

Multiple Charged Constituents in Suwannee River Natural Organic Matter

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1. INTRODUCTION

Natural organic matters are complex biogeochemical non-repetitive materials existing in such a vast amount, that their quantity easily exceed the amount of functional biomolecules (1, 2). And furthermore their continuous reformation, degradation and diagenesis, which are governed by the rather fundamental restrains of thermodynamics and kinetics, result their extreme intricacy. Therefore the molecular level understanding of such supermixtures is extremely difficult. However the proper characterization of such a vast system is feasible only if minimal–invasive sampling techniques (3,4) and afterwards several high-end analytical techniques are combined, since generally applied methods, for instance elemental analysis, IR spectroscopy describe them only with an averaged characteristic (5). To verify this, surplus information were presented here, by utilizing FT-ICR/MS and Q-TOF-IM/MS and integrated mathematical data analysis. FT-ICR measurements give sufficient mass accuracy and resolution even in broadband mode (6), enabling postulate exact molecular formulae and determine the charge-state (a singly (or doubly) charged $^{13}\text{C}^{12}\text{C}_{n-1}$ ion will be higher in m/z by 1 (or 0.5) than the corresponding $^{12}\text{C}_n$ nuclide). Therefore application of such a method to resolve and describe individual humic constituents is preferable. Despite of ultra-high resolution, non-adequate settings of any kind of experimental settings might mislead the general result of the characterization (7-9). Since the occurrences of poly-carboxylic acids and -hydroxyl groups, and their relative high abundances had been shown in these type of mixtures (10-12), the formation of multiply charged negative ions in the ESI experiments would have been expected. Though it should be noted, that the denoted properties of large molecules might change

during their transformation from polyelectrolyte state in solution to the detected ions in the gas phase. On the other hand, till now, the presence of probable multiple charged constituents were only assumed, moreover sometimes depicted as “bumps”, but without further details (9, 13).

2. MATERIALS AND METHODS

The sample, SRDOM was dissolved in methanol directly before the analysis. Ultra-high resolution mass spectra of the sample, was acquired at the Helmholtz Zentrum München with a Bruker APEX Qe Fourier transform mass spectrometer (FTICR-MS) equipped with a 12 Tesla superconducting magnet and an Apollo II electrospray source. The sample was injected with direct infusion with the flow rate of 2 $\mu\text{L}/\text{min}$. The spectrum was obtained in negative mode with the number of data points per measurements of 4 MW (time domain size) with the typical resolution of $5 \cdot 10^5$. The number of scans was 11000 for 1 $\mu\text{g}/\text{mL}$ sample concentration. The spectrum was externally calibrated on arginin clusters and then internally recalibrated with fatty acids. Once the exact masses of the molecules had been determined, their molecular formulae were batch-calculated by a software tool, written in-house and provided by E.V. Kunenkov. Ion mobility (IM) measurements were carried out in negative modus, on a Waters Synapt High Definition MS system in the Waters Application Laboratory (Manchester).

3. RESULTS AND DISCUSSION

Herein, the obtained high resolution mass spectra of SRDOM (Suwannee river dissolved organic matter) and SRFA (Suwannee river fulvic acid) revealed beside the usually characterized single charged constituents further multiple charged species, and were described for the first time. In the spectrum of 1 $\mu\text{g}/\text{mL}$ SRDOM, beside the regular pattern of singly charged nominal masses, a second series with 0.5 Da mass width, between 250-560 m/z mass range were visible. Since ESI negative ion 12 Tesla FTICR mass spectra of SRDOM resolved over 120.000 peaks, with the average resolution of ≥ 300.000 molecular formula assignment with a software tool, written in-house, with less than 0.5 ppm was possible, resulting over 6000 assigned molecular formulae. The most significant mass differences between $\text{C}_c\text{H}_h\text{O}_o\text{N}_n\text{S}_s$ and $^{13}\text{C}_{c-1}\text{H}_h\text{O}_o\text{N}_n\text{S}_s$ were $\Delta m = 1$ Da (singly charged) and 0.5 Da (doubly charged). Afterwards, the charge sorted constituents were plotted based on their H/C and O/C elemental ratios in van Krevelen diagrams.

The peculiar presentation of assigned molecular formulas, enables to individually describe certain structural properties of the observed constituents within this

supermixtures. Although we have to note that, in several cases, differentiation between charge states were not possible since constituents with $C_xH_yO_z^-$ and $C_{2x}H_{2y}O_{2z}^{2-}$ elemental composition, denote the same position in the van Krevelen diagram. But still, one of the most remarkable difference between charge states is that, molecules assigned as doubly charged, are densely situated in higher O/C field than the single charged ones.

Beside FTICR measurements, ion mobility experiments were also conducted in order to support the observed results (14). SR DOM sample were injected without preliminary separation in different concentration (100-0.5 $\mu\text{g/mL}$) resulted similar results independent of the applied concentration. ESI negative ion TOF-Ion Mobility mass spectra and drift time vs. m/z distribution of SRDOM, obtained on Synapt HDMS system, confirmed also the existence of multiple-charged constituents through visible separation of molecules, based on their charge-states. In the drift map, at least two distribution of ions were presented: group of doubly charged constituents with shorter drift time and a group of singly charged molecules with longer drift time.

The efficiency of an automated formula assignment, developed by E. V. Kunenkov, was also tested successfully. For this purpose, beside the complete FTICR mass spectra, an expanded m/z region of 348.8-350.6 was selected, and the possible molecular formulas were individually assigned (51 constituents, belonged to 10 different series), with built in software of Bruker Data Analysis 3.4). Mass accuracy for all assignments were ≤ 90 ppb.

4. CONCLUSIONS

Overall, our results show for the first time that expanded and carefully selected experimental (measurement and data analysis) settings and combined high-end analytical tools can eventuate the extension of the existing knowledge on molecular level understanding of such a supermixtures.

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