Size exclusion fractionation: what does it tell us about molecular weights and structures of humic substances?

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1. INTRODUCTION

Understanding multilevel complex systems of many interacting objects is a key problem of modern environmental chemistry. Typical examples of such systems are humic substances (HS). The building blocks of HS are products of stochastic synthesis, and produce an assembly of structurally close, but not identical molecules. Properties of such an assembly are consistent with steady state dynamics of humification process and bear genetic information on HS formation (Cabaniss et al, 2005). It might be encoded by molecular weight distributions (MWD), structural patterns, and their relationships.

Size-exclusion chromatography (SEC) is a powerful technique for both fractionation of complex mixtures and determination of their MWD. It is traditionally used in HS analysis (De Nobili et al. 1989). However, SEC of HS is complicated with non-size exclusion effects due to polyelectrolyte nature of humic molecules. In-depth interpretations of the SEC results are needed to adequately treat these data for HS. In this work, we used Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry, which is the most powerful method for studying complex mixtures, to complement data of SEC.

The goal of this work was to develop in-depth interpretations of the molecular weight and structural data provided by SEC through studies on structure/molecular properties versus molecular weight relationships for humic
materials from different sources using both on-line and off-line SEC-UV/DOC analysis and off-line FTICR MS of the obtained fractions.

2. MATERIALS AND METHODS

**Humic materials:** Standards of the International Humic Substances Society (IHSS): fulvic and humic acids isolated from the Suwannee River (SRFA and SRHA, respectively) and DOM were used as source compounds. In addition, peat humic and fulvic acids and their non-fractionated mixture were used, respectively, PHA-T598, PFA-T598, and PHF-T598.

**SEC:** Liquid chromatographic system with tandem UV-DOC detection was used for preparative fractionation of HS as described by Perminova et al. (1998). Solutions of humic materials were prepared in phosphate buffer with different pH and ionic strength. HS concentration was 50 mg/l. SEC system consisted of HPLC pump, autosampler, glass column, and equipped with UV and DOC detectors. Gel Toyopearl TSK HW-55S gel was used for separation.

**FTICR MS:** Bruker mass spectrometer (12 Tesla) with electrospray ion source in negative ionization mode was used for HS analysis. 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 of source samples. Different data filtering techniques were also applied to exclude a data which may be irrelevant and to check robustness of obtained results.

**UV Analysis:** Absorbance of each fraction was measured at 254 nm and normalized to the content of organic carbon to yield SUVA-values.

3. RESULTS AND DISCUSSION

The studies on non-size exclusion effects of SEC analysis of HS have shown a remarkable role of pH and ionic strength for resulting data. The optimum conditions differed greatly for FA and HA fractions of the same humic material as well as for aqueous versus peat, soil, and coal materials. A use of ammonium hydrocarbonate (AC) buffer versus commonly used phosphate buffer (PB) has yielded very close SEC-profiles of the humic materials studied. This allows us for recommending this buffer for direct FTICR MS studies. Five humic materials of aquatic and peat origin were fractionated into narrow
fractions using both SEC-buffers. These fractions were further analyzed using SEC-UV/DOC and FTICR MS to determine molecular weight, elemental composition and absorptivity (SUVA) for all fractions.

The SUVA versus MW relationships were calculated using SEC-UV/DOC data, and H/C versus MW relationships were calculated using FTICR MS data for the same samples. The comparative analysis showed a general consistency in the structure versus MW relationships obtained using both independent methods.

4. CONCLUSIONS

The demonstrated consistency of the structure versus MW relationships obtained using two independent methods - SEC-UV/DOC and FTICR MS - gives a good promise for developing novel in-depth interpretations of the SEC data on HS and for their use in humification models.

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