Comparison of FTICR Data on the Suwannee River Humic and Fulvic Acids

Erast V. Kunenkov[1], Alexey S. Kononikhin[2], Andras Gaspar[3], Philippe Schmitt-Kopplin[3], Irina V. Perminova[1], Andrey V. Garmash[1], Norbert Hertkorn[3], Igor A. Popov[2], Eugeniy N. Nikolaev[2]

[1] Department of Chemistry, Lomonosov Moscow State University, 119992 Moscow, Russia
[2] Laboratory of Biomacromolecules, Emmanuel Institute of Biochemphysics of RAS, 117977 Moscow, Russia
[3] GSF Research Center for Environment and Health, Institute of Ecological Chemistry, 85764 Neuherberg, Germany

Humic substances (HS) are complex mixtures formed during decay of plant and animal materials. HS are operationally classified into fulvic acid (FA) and humic acid (HA) fractions according to their solubility in weak acids and bases: HA precipitates at pH < 1-2 and represents higher molecular weight, carbonized fraction of HS, while FA is more oxygenated, lower molecular weight fraction of HS. Given the ultrahigh resolution of FTICR mass spectrometry that posits it as a most powerful tool for analysis of the complex mixtures, its use can be particularly advantageous for revealing differences between HA and FA on the molecular level. This, in turn, can lead to development of HS classification based on the structural features of the constituting compounds.

The goal of this study was to compare FTICR data on humic and fulvic acids - two operationally defined fractions of humic substances from the Suwannee River. Another goal of this study was to compare FTICR data obtained for the same humic sample using different FTICR spectrometers. To reach this goal, FTICR data were obtained using 7 Tesla LTQ FT (Finnigan) and 12 Tesla Bruker (Bruker Daltonics) mass spectrometers with electrospray ion source in negative ionization mode. We used well characterized samples of Suwannee River Fulvic (SRFA) and Humic (SRHA) Acids of the International Humic Substances Society (IHSS) as source compounds. Two solvents were used: 1:1 water-methanol solution and 1:4 water acetonitrile solution. Concentrations of HS samples ranged between 0.1 to 1 mg/mL. Self-designed software FIRAN was used for FTICR mass spectra processing. This software was applied to: 1) calculate differential mass spectra; 2) create Kendrick mass plots; 3) create van Krevelen diagrams; 4) estimate elemental composition. Different data filtering techniques were applied to exclude data, which may be irrelevant and to check robustness of the obtained results.

The plot of Kendrick mass defect versus nominal mass obtained for HA and FA tested shows that FA ions have smaller Kendrick mass defects as compared to HA. This could be indicative of higher oxygen content in FA structure. This conclusion was confirmed by the corresponding van Krevelen diagrams, which demonstrated higher O/C and lower H/C ratios for FA as compared to HA. Data filtering based on the results of isotope simulation provided for much better distinction between HA and FA ions. A use of original computational approach based on construction of differential mass spectra, has allowed us to find prevalent differences between ion masses and evaluate variations in composition of HS. Unlike conventional approaches, this processing technique did not reveal any significant differences between FA and HA samples even in case of data filtering enabled. This may be interpreted in a way that the molecules of fulvic and humic fractions are consisted of different "cores", but have similar structural variations in their "environment".

Estimated elemental composition of HS samples was found to be insensitive to data filtering and had little sensitivity to experimental conditions. For SRFA, gross elemental compositions were estimated from three mass spectra obtained using different solvents and different spectrometers. All three results were close one to another and laid in the following ranges:: C: 54.3 − 54.7%, H: 4.0 − 5.0%, O 39.3-41.7%, and all of them were close to results of elemental analysis (C: 52.34, H − 4.36, O 42.98): At the same time, for SRHA, elemental compositions from FTICR mass spectra led to noticeably higher carbon content (62.9 %) and lower oxygen content (31.2) as compared to the results of elemental analyses (C - 52.63% and O-42.04%). This data might be indicative of different ionization efficiency of FA and HA fractions: whereas SRFA was almost completely ionized by electrospray ion source, only a portion of more reduced HA molecules was ionized under these conditions.