Comprehensive characterization of natural organic matter by MALDI- and ESI-Fourier transform ion cyclotron resonance mass spectrometry

Dong Cao^{a,1}, Huogao Huang^{b,1}, Ming Hu^{c,1}, Lin Cui^a, Fanglan Geng^a, Ziyu Rao^a, Hongyun Niu^a, Yaqi Cai^a, Yuehui Kang^{a,*}

^a State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Science, Chinese Academy of Sciences, P.O. Box 2871, 18 Shuangqing Road, Haidian District, Beijing 100085, China

^b Department of Endocrinology and Rheumatology, Navy General Hospital, PLA, 6 Fucheng Road, Haidian District, Beijing 100048, China

^c Central Lab Navy General Hospital, PLA, 6 Fucheng Road, Haidian District, Beijing 100048, China

HIGHLIGHTS

- MALDI-FT-ICR-MS was firstly employed for molecular characterization of NOM.
- 1,8-Bis(dimethyl-amino)-naphthalene (DMAN) was used as matrix.
- Mass spectra of NOM generated by MALDI and ESI methods were compared.
- Complementary molecular information of NOM was provided by MALDI.

GRAPHICAL ABSTRACT

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ABSTRACT

Natural organic matter (NOM) is a complex and non-uniform mixture of organic compounds which plays an important role in environmental processes. Due to the complexity, it is challenging to obtain fully detailed structural information about NOM. Although Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) has been demonstrated to be a powerful tool for providing molecular information about NOM, multiple ionization methods are needed for comprehensive characterization of NOM at the molecular level considering the ionizing selectivity of different ionization methods. This paper reports the first use of matrix assisted laser desorption/ionization (MALDI) method coupled with FT-ICR-MS for molecular characterization of NOM within a mass range of 200–800 Da. The mass spectral data obtained by MALDI were systematically compared with data generated by electrospray ionization (ESI). It showed that complementary molecular information about NOM which could not be detected by ESI, were provided by MALDI. More unsaturated and aromatic constituents of NOM with lower O/C ratio (O/C ratio < 0.5) were preferentially ionized in MALDI negative mode, whereas more polar constituents of NOM with higher O/C ratio were preferentially ionized in ESI negative mode. Molecular anions of NOM appearing at even m/z in MALDI negative ion mode were detected. The results show that NOM molecules with aromatic structures, moderate O/C ratio ($0.7 > \text{O/C ratio} > 0.25$) and lower H/C ratio were liable to form molecular anions at even m/z , whereas those with higher H/C ratio are more likely to form deprotonated ions at odd m/z . It is speculated that almost half of the NOM molecules identified by MALDI may be aromatic or condensed aromatic compounds with

* Corresponding author. Tel.: +86 10 62849729; fax: +86 10 62849729.

E-mail address: yhkang@rcees.ac.cn (Y. Kang).

¹ The authors contributed equally to this work.

special groups which are liable to absorb electron from other molecules to generate free radical anions during MALDI ionization.

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1. Introduction

Natural organic matter (NOM) is a complex and poorly defined organic mixture present in the environment. Many studies have reported that NOM may affect the toxicity of nanoparticles by forming NOM-nanoparticle composites [1], may interact with inorganic contaminant in the environment to affect its environmental behavior [2], and may be a source of new contaminants during the chlorination processes of drinking water [3,4]. Because NOM molecules play a pivotal role in the cycling and transport of elements in the ecosphere and have other effects on the environmental quality, it is important for environmental chemists to better understand the molecular composition and transformation of NOM.

However, due to its complexity and special properties, it is difficult to separate NOM using chromatographic methods. As a result, the extent of molecular information about NOM is largely restricted. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), due to its ultrahigh resolution and mass accuracy, can provide molecular elemental information about NOM without chromatographic separation [5,6], and has been used for accurate characterization of NOM [7–9]. Electrospray ionization (ESI) is the most commonly used ionization method for FT-ICR-MS characterization of NOM because it is one of the softest ionization methods and can be easily coupled with FT-ICR-MS [10,11]. However, during ESI analysis, only some polar and moderately polar molecules of NOM can be ionized by ESI, and most of non-polar compounds cannot be detected [11]. Recently, some reports combined ESI and APPI ionization methods to characterize DOM and compared the obtained mass spectra [12,13]. The results suggested that APPI tended to ionize smaller and less polar molecules, whereas ESI produces ions that in general contain more oxygen atoms. Moreover, Hertkorn et al. used different ionization methods (ESI, APCI and APPI) to characterize Suwannee River fulvic acid (SuwFA) [8]. The results indicated that molecules with lower O/C ratio were preferentially ionized in the negative APCI mode, and molecules with higher O/C ratio were preferentially ionized in the negative ESI and APPI mode. Thus, in order to obtain comprehensive molecular information about NOM, multiple ionization methods should be combined. Laser desorption/ionization (LDI) and matrix assisted laser desorption/ionization (MALDI), two other soft ionization methods, are also used for NOM characterization. However, LDI might generate excessive fragmentation of NOM during the ionization process because it is a relatively energetic ionization method [14,15]. MALDI, with the addition of external matrices, can facilitate energy dispersion and enhance sample ionization, so this method tends to generate intact molecular ions with minimal or no fragmentation [16,17]. Mugo et al. [17] had tested an array of MALDI matrices for humic substances detection. The results showed that 2,5-dihydroxybenzoic acid (DHBA) was suitable for humic substances detection, because it exhibited superior ionization efficiency, low noise, and few matrix interfering ions at the mass range of interest. Furthermore, the results also indicated that compared with LDI, fragmentation of high-MW ions was minimized by using MALDI because the laser energy was absorbed by the matrix and only partially transferred to the sample during the ionization process [17]. However, molecular elemental information about humic substance was not obtained by Mugo's study because a lower resolution mass analyzer was used.

In this study, 1,8-bis(dimethyl-amino)-naphthalene (DMAN) was used as the matrix for molecular-level characterization of NOM with mass range of 200–800 Da by MALDI-FT-ICR-MS. Meanwhile, a systematically molecular visual comparison of mass spectra generated by MALDI and ESI were performed.

2. Experimental

2.1. Chemicals and samples preparation

Suwannee River natural organic matter (SuwNOM) was purchased from the International Humic Substances Society. 1,8-Bis(dimethyl-amino)-naphthalene (DMAN, $\geq 98\%$) was obtained from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water was prepared using a Milli-Q system (Millipore, Milford, MA, USA). Methanol (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, New Jersey). Other chemicals were analytical grade.

0.5 mg mL⁻¹ of NOM solution was prepared by dissolving NOM in methanol; DMAN was dissolved in 50% methanol aqueous solution to form 1 mg mL⁻¹ of matrix solution. For MALDI-FT-ICR-MS analysis, 0.5 mg mL⁻¹ of NOM was mixed with 1 mg mL⁻¹ of DMAN matrix at equal volumes, and then the solution was vortexed for 20 s at room temperature. Subsequently, 1 μ L of the final solution was directly spotted on a 384-well MALDI target plate (AnchorChipTM target plate, provided by Bruker Daltonics, Germany), followed by drying at room temperature for MALDI-FT-ICR-MS analysis. For ESI-FT-ICR-MS analysis, 0.25 mg mL⁻¹ of NOM methanol solution was directly infused into the ESI source unit at a flow rate of 120 μ L h⁻¹.

2.2. Instrumentation

Ultrahigh resolution mass spectra were acquired using a Bruker Solarix Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) equipped with a 15.0 T superconducting magnet and a dual-mode ESI/MALDI ion source. For ESI analysis, samples were continuously infused into the ESI unit by syringe infusion at a flow rate of 120 μ L h⁻¹. ESI needle voltage was set to -3.8 kV. For MALDI analysis, samples spotted on the target plate were irradiated by a 355 nm and 1000 Hz solid-state Smartbeam Nd:YAG UV laser, and laser power was set at 20%. 100 laser shots were summed for each mass spectrum. All samples were analyzed in negative ionization mode with broadband detection. Ions were accumulated in a hexapole ion trap for 2.0 s before being introduced into the ICR cell. 4M words of data were recorded per broadband mass scan. The lower mass limit was set to m/z = 200 Da, the upper mass limit was set to m/z = 1500 Da. 20 mass spectra were averaged per sample. The spectra were externally calibrated with 10 mM of sodium formate solution in 50% isopropyl alcohol using a linear calibration, and then internally recalibrated using an in-house reference mass list. After internal calibration the mass error was less than 500 ppb over the entire mass range. Peaks were identified with Bruker Data Analysis software.

2.3. Molecular formula assignment

All possible formulas were calculated with Formula Calculator software based on the requirement that the mass error between the measured mass, and the calculated mass for a given chemical

formula is less than 0.5 ppm, and the signal-to-noise ratio (S/N) of mass peaks exceeds 5. Because NOM mainly contains C, H, and O elements, whereas N, P and S elements are found in a lower level [18], in the present work, only C, H, and O elements were considered in the calculation: ^{12}C (1–100), ^1H (1–200), ^{16}O (0–50), and ^{13}C (0–1). The elemental ratios of $\text{H/C} < 2.0$ and $\text{O/C} < 1.2$ were used as further restrictions for formula calculation. Once formulas had been assigned, the obtained mass lists were transformed into Excel format and divided into different categories for interpretation.

For visually graphic displays of the mass spectral data, the following parameters were calculated according to the previous reports: H/C ratio and O/C ratio, parameters for constructing Van Krevelen diagram [19]; Kendrick mass (KM) and Kendrick mass defect (KMD) for the purpose of identifying CH_2 -, CO_2 -, O-, CO -, H_2O -, and H_2 -homologous molecular formulas series [7,20]; the modified aromaticity index (AI_{mod}) was calculated to estimate the fraction of aromatic and condensed aromatic structures [9,18]; the nominal oxidation state of carbon (NOSC) was calculated from the formulas to indirectly show the polarity of NOM constituents detected by FT-ICR-MS [9].

3. Results and discussions

DMAN is a classic “proton sponge” compound which was introduced by Alder et al. [21], and was used as the matrix for fatty acids detection and metabolomics studies by MALDI-MS [22,23]. Recently, DMAN was also applied as the matrix for perfluorinated acidic compounds detection by our group [24,25]. The primary advantage of a DMAN matrix is that it has no matrix ion interference in low mass region, and it is well-suited for detection of small acidic compounds. Because the primary constituents of NOM are acidic compounds, DMAN should be an ideal matrix for NOM characterization by MALDI-FT-ICR-MS. The details about optimizing analytical variables of DMAN matrix, such as concentration, solvent composition and laser energy, will be discussed in

another manuscript being prepared by our group. In this paper, we mainly focus on the molecular elemental information about NOM provided by MALDI-FT-ICR-MS.

The mass spectra of NOM detected separately by MALDI- and ESI-FT-ICR-MS are displayed in Fig. 1. It is evident that the data followed an approximately Gaussian distribution, which was a remarkable feature of FT-ICR mass spectra of NOM widely reported in previous studies [20,26,27], and compound detection was exhibited in both mass spectra within the 200 to 800 m/z range (shown in Fig. 1A and B). Over 8300 peaks with signal-to-noise of 5 or greater were detected by MALDI-FT-ICR-MS. However, in ESI negative mode, only about 6800 peaks were detected. The peak resolving power was calculated to be over 400 000 at m/z 400. Fig. 1C and D separately displayed the scale-expanded segment at nominal m/z 447 in each spectrum. In the m/z range from 447.0000 to 447.3000, only 10 peaks assigned molecular formulas were detected by ESI, but with MALDI, 17 assignable molecular formulas were observed. The molecular formulas that could be identified by MALDI and ESI were annotated with black, while the molecular formulas that could be identified by MALDI or ESI were annotated with blue or red, respectively (Fig. 1C and D). All proposed molecular formulas at nominal m/z 447 are listed in Table 1. It shows that the commonly identified molecules contain carbon, oxygen, and hydrogen compounds ranging from C_{16} to C_{24} , O8 to O15, and H12 to H32, whereas for unique identified molecules, the elemental contents varied from C_{23} to C_{29} , O5 to O10, H12 to H36 for MALDI, and C_{17} to C_{19} , O20 to O28, H12 to H14 for ESI. This result suggests that constituents of NOM with lower O/C ratio are preferentially ionized in MALDI negative mode, whereas in ESI negative mode, constituents of NOM with higher O/C ratio (as compared with MALDI uniquely identified compounds) are preferentially ionized. The selectivity of ESI in negative mode for NOM observed in our work coincides with a previous study reported by Hertkorn et al. [8].

Fig. 1. 15.0 T FT-ICR mass spectra of SuwNOM in MALDI (A) and ESI (B) negative mode for the m/z from 200–800 Da; the scale-expanded m/z segment at nominal m/z 447 Da in MALDI- (C) and ESI- (D); FTICR mass spectra, at nominal m/z 439 Da (E, MALDI odd m/z), and at nominal m/z 440 Da (F, MALDI even m/z) in MALDI-FT-ICR mass spectra. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

Table 1 $C_nH_mO_q$ identified by MALDI- and ESI-FT-ICR-MS.

MALDI				ESI			
Measured m/z	Calculated m/z	Error (ppm)	Proposed molecular formula	Measured m/z	Calculated m/z	Error (ppm)	Proposed molecular formula
447.02051	447.02051	0	C19H12 O13	447.02047	447.02051	−0.08	C19H12 O13
447.04177	447.04164	0.29	C16H16 O15	447.04169	447.04164	0.11	C16H16 O15
447.05684	447.0569	−0.13	C20H16 O12	447.05689	447.0569	−0.02	C20H16O12
447.09320	447.09328	−0.17	C21H20 O11	447.09324	447.09328	−0.08	C21H20 O11
447.12964	447.12967	−0.06	C22H24 O10	447.12967	447.12967	0	C22H24O10
447.16602	447.16605	−0.06	C23H28 O9	447.16604	447.16605	−0.02	C23H28 O9
447.20236	447.20244	−0.17	C24H32 O8	447.20242	447.20244	−0.04	C24H32O8
447.03570	447.03570	0	C23H12O10	447.07803	447.07802	0.02	C17H20O14
447.05097	447.05102	−0.11	C27H12 O7	447.11451	447.11441	0.22	C18H24 O13
447.07208	447.07215	−0.15	C24H16O9	447.15087	447.1508	0.15	C19H28 O12
447.08733	447.08741	−0.17	C28H16 O6				
447.10846	447.10854	−0.17	C25H20 O8				
447.12371	447.12379	−0.17	C29H20 O5				
447.14483	447.14492	−0.20	C26H24 O7				
447.18115	447.18131	−0.35	C27H28O6				
447.21756	447.21769	0.29	C28H32 O5				
447.23879	447.23882	0.08	C25H36 O7				

Note: Proposed molecular formulas denoted with boldface letters representing the molecules commonly identified by ESI and MALDI.

On the other hand, because in most cases the majority of peaks at even m/z are assigned to the ^{13}C isotope of peaks in high resolution mass spectra of NOM, only peaks at odd m/z were considered for CHO-molecular formulas assignment in the

previous studies [5,19]. However, interestingly, during processing mass spectra in MALDI negative mode in our study, a significant portion of peaks at even m/z can also be assigned as CHO-molecules except for the ^{13}C isotope peaks. Furthermore,

Table 2 $C_nH_mO_q$ identified by MALDI-FT-ICR-MS. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

MALDI Odd		MALDI Even		$\bar{A}m$ (Da)	Average $\bar{A}m$ (Da, $n=7$)
Measured m/z	Proposed Molecular formula	Measured m/z	Proposed Molecular formula		
439.03065	C21 H12 O11	440.03847	C21 H12 O11	1.00782	
439.04594	C25 H12 O8	440.05362	C25 H12 O8	1.00768	
439.06702	C22 H16 O10	440.07480	C22 H16 O10	1.00788	
439.08229	C26 H16 O7	440.08999	C26 H16 O7	1.00770	1.00782
439.10336	C23 H20 O9	440.11128	C23 H20 O9	1.00792	
439.13979	C24 H24 O8	440.14773	C24 H24 O8	1.00794	
439.17618	C25 H28 O7	440.18396	C25 H28 O7	1.00778	
439.01539	C17 H12 O14	440.00199	C20 H8 O12		
439.05177	C18 H16 O13	440.01757	C24 H8 O9		
439.08818	C19 H20 O12				
439.11853	C27 H20 O6				
439.12448	C20 H24 O11				
439.15506	C28 H24 O5				
439.16086	C21 H28 O10				
439.19738	C22 H32 O9				
439.21251	C26 H32 O6				

Table 3
Percentages of $C_nH_mO_q$ identified by MALDI- and ESI-FT-ICR-MS.

Ionization method	Number of $C_nH_mO_q$	Total number of $C_nH_mO_q$	Percent (%)
ESI and MALDI			
ESI Only	937	3305	28.35
MALDI Only	1159		35.07
ESI and MALDI	1209		36.58
MALDI even and odd			
MALDI odd only	1271	2468	51.50
MALDI even only	100		4.05
MALDI even and odd	1097		44.45

compared with the CHO-molecular formulas at odd m/z , most of the peaks at even m/z have the identical molecular formulas with the peaks at odd m/z but have an average difference of 1.00782 between even and odd m/z values. This difference is quite close to the atomic mass of hydrogen (1.007825 Da), so we can deduce that each peak at even m/z having the same molecular formula with that at odd m/z is molecular ion $[M+e]^-$ (mass of electron (e) is 0.000549 Da), and the corresponding peak at odd m/z is deprotonated ion $[M-H]^+$ (mass of hydrogen proton (H^+) is 1.007276 Da). This is a significant finding for NOM characterization. Although for data process simply, radical anions are not wanted, these radical anions can also be well identified due to the ultrahigh resolution of FT-ICR-MS. Meanwhile, these radical anions are beneficial for NOM characterization. Firstly, the proposed molecular formulas assigned by $[M-H]^+$ ions at odd m/z can be further confirmed by their $[M+e]^-$ ions at even m/z ; secondly, the molecules being liable to form $[M+e]^-$ ions are very likely to be aromatic or condensed aromatic compounds with special substituent groups which have higher electron affinity and are more likely to absorb electron from other molecules to produce free radical anions during the MALDI ionization process in spite of a matrix being used. Photoexcitation and photoionization may be the mechanism for the formation of radical anions [28]. The scale-expanded segment of mass spectra at nominal m/z 439 Da and 440 Da in MALDI negative mode are displayed in Fig. 1 E and F. The blue or red annotations separately represent the unique molecular formulas assigned by MALDI odd or even m/z peaks, and the black annotations represent the common formulas assigned by both m/z peaks. Table 2 shows the assigned molecular formulas at nominal m/z 439 Da and 440 Da. Molecules shown in green and red progress

as follows: as the number of CH_4 group increases by one, the number of oxygen atoms decreases by one; molecules noted in green progress from C20 through C26, while molecules noted in red progress from C24 through C28. Through comparison of the proposed molecular formulas, our preliminary conclusion is that for identical classes of molecules, we can state three rules based on the observation that the number of CH_4 and O groups ($+CH_4/-O$) affect whether a molecule is detected by MALDI at even m/z peaks, odd m/z peaks, or both: (1) the molecules with lower H/C ratio and higher O/C ratio (such as $C_{20}H_8O_{12}$, $C_{24}H_8O_9$) are more likely to form $[M+e]^-$ ions during the MALDI process (only even m/z peaks appeared); (2) the molecules with higher H/C ratio and lower O/C ratio (such as $C_{26}H_{32}O_6$, $C_{27}H_{20}O_6$, $C_{28}H_{24}O_5$) preferentially form $[M-H]^+$ ions during the MALDI process (only odd m/z peaks appeared); (3) molecules which fall in between those two extremes may be detected in either odd or even mode. For the class of molecules only observed at odd m/z (noted in black on Table 2), no obvious features were observed in CHO-composition, which indicates those molecules may be some special aromatic or condensed aromatic compounds with fewer special substituent groups.

To extend these observations to the whole mass range of detected ions, the data generated by MALDI and ESI were automatically processed with formula assigned software, and different parameters were calculated for visually graphic comparison of the two types of mass spectral data. Collectively, a total of 3305 CHO-molecular formulas were assigned for MALDI and ESI negatively odd m/z ions in the mass range from 200 to 800 Da, of which 1209 (36.58%) formulas were commonly assigned by both ionization modes (Table 3). The number of unique CHO-molecular formulas assigned by MALDI is 1159 (35.07%), and only 937 (28.35%) unique CHO-molecular formulas were assigned by ESI. This result indicates that the MALDI ionization mode can provide molecular information about NOM which ESI cannot detect. Combining different ionization modes (such as MALDI, ESI, APPI and APCI) is needed to generate more complete molecular information about NOM. For CHO-molecular formulas assigned by MALDI at odd and even m/z peaks, a total of 2468 formulas were assigned by both m/z ions, whereas 1271 (51.50%) formulas were uniquely observed by MALDI odd m/z ions and only 100 (4.05%) by MALDI even m/z ions. The number of commonly observed formulas is 1097 (44.45%) (Table 3). This observation indicates that about half the NOM molecules identified by MALDI may contain special substituent groups which are liable to generate free radical anions.

Fig. 2. Van Krevelen diagrams for molecular formulas observed in NOM by MALDI and ESI ionization mode (A), and formulas observed by MALDI at even and odd m/z (B).

To further compare the selectivity of MALDI and ESI for NOM molecules, Van Krevelen diagrams were constructed for each formula by using the molar ratio of hydrogen to carbon (H/C ratio) as the ordinate and the molar oxygen to carbon ratio (O/C ratio) as the abscissa. Fig. 2A shows the Van Krevelen diagrams of the formulas observed by MALDI and ESI. A notable feature of selectivity of MALDI and ESI for NOM molecules was visually observed. In general, NOM molecules with relatively lower O/C ratio (O/C ratio < 0.5) were preferentially ionized by negative-ion MALDI, of which more than three-fourths of total numbers were not ionized by negative-ion ESI. However, those molecules with higher O/C ratio (O/C ratio > 0.5) were commonly ionized by MALDI and ESI or uniquely detected by ESI. Van Krevelen diagrams for molecular formulas observed by MALDI at even and odd m/z peaks (Fig. 2B) suggested that NOM molecules with moderate O/C ratio ($0.7 > \text{O/C ratio} > 0.25$) and lower H/C ratio were liable to form molecular anions during MALDI ionization process.

For purposes of visual resolution and identification of compound classes, types, and carbon distributions, plots of Kendrick mass defect vs. nominal Kendrick mass for mass spectral peaks detected by MALDI and ESI were constructed and shown in Figs. 3–5 and Figs. S1–S3 in the Supporting information. Kendrick mass (F) and Kendrick mass defect (F) ($F = \text{CH}_2, \text{CO}, \text{COO}, \text{H}_2\text{O}, \text{H}_2, \text{O}$) were calculated according to previous reports [19,20]. Generally:

$$\text{Kendrick mass (F)} = \text{observed mass} \times [(\text{nominal mass of F})/(\text{exact mass of F})] \quad (1)$$

$$\text{Kendrick mass defect (F)} = (\text{nominal mass} - \text{Kendrick mass (F)})/2 \quad (2)$$

Each of the plots showed that the relatively large molecules (800 Da > MW > 600 Da) were preferentially ionized by the ESI mode relative to MALDI (Figs. S1A, S2A and S3A in the Supporting information, and Figs. 3A, 4A, 5A). The scale-expanded Kendrick

Fig. 3. H₂O-based Kendrick mass defect vs. H₂O-nominal Kendrick mass for odd m/z detected by MALDI and ESI (A,B), and even and odd m/z detected by MALDI (C,D).

Fig. 4. O-based Kendrick mass defect vs. O-nominal Kendrick mass for odd m/z detected by MALDI and ESI (A,B), and even and odd m/z detected by MALDI (C,D).

mass defect or nominal Kendrick mass segments of plots revealed some remarkable features (Figs. S1B, S2B and S3B in the Supporting information, and Figs. 3B, 4B, 5B). In the CH_2^- , CO_2^- or H_2^- -based Kendrick mass plots (Figs. S1B and S2B in the Supporting information, and Fig. 5B), the results showed that some compounds of identical class are uniquely ionized by MALDI in negative mode, and alternation of the numbers of CH_2^- , CO_2^- or H_2^- -groups had no effect on the ionization selectivity of MALDI for those compounds. By contrast, these plots do not show any compounds of identical class being uniquely ionized by ESI. We speculate that those compounds uniquely ionized by MALDI may be non-polar compounds, so they are more difficult to ionize by ESI, although polar portions of those compounds may be affected by the alternation of the number of CH_2^- , CO_2^- or H_2^- -groups. Interestingly, in the CO-based Kendrick mass plots (Fig. S3B in the Supporting information), some compounds of identical class being uniquely ionized by ESI were also found, except those compounds of identical class uniquely ionized by MALDI. The

more notable features were observed in the H_2O -based Kendrick mass plots (Fig. 3B). The plots show that the molecules within a class are more liable to be ionized by MALDI by decreasing the number of H_2O -groups. Conversely, by increasing the number of H_2O -groups, the molecules will be preferentially ionized by ESI. We speculate that the number of H_2O -groups may be related to the polarity of molecules. With increasing the number of H_2O -groups, the polarity of molecules will increase. So molecules with higher number of H_2O -groups are more polarity, which lead them to being preferentially ionized by ESI, but those with lower number of H_2O -groups may be non-polar compounds which are liable to be ionized by MALDI. Similar trends are also observed in the O-based Kendrick mass plots (Fig. 4B). That is to say, identical classes of molecules with more O-groups will be more likely to ionize by ESI, but those with fewer O-groups will be preferentially ionized by MALDI. These remarkable observations further suggest that NOM molecules with lower O/C ratio are more likely to be ionized by MALDI.

Fig. 5. H₂-based Kendrick mass defect vs. H₂-nominal Kendrick mass for odd m/z detected by MALDI and ESI (A,B), and even and odd m/z detected by MALDI (C,D).

Analysis of plots of Kendrick mass defect vs. nominal Kendrick mass for even- and odd-mass ions detected by MALDI (Figs. S1C and D, S2C and D, S3C and D in the Supporting information, and Figs. 3C and D, 4C and D, 5C and D) shows that some compounds of identical class are uniquely observed at odd m/z in MALDI mode, and alternation of the numbers of CH₂, CO₂- or CO-groups cannot lead these molecules to form molecular ions at even m/z . However, in the plots of H₂O- and H₂-based Kendrick mass defect vs. nominal Kendrick mass (Figs. 3D, 5D), notable features were observed. Identical classes of molecules with more H₂O- or H₂-groups will be more likely to form deprotonated ions at odd m/z , but those with fewer H₂O- or H₂-groups will more readily form molecular ions at even m/z by MALDI. This observation suggests that NOM molecules with higher H/C ratio are more likely to form deprotonated ions at odd m/z , whereas those with lower H/C ratio are more likely to form molecular ions at even m/z .

To estimate the aromatic and condensed aromatic structures of NOM ions detected by MALDI and ESI, a modified aromaticity index (AI_{mod}) was calculated from the formulas according to previous works [9,18]. For C_nH_mO_q compounds of NOM, AI_{mod} values are calculated as follows:

$$AI_{mod} = (1 + n - 0.5 \times q - 0.5 \times m) / (n - 0.5 \times q)$$

Aromatic and condensed aromatic structures can be identified with threshold criteria of AI_{mod} > 0.5 and AI_{mod} ≥ 0.67 (as shown in Fig. 6). For the molecules uniquely detected by MALDI (Fig. 6A), 76.3% of them contain aromatic structures, including a large proportion of condensed aromatic structures (41.2%). By contrast, for the molecules uniquely detected by ESI (Fig. 6B), only 23.7% of them involve aromatic structures, and of those, only a small proportion of condensed aromatic structures (9.6%) are included. The compounds commonly detected by ESI and MALDI

Fig. 6. Element ratio plots for FT-ICR-MS ions of NOM detected by MALDI only (A), ESI only (B), MALDI and ESI commonly (C), MALDI at odd m/z only (D), MALDI at even m/z only (E) and MALDI at odd and even m/z commonly (F). Aromatic and condensed aromatic structures can be identified with threshold criteria of $Al_mod > 0.5$ (red dots) and $Al_mod \geq 0.67$ (blue dots). (For interpretation of the references to color in the legend, the reader is referred to the web version of this article.)

show roughly similar trends with those uniquely detected by ESI (Fig. 6C). Another distinction for these aromatic molecules is that nearly all of the molecules uniquely detected by MALDI involving aromatic structures have low O/C ratios (O/C ratios < 0.5), but for those uniquely detected by ESI or commonly detected by ESI and MALDI, the majority of them have higher O/C ratios ($1 > O/C \text{ ratios} > 0.5$). These observations suggest that aromatic and condensed aromatic compounds with low O/C ratio (O/C ratios < 0.5) in NOM are preferentially ionized by MALDI but cannot be ionized by ESI. Only a small proportion of aromatic and condensed aromatic compounds with higher O/C ratios ($1 > O/C \text{ ratios} > 0.5$) can be ionized by ESI. We speculate that the aromatic structures can partly absorb laser energy and give rise to ion formation more easily during the MALDI ionization process. Furthermore, aromatic structures and lower O/C ratio may lead the compounds to having lower polarity, both of which may lead those compounds with aromatic and condensed aromatic structures and low O/C ratio (O/C ratios < 0.5) to being difficult to ionize by ESI but uniquely ionized by MALDI.

For analysis of the molecules detected by MALDI at even and odd m/z peaks, the elemental ratio plots are displayed in Fig. 6D–F. The plots reveal that of the molecules detected by MALDI at both odd and even peaks, 69.2% had $Al_mod > 0.5$, that is, had aromatic or condensed aromatic structures (Fig. 6F). Furthermore, 97.9% of the molecules uniquely detected by MALDI at even m/z peaks contain aromatic or condensed aromatic structures (as shown in Fig. 6E). This result suggests that most of the molecules of NOM with aromatic or condensed aromatic structures are liable to obtain electrons and form molecular anions in negative-ion MALDI.

Nominal oxidation state of carbon (NOSC), first introduced by Riedel et al. [9] as a formal parameter for interpretation of ultrahigh precision mass spectrometry data, can provide

information on the average oxidation state of all carbons per standard calculations. Meanwhile, the parameter can also give an indirect measure for the polarity of a compound. For $C_nH_mO_q$ compounds of NOM, NOSC can be estimated from the chemical formula using the following equation:

$$NOSC = 4 - [(4n + m - 2q)/n]$$

To further investigate whether the oxygen content or the degree of unsaturation of molecules in NOM is responsible for the ionizing selectivities of MALDI and ESI, plots of DBE vs. NOSC were constructed and shown in Fig. 7. Based on these plots, it is apparent that molecules with the highest NOSC values are detected primarily only by ESI, which suggests that ESI shows an ionizing preference for more oxygenated molecules with greater polarity (see Fig. 7A). Conversely, most of molecules with lower NOSC values but higher DBE values can only be detected by MALDI. This indicates that less oxygenated and more unsaturated molecules are more liable to be ionized by MALDI. These results agree well with our expectation. In the plot of DBE vs. NOSC for formulas observed by MALDI at even and odd m/z peaks (Fig. 7B), molecules with identical DBE values but with higher NOSC values are seen to be liable to form molecular anions at even m/z . It suggests that more oxygenated analytes may be more likely to contain special substituent groups which are preferential to form free radical anions by obtaining electrons during MALDI ionization. Furthermore, with the values of DBE increasing, the number of molecules which can form molecular anions increases gradually. This suggests that molecules with a higher degree of unsaturation are more likely to form molecular anions. As the degree of unsaturation increases, the aromaticity may also increase. So we can speculate that molecules with more aromaticity are liable to form molecular anions. Al_mod plots as shown in Fig. 6D–F demonstrate this trend.

Fig. 7. Plots of DBE vs. the average nominal oxidation state of carbon (NOSC) in each formula observed by MALDI and ESI (A) and by MALDI at even and odd m/z (B).

4. Conclusions

NOM is so complex that multiple ionization methods should be combined to gain more comprehensive molecular information of NOM, because different ionization methods, such as ESI, APCI, APPI and MALDI, have different ionizing selectivities for NOM molecules with different properties (such as polarity and aromaticity). In this study, MALDI coupled with FT-ICR-MS was first employed for characterization of NOM within a mass range of 200–800 Da in molecular level. Furthermore, the obtained mass spectral data by MALDI were fully compared with data generated by ESI. Significant ionization selectivities of MALDI and ESI for different NOM molecules were observed. As expected, complementary molecular information about NOM that ESI cannot detect was provided by MALDI. It is notable that more unsaturated and aromatic constituents of NOM with lower O/C ratio (O/C ratio < 0.5) are preferentially ionized in MALDI negative mode, whereas polar constituents of NOM with higher O/C ratio are preferentially ionized in ESI negative mode. The ionization selectivities of MALDI and ESI mainly attribute to the difference of ionization mechanism of these two techniques. The principles of ionization with ESI and MALDI have been well reviewed by Cole [29], Zenobi and Knochenmuss [28] and Dreisewerd [30] separately. In general, ESI ionizes molecules by transferring pre-existing ions from solution phase to gas phase. Therefore, molecules that exist as ions in solution or those with acidic or basic groups capable of forming deprotonated or protonated ions are liable to ionize by ESI. Whereas, MALDI ion formation includes a number of chemical and physical pathways, such as gas-phase photoionization, excited-state proton transfer, thermal ionization, and desorption of preformed ions. Thus, molecules with unsaturated or aromatic core structures which are liable to absorb UV laser energy are preferentially ionized by MALDI. Moreover, most of the molecular anions that appeared at even m/z in MALDI negative ion mode were analyzed. The analysis of those molecules indicated that molecules of NOM with Lower H/C ratios and moderate O/C ratios ($0.7 > \text{O/C ratio} > 0.25$) as well as containing aromatic or condensed aromatic structures are liable to form molecular anions during MALDI ionization. In summary, it is notably demonstrated that MALDI is another useful ionization method for providing more complementary molecular information about NOM. For purposes of comprehensive characterization of NOM at the molecular level, more than one ionization methods should be applied.

Conflict of interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aca.2015.01.051>.

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