Understanding the Structural Complexity of Dissolved Organic Matter: isomeric diversity

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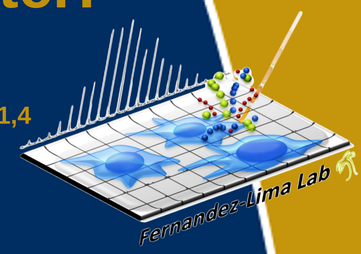


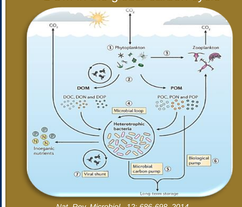
Overview

In the present work, we addressed the isomeric complexity of DOM samples collected from Pantanal (PAN) National Park (Brazil) using TIMS-FT-ICR MS. An average of 3000 chemical assignments were identified in a single infusion experiment. A high isomeric complexity (4-10 isomers per m/z signal) was found at nominal mass. An upper estimate of the number of isomers per chemical formula was provided based on unique neutral loss fragmentation patterns and core fragments resulting from the FT-ICR MS/MS analysis at nominal mass isolation.

Introduction

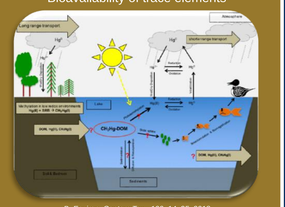
Dissolved Organic Matter (DOM): Complex mixture resulting from the degradation of bacteria, algae, and plants.

DOM in the global carbon cycle



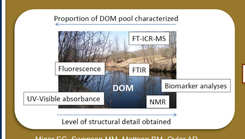
Nat. Rev. Microbiol., 12: 686-698, 2014

Bioavailability of trace elements

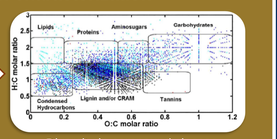


B. Environ. Contam.Tox., 100: 14–25, 2018

Bulk vs Molecular level characterization



Minor EC, Swenson MM, Mattson BM, Oyler AR. Environmental Science: Processes & Impacts. 2014;16(9):2064-79.



Diversity in compound classes

- Analysis of DOM is challenging due to its high structural heterogeneity and functionalities, isomeric complexity and wide range of molecular weights.
- Trapped Ion Mobility Spectrometry offers a promising alternative toward a more reliable isomeric characterization of DOM when coupling TIMS to FT-ICR MS

References

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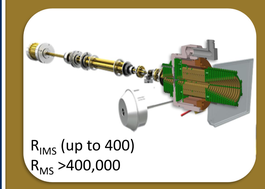
Experimental

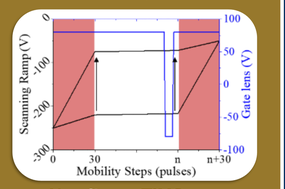
Sample preparation

- The PAN samples were collected from the Paraguay River (PAN-L) and a wetland channel in Pantanal National Park, Brazil (PAN-S).
- DOM was dissolved in 50:50 v/v CH_3OH/H_2O (1 ppm).

TIMS-FT-ICR MS

Custom built TIMS-FT-ICRMS 7T Solarix spectrometer equipped with an infinity ICR cell (Bruker Daltonics Inc., MA).





Non linear TIMS scan

- TIMS cell fill/trap/elute/quench sequence: 9/3/9/3 ms
- 1000 IMS scans per MS spectrum
- Voltage difference across the ΔE gradient: 5.0 V

FT-ICR MS/MS analysis

FT-ICR MS/MS experiments: quadrupole isolation at nominal mass and CID energies of 15-20 eV.

Analytical workflow

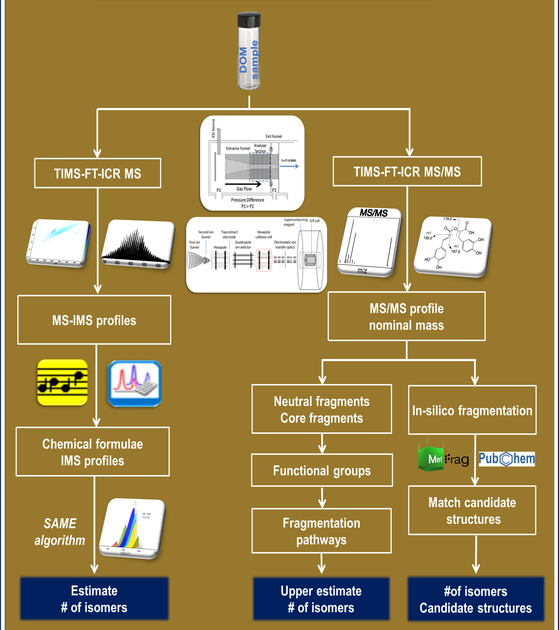


Figure 1. Workflow used to estimate the number of isomers in PAN sample

Results TIMS-FT-ICR MS

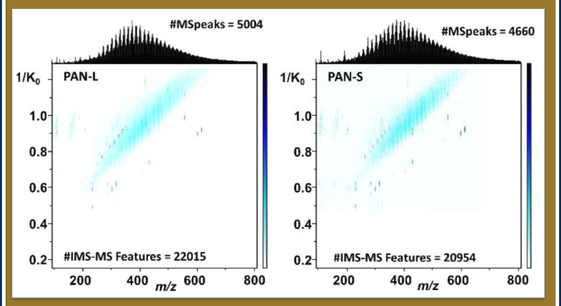


Figure 2. MS and 2D-IMS-MS contour plots of PAN samples

- 3,066 and 2,830 compounds identified in PAN-L and PAN-S samples respectively based on the general formula: $C_xH_yN_{0-3}O_{0-19}S_{0-1}$.
- Around 80% of compounds corresponded to highly conjugated oxygen species (O_1-O_{20}).

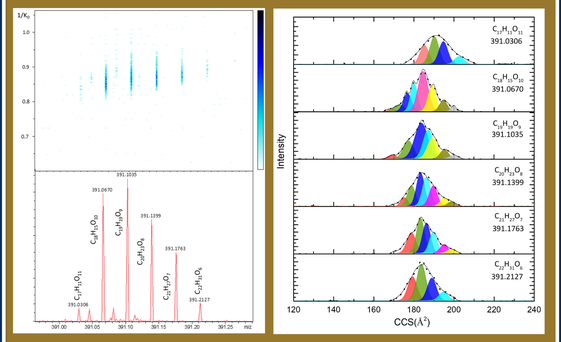
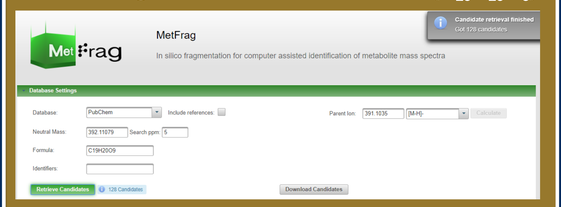
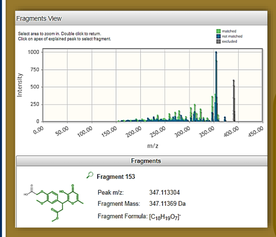


Figure 3. 2D-IMS-MS, MS and IMS projections at nominal mass 391 m/z.

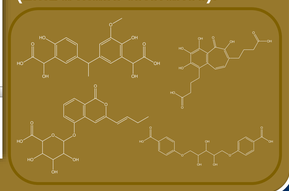
Large isomeric diversity at nominal mass and per chemical formula (average of 6-10 structural and conformational isomers per chemical formula)

In silico fragmentation of m/z 391.1035 ($C_{19}H_{19}O_9$)





96 candidate structures based on accurate masses of precursor and fragment ions (1mDa mass tolerance).



Results FT-ICR-MS/MS

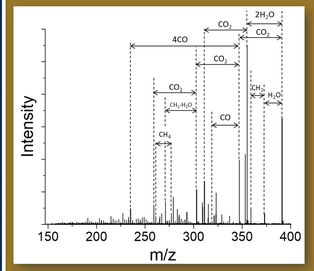


Figure 4. FT-ICR MS/MS spectrum of q-isolated 391 m/z precursor ion and subjected to CID.

- Neutral losses can be directly associated with functional groups and the overall structure of the precursor ion.
- The number of pathways could provide an upper estimate of the number of structural isomers.
- 260 structural isomers based on core fragments and unique fragmentation pathways.

Precursor ion m/z	Core Fragment m/z	Structural isomers
391.1035 $C_{19}H_{19}O_9$	161.0607 $C_{10}H_9O_2$	13
	163.0763 $C_{10}H_{11}O_2$	7
	165.0192 $C_8H_5O_4$	3
	165.056 $C_9H_9O_3$	2
	167.0349 $C_8H_7O_4$	1
	171.0814 $C_{12}H_{11}O$	23
	173.0607 $C_{11}H_9O_2$	23
	175.0400 $C_{10}H_7O_3$	15
	183.0450 $C_{12}H_7O_2$	40
	183.0814 $C_{13}H_{11}O$	25
	185.0607 $C_{12}H_9O_2$	29
	187.0400 $C_{11}H_7O_3$	25
	201.0192 $C_{11}H_5O_4$	25
	202.9984 $C_{10}H_3O_5$	15
	205.0140 $C_{12}H_5O_4$	7
	241.0140 $C_{13}H_5O_5$	7

$$[C_{19}H_{20}O_9\text{-}H]^- \begin{cases} [C_{10}H_9O_2]^- + \{5CH_2;\ CO;\ 3CO_2\} \\ \vdots \qquad\qquad \vdots \\ [C_{13}H_5O_5]^- + \{2CH_2;\ 2CH_4;\ H_2O;\ CO;\ CO_2\} \end{cases}$$

Conclusions

- A single infusion TIMS FT-ICR MS experiment permitted the identification of around 3,000 chemical components in PAN samples based on mass accuracy and assuming a total general formula of $C_xH_yN_{0-3}O_{0-19}S_{0-1}$.
- TIMS measurements provided structural and conformational isomeric content per chemical formula (e.g., 4-10 isomers).
- A further estimation of the number of structural isomers was possible based on unique neutral loss fragmentation patterns and core fragments from tandem MS/MS.
- Overall data suggested that multiple structural isomers could share very closely related CCS, which will require the use of ultrahigh resolution TIMS mobility scan functions in tandem with MS/MS.

Acknowledgments

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