

Sample	(A)	(B)	(C)	(D)	(E)	(F)
Number of formulas	1450	3340	772	1344	1855	2915
m/zwa	480	557	507	457	378	526
Cwa	26.6	34.4	29.8	27.8	17	30
Hwa	23.7	20.8	18.8	21.9	10.6	22.6
Nwa	3.2	2	2.6	2.9	2.4	2.6
Owa	3.9	4.4	5.5	2.8	6.6	6
Swa	0.9	0.8	0.2	0.5	0.8	0.4
H/Cwa	0.93	0.59	0.61	0.86	0.59	0.73
O/Cwa	0.15	0.14	0.19	0.13	0.46	0.19
N/Cwa	0.15	0.06	0.09	0.13	0.16	0.11
S/Cwa	0.04	0.02	0.01	0.02	0.06	0.02
DBEwa	17.4	26	22.7	19.3	13.9	20.9
Almod,wa	0.62	0.76	0.79	0.68	0.86	0.72
CHO (%)	8.8	0.1	6.8	22.5	11.3	0.2
CHON (%)	21	54.6	59.4	47.7	26.1	37.5
CHOS (%)	3.4	3.3	14.1	0	23.7	14.1
CHONS (%)	52.6	34	12.3	27.5	35.4	38.8
Others (%)	14.1	8.1	7.4	2.4	3.5	9.4
CAS (%)	24.4	49.3	36.9	32.9	33.8	19.6
Lignin/CRAM (%)	50.1	26.7	38.5	45.4	47.1	47.4
Protein (%)	3.7	0.3	10.8	0.4	0	12.5
Carbohydrate (%)	0	0.8	1.2	0	0.7	2.6
Lipid (%)	1.1	1	0.4	0.7	0.3	0.7
UnsatHydroC (%)	20.7	15	9.6	20.7	5.5	7.5
Tannin (%)	0	7	2.6	0	12.7	9.7

while CHOS-based compounds were scarce. Using the Agilent PPL cartridge, the lignin/CRAM and CAS contents were high. These organic compounds have aromatic properties, relatively high hydrophobicity, and relatively low decomposability. Nitrogen and sulfur are present in some groundwater, in very small amounts; NOM mostly comprises organic substances with low decomposability. Using the Phenomenex C18 cartridge, most of the NOM in the groundwater was in the form of lipid bodies (57.58%). Therefore, in order to more accurately measure the distribution of properties of spring water including natural organic matter, a PPL cartridge was selected and used.

The Van Krevelen diagrams presented in Figure 3 were constructed for 6 of 46 mineral spring water samples. As the samples were all collected at the same time, they were compared by region rather than period. There was a difference in protein and tannin contents by region. The NOM samples mainly comprised lignins and CAS compounds (42.1~94.9%), which are chara-cteristic of refractory organic matter. In Jeju, protein content was particularly high, likely due to the abundance of an amino acid-based substance containing sulfur. Detailed Comparison of these results with existing literature is necessary to gain further insight.

Conclusions

The chemical composition of NOM in water samples obtained from various sites in Korea are reported. A method for pretreating spring water samples prior to MS analysis was introduced. MS can provide higher resolution data than other systems, which facilitates analysis of natural organic substances in spring water. MS can also be combined with other analysis methods.

Water quality has a significant impact on human health. The methods described herein were designed to benefit human health by allowing detailed information on natural organic substances in water to be obtained.

Acknowledgments

This work was supported by the Korea Basic Science Institute (KBSI) under the R&D program (Project No. PN2020111 and C030222).

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