Spectroscopic Characterization of Structural and Functional Properties of Natural Organic Matter Fractions

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Introduction

Natural organic matter (NOM) consists of complex organic materials occurring in all terrestrial and aquatic ecosystems. It contains a variety of organic compounds or components such as humic acid, fulvic acid, polyphenols, and carbohydrates, and these components of NOM each possess highly variable molecular sizes, structures, redox-active functional groups, metal-complexing sites, and polyelectrolytic characteristics (MacCarthy, 1991, Gu et al. 1996). NOM functions in the environment also seem very prominent since NOM can act as scavengers for various kinds of inorganic and organic contaminants. The interactions between NOM and contaminants are known to depend on the physico-chemical structure and functional properties of humic macromolecules, whose attributes must be well characterized to elucidate their specific environmental functions.

Much of the effort to physically and chemically characterize NOM has focused on humic acid and fulvic acid of aquatic and terrestrial origin (Leenheer, 1995, Senesi, 1990). However, few systematic studies have been performed on characterizing the subcomponents or fractions of NOM with varying chemical and structural properties. The objective of this research is to more fully characterize, through use of chemical and spectroscopic techniques including UV-VIS, ^{13}C NMR, FTIR, fluorescence, and ESR, the humic substances fractionated from a whole aquatic NOM and contrast its characteristics to those found in a soil-derived humic acid.

Materials and Methods

NOM Fractions - An aquatic NOM was obtained from a wetland pond in Georgetown, South Carolina (Gu et al. 1994, 1995). It was fractionated into two fractions, namely polyphenolic-rich (NOM-PP) and carbohydrate-rich (NOM-CH) fractions, on the basis of their adsorptive behavior on a cross-linked polyvinyl pyrrolidone (PVP) polymer under acidic conditions. In addition, a soil HA was obtained from the International Humic Substances Society (IHSS) for comparison.

Spectroscopic analysis - UV/VIS absorbance was measured by means of a Hewlett-Packard 8453 spectrophotometer (scan range 190-1,100 nm). Each sample analyzed contained 5mg C/L NOM at pH ~7.0. The ^{13}C NMR spectra were collected with an NT-200 NMR spectrometer and operated at the ^{13}C frequency of 50.3 MHz. The FT-IR spectra were collected with a Nicolet Magna 760 FTIR spectrophotometer equipped with an MCT (Hg-Cd-Te) detector on a spectral range of 400-4000 cm^{-1}. Fluorescence spectra in synchronous-scan mode were determined on aqueous solutions of NOM at a concentration of 20 mg C/L in 0.01M KCl at pH 7.0. Spectra were recorded by using a Fluorolog 4 fluorescence spectrophotometer (Johin-Yvon-SPEX instruments Inc.) scanned from 300 to 600 nm while maintaining $\Delta\lambda$=20nm. ESR spectra were measured on liquid samples using a Bruker EMX Spectrometer equipped with a TM110 cavity. The EPR was operated at a peak-to-peak modulation amplitude of 2.0 Gauss, microwave attenuation of 25 db, modulation frequency 100 kHz, conversion time 10.24 ms, and a time constant of 1.28 ms.

Results and Discussion

Figure 1 shows the UV-VIS spectra of NOM-PP, NOM-CH and Soil HA. In general, humic substances have strong absorbance in the UV-visible range (from 190–800 nm), particularly in the UV region, because of the presence of aromatic chromophores and/or other organic compounds. In fact, the UV absorptivity at 260 nm (ABS_{260}) is commonly used to determine the relative abundance of aromatic C=C content of NOM since π-π* transitions in substituted benzenes or polyphenols occur in this wavelength region. Although the spectra appear to be broad and featureless, the absorption intensity vary greatly among the three NOM fractions with NOM-PP > Soil HA > NOM-CH. The spectra of NOM-CH fraction exhibits about two-fold lower UV absorbance in comparison with the NOM-PP and soil HA fractions. These observations suggest that both NOM-PP and soil HA contain a relatively high amount of aromatic or polyphenolic organic compounds relative to the NOM-CH fraction.

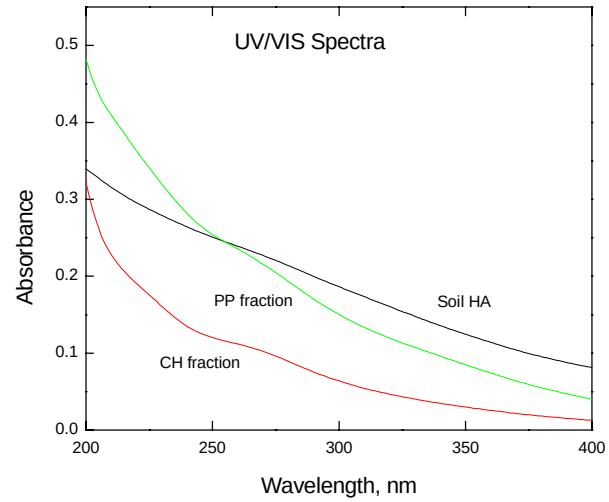

Figure 1. The UV/Vis spectra of three NOM fractions.

The ^{13}C-NMR spectra (Figure 2) indicate the soil HA contains a relatively high aromatic carbon (120-140 ppm) but low carboxyl and heteroaliphatic C-O and C=O (or ketone) functional groups (160-180ppm and 40-80 ppm) in comparison with the NOM-PP or NOM-CH fractions. These spectral characteristics of NOM fractions are similar to those of fulvic acid and humic acid from Ogeechee and Suwannee River as reported by Malcolm (1985) and other investigators (Frδnd, et al. 1989, Hatcher, 1981, Leenheer, 1987). These results are in general consistent with the UV/Vis data (Figure 1). Soil HA showed a high aromaticity (by NMR analysis) and a high UV/Vis absorbance (at a wavelength >~250 nm) in comparison with the aquatic NOM-PP and NOM-CH fractions. However, Soil HA exhibited a lower UV absorbance (at < ~250 nm) than that of the NOM-PP fraction. These observations are probably related to its highly polymerized aromatic structure and a high molecular size of soil-HA macromolecules. Results of the NMR and UV/Vis analyses are also generally agreeable with the FTIR spectral data (not shown), which show the abundance of aromatic C=C functional groups in the order of Soil HA > NOM-PP > NOM-CH fractions. On the other hand, the NOM-CH fraction exhibits a relatively higher amount of –C=O and carboxyl functional groups (at ~1730 cm^{-1}) than the Soil HA and NOM-PP fractions.

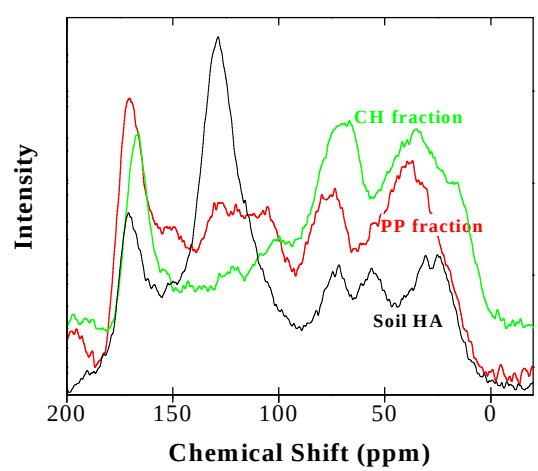

Figure 2. ^{13}C-NMR spectra of NOM fractions.

The results of synchronous scan fluorescence spectroscopy complement the above UV/Vis, NMR, and FTIR spectral analyses. Spectra of soil HA (Figure 3) show strong fluorescence intensity in the long wavelength

region (at ~470 nm) and closely resemble those spectra typically representative of soil HA (Pullin, 1995, Senesi, 1990, 1991). In other words, it shows a high degree of conjugated aromatic π-electron system with electron-withdrawing functional groups; e.g. carboxyl, carbonyl which are responsible to shift fluorescence to lower energy level or longer wavelength. In comparison, the fluorescence maximums for the NOM-PP and NOM-CH fractions occurred at a shorter wavelength (~390 nm) and are indicative of relatively simple and less-conjugated aromatic structural features, with the conjugated chromophores and electron-donating substituents (such as hydroxyl, methoxyl, and amino groups) contributing to the fluorescence in shorter wavelength regions. As can be expected, the NOM-CH fraction showed the lowest fluorescence intensity because of its low aromatic carbon content.

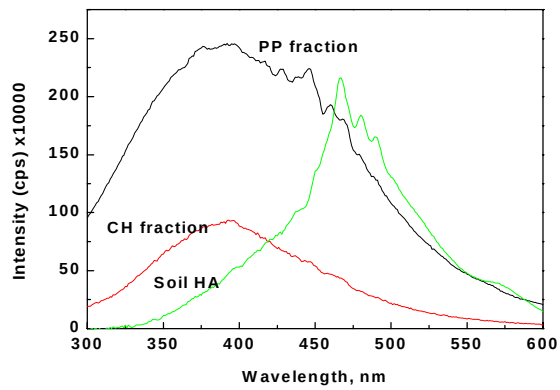

Figure 3. Synchronous scan fluorescence spectra of NOM fractions.

The ESR studies provide additional information on the chemical properties and reactivity of NOM. The ESR spectra of the three NOM fractions are qualitatively similar, showing a sharp, narrow single line resonance at g = 2.0018 ~ 2.0051 with line width of ~4–5 Gauss. These properties are typical of humic substances of any nature and source and are believed to be as a result of free radicals of semiquinonic organic compounds conjugated with extended aromatic system (Senesi, 1990). Previous studies also indicated that organic free radical concentrations are correlated directly with the degree of aromaticity and molecular complexity (Riffaldi and Schnitzer, 1972, Senesi, et al. 1977, 1990). Similarly, we found that the spin counts of the three NOM fractions are in the order of NOM-PP > Soil HA >> NOM-CH fractions, and the results are therefore consistent with the NMR and FTIR spectroscopic analyses. Relatively high free radical contents in the Soil HA and NOM-PP fractions are well correlated with their aromatic carbon contents by comparing with the NOM-CH fraction, which showed the lowest aromatic carbon content (Figure 2). It is thus anticipated that the Soil HA and NOM-PP fractions may play more active roles in many of the environmental reactions such as in the reduction and complexation of heavy metals and radionuclides.

Acknowledgements

This research was sponsored by the Office of Biological and Environmental Research, U.S. Department of Energy, under contract DE-AC05-00OR22725 with Oak Ridge National Laboratory (ORNL). We are grateful to Drs. H. Pan, E. Hustedt, and S. Dai for assistance in the ^{13}C-NMR, ESR, and fluorescence spectroscopic analyses.

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