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**CHARACTERIZATION OF HUMIC SUBSTANCES AND NON-IDEAL
PHENANTHRENE SORPTION AS AFFECTED BY CLAY-HUMIC
INTERACTIONS**

A Dissertation Presented

by

Kaijun Wang

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2005

Plant, Soil and Insect Sciences

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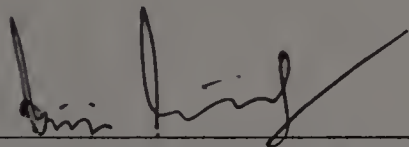
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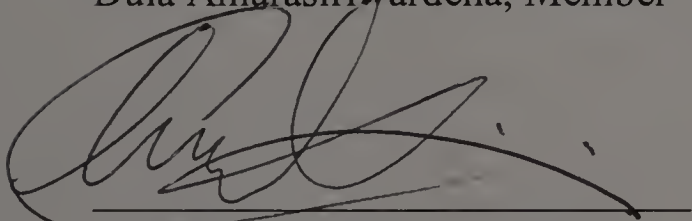
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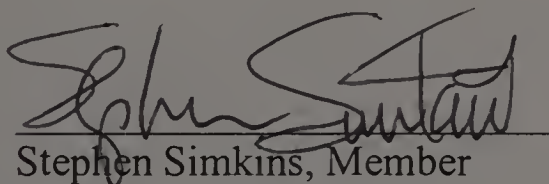
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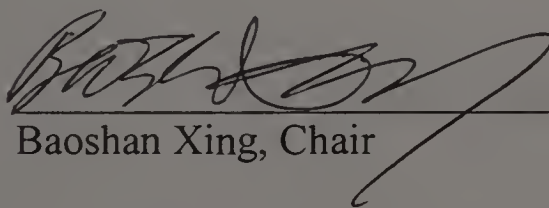
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DEDICATION

To my parents

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I am exceedingly grateful for the leadership and direction of Dr. Baoshan Xing throughout this graduate program. His wisdom, vision, and experience greatly benefit me in my study. I appreciate his never-ending friendship and encouragement, and I am fortunate to have worked with him.

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ABSTRACT

THE CHARACTERIZATION OF HUMIC SUBSTANCES AND NON-IDEAL PHENANTHRENE SORPTION AS AFFECTED BY CLAY-HUMIC INTERACTIONS

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Humic substances (HS) are major components of soil organic matter (SOM). Advances on characterization of HS and their interaction with minerals can provide a more fundamental understanding of HS functions in soils. The objectives of this research were to investigate potential fractionation of humic acid (HA) upon adsorption on minerals and to determine any variation on structure and sorption properties of humin extracted by different methods. Nuclear magnetic resonance (NMR) spectra, for the first time, provided direct evidence that HA was fractionated during adsorption on mineral surfaces. Aliphatic fractions of HA were preferentially adsorbed while aromatic fractions were more likely to be left in solution. The bound HA fractions had higher sorption linearity (N) and affinity (K_{OC}) for phenanthrene than the source HA. For montmorillonite and kaolinite, the K_{OC} values of adsorbed HA were up to several times higher than that of the source HA as a result of fractionation. Extraction procedures had

substantial influences in structure and sorption characteristics of humin. Humin from 0.1 M NaOH exhaustive extraction and 6 M HF/HCl extraction at 60 °C had relatively more aliphatic components as compared with 1 M HF extracted humin. The treatment of 6 M HF/HCl at 60 °C reduced more than 50% carbohydrate components (50-108 ppm) and enriched amorphous poly(methylene) domains. The NaOH exhaustively extracted humin had the most nonlinear sorption isotherms and the HF extracted humin had the lowest K_{OC} . We concluded that both NaOH and NaOH-HF procedures were appropriate approaches for humin extraction but the extraction with 6 M HF/HCl at 60 °C would be discouraged for use due to structural modifications of humin both chemically and physically. Segmental mobility of HA in solution was also explored by NMR relaxation. Proton spin-lattice relaxation time (T_1) of HAs ranged from 0.3 to 0.8 s in d_6 -DMSO, and from 0.26 to 2.3 s in 0.5 M NaOD at 300 MHz. Proton correlation time of HA in solvents was in an order of 10^{-10} s. Carbohydrate was identified as the largest and the most immobile components while aliphatic and aromatic fragments were relatively smaller and more mobile in aqueous solution.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Overview

Soil organic matter (SOM) is a major pool of carbon in the global carbon cycle, composing of $14-15 \times 10^{17}$ g of C (Eswaran et al., 1993). It can be divided into two classes of compounds, non-humic materials and humic substances (HS). Non-humic materials are the recognizable plant debris and they are identifiable classes of organic macromolecules, such as proteins, polysaccharides, nucleic acids, carbohydrates, and peptides. Humic substances are highly transformed, amorphous, structurally complex large to macromolecules which have a yellow to black appearance as a consequence of the breakdown of plant and animal residues mainly by microbial activity (Jones and Bryan, 1998). HS are present in soils, sediments and natural waters. They constitute 60-70% of soil organic matter and 30-50% of surface water organic matter and are the most abundant of organic macromolecules in nature (Woodwell et al., 1978; Thurman and Malcolm, 1981; Hayes, 1997). HS can be generally divided into three classes based on solubility characteristics: humic acid (HA), fulvic acid (FA) and humin (Aiken et al., 1985). FA is the fraction soluble in water under all pH conditions. HA is the fraction that is not soluble in acid conditions but becomes soluble at $\text{pH} > 2$ (used by water scientist) or $\text{pH} > 1$ (used by soil scientist). Humin is the fraction insoluble at any pH.

Although the HS contents in mineral soils are generally less than 5%, they have great influences on soil properties and performance, and environmental quality

(Stevenson, 1994). HS serve as a major nutritional source of N, P, and S for plants, a source of energy for microorganisms. It profoundly affects crop yields and the activities of soil microorganisms. In addition, HS are vital to the formation and stabilization of soil aggregates, thereby improving soil aeration and capacity of moisture retention. HS are also of environmental significance. They play significant roles in detoxification of hazardous compounds and in the transport and binding of organic and inorganic contaminants. Thus, their properties and quantity directly or indirectly affect our environment and human health (Xing, 1998).

1.2 Composition investigation of humic substances

HS are the most abundant organic macromolecules found in nature. Their chemical composition and structure are fundamental for us to understand their role in the structure and function of ecosystems. Various spectroscopic procedures have been employed for investigations of the compositions and structures of HS (Hayes, 1997). These techniques include infrared (IR) and diffuse reflectance Fourier transform infrared (DRIFT) spectra (MacCarthy and Rice, 1985; Neimeyer et al., 1992), Raman spectroscopy (Liang et al., 1996), ultra violet-visible spectroscopy, electron spin resonance spectroscopy (Senesi and Steelink, 1989), X-ray photoelectron (Bloom and Leenheer, 1989), and nuclear magnetic resonance spectroscopy (NMR) (Preston, 1996; Preston et al., 1997; Xing et al., 1999).

Among all the above spectroscopic techniques, NMR has been shown to be the most powerful spectroscopic method for the characterization of HS. NMR takes advantages of the magnetic properties of an observed nucleus, which is sensitive not

only to its chemical environment, but also to physical interactions with its environment. Both can be examined by measuring specific NMR parameters such as chemical shifts, coupling constants, and relaxation times. Therefore, NMR can be used to determine functionality, elucidate molecular compositions, investigate the molecular motion and mobility of different components, and study the interactions of HS with organic or inorganic materials. In recent years, one dimensional carbon and proton spectra of HS have been used extensively to characterize the HS structure, and substantial progress has been made after the introduction of techniques such as cross-polarization nuclear induction spectroscopy and cross polarization magic-angle spinning (CPMAS) NMR (Hatcher et al., 1980; Guggenberger et al., 1995; Schmidt et al., 1997; Xing and Chen, 1999; Smernik and Oades, 2000; Mao et al., 2000). The chemical group assignment is primarily based on the range of chemical shifts observed in 1-D spectra by reference to those of known compounds.

The complex, heterogeneous nature of HS results in serious overlapping, which leads to uncertainty in mapping the H-C-O framework by using 1D NMR approach alone. The application of multidimensional NMR in HS research not only minimizes the ambiguities inherent in chemical shift-based assignment of chemical groups but also greatly facilitates the determination of structures and conformations of extended molecular fragments present in complex macromolecules (Fan et al., 2000). In recent years, some studies have begun to take the advantage of 2D NMR techniques for structural characterization of HS (Simpson et al., 1997, 2001a, 2001b; Chien and Blears, 1998; Kingery et al., 2000; Fan et al., 2000; Simpson, 2001). Results obtained with these NMR experiments provided substantial improvements in functional group

assignment capabilities and the potential for remarkable progress in the determination of the chemical structure of HS.

1.3 Investigation of proton relaxation and segmental mobility of humic acid with solution NMR

In NMR experiment, the thermal equilibrium of a spin system is disturbed by irradiation at the resonance frequency. The system relaxes after perturbation until it once again reaches equilibrium. In contrast to electronic, vibrational and rotational excited state, the relaxation of nuclear systems is very slow, especially for the nuclei with spin quantum number $I = \frac{1}{2}$. There are two different relaxation processes: spin-lattice relaxation and spin-spin relaxation. The spin-lattice relaxation time (T_1) is a first-order time constant that characterizes how fast a nuclear spin gives up energy to its surroundings, referred to as the lattice. It is essential to know nuclear relaxation rates ($1/T_1$) to obtain reliable spectra. For example, to obtain quantitative spectra, we must be sure the pulse delay is long enough (usually at least 5 times longer than the longest T_1) that all the nuclear spins return to their equilibrium state (Braun et al., 1998). Shorter pulse delay results in diminished intensity of spectral band. So, even for routine work at least a qualitative knowledge of this parameter is necessary for choosing a reasonable pulse repetition time and important for setting up Nuclear Overhauser Enhancement (NOE) experiments. More importantly, the spin-lattice relaxation time (T_1) is a sensitive and powerful probe of molecular structure and dynamics. Most relaxation studies relate the relaxation parameters to the shape, size, mobility, and dynamic processes of the molecules or molecular fragments. For instance, an important application of relaxation

processes is to discriminate mobile fractions from immobilized fractions in heterogeneous samples even on species which have the same chemical shift (Veeman, 1997).

According to definition, the spin-lattice relaxation, involving the transfer of magnetization between the magnetic nuclei (spins) and their environment (the lattice), is induced by fluctuations of magnetic field at spins' resonance frequency due to molecular motion. Therefore, T_1 is a reflection of the rotation or reorientation of the molecule in solution. The theoretical description of dipolar relaxation for an assembly of molecules under the motional narrowing limit leads to a proportionality relationship: $1/T_1 \propto \tau_r$. In the extreme narrowing limit ($\omega_0 \tau_r \ll 1$, ω_0 is Larmor frequency), this can be expressed as a useful rule: the faster a molecule moves, the greater is T_1 (Friebolin, 1998). Here τ_r is the correlation time, which is the average time for a molecule to reorient by one radian for random molecular tumbling (Harris, 1983). Large molecules generally tumble more slowly in solution and have more time to interact with the lattice; thus, they require a shorter time (here is T_1) for their nuclear spins to return to the equilibrium state. The opposite effect takes place with small molecules, which tend to tumble quickly and spend less of their time interacting with the lattice, resulting in longer T_1 (Bortiatynski et al., 1997).

A number of mechanisms by which spins can relax have been established: dipole-dipole interactions (nuclear-nuclear and electron-nuclear), spin-rotation, scalar coupling, and chemical shift anisotropy (Levy et al., 1973), among which, the dipole-dipole interactions are the most important mechanisms for normal organic molecules in solution. For proton spin-lattice relaxation in solutions, the dominant relaxation

mechanism for energy transfer is usually the dipole-dipole interactions (Van der Maarel et al., 1987; Carper and Keller, 1997).

Unlike the chemical shift, which can be obtained from a single measurement, the determination of relaxation times requires a series of measurements. The common methods for T_1 determination are: (1) inversion recovery (180° -t- 90° -AT)_n, (2) progressive saturation [90° -(t- 90°)_n], and (3) saturation recovery (90° -HSP-t- 90°)_n (HSP stands for a homogeneity spoiling pulse) (Wehrli et al., 1988). The progressive saturation experiments put more stringent demands on instrumentation and are thus more susceptible to systematic errors. In particular, they are not very tolerant to offset-related pulse imperfection. It is why the progressive saturation method has largely been abandoned for high-resolution T_1 determinations. Saturation recovery T_1 experiments need a field gradient pulse for dispersal of the transverse magnetization. Both methods suffer from the drawback that 90° pulses cause mixing effect. If mixing effects do not decay out prior to the monitoring pulse, the resulting spectrum may exhibit phase and intensity anomalies (Dais and Perlin, 1987). Because there is no guarantee that these two methods provide reliable time, they should be avoided. The most popular, and also a very accurate, experimental method for measuring T_1 is the inversion recovery pulse sequence, even though it requires time-consuming procedures to measure enough data points.

Few relaxation studies have been conducted in the field of humic chemistry. Wilson et al. (1981) pointed out that T_1 values for ^{13}C in organic compounds could vary from less than 1 s to several minutes. Preston and Blackwell (1985) conducted ^{13}C relaxation study on a humic and a fulvic acid from a mineral soil in 0.5 M deuterated

sodium hydroxide (NaOD) using progressive-saturation techniques. They found that the T_1 values ranged from 0.2 to 2.0 s, with maximum values for carboxyl and methoxyl carbons. Based on T_1 values for ^{13}C on different functional groups, Newman and Tate (1984) estimated the fragmental mobility of alkaline soil extracts in solution. They concluded that aromatic and aliphatic carbon signals recovered at similar rates, supporting the model in which aromatic structures, carbohydrates, amino acids and polymethylene chain were linked together to form flexible macromolecules. In addition, relaxation study using liquid-state NMR has been adopted to monitor the interactions between HS and organic chemicals in recent years. Bortiatynski et al. (1997) and Nanny et al. (1997) successfully examined noncovalent interactions between humic substances and ^{13}C labeled organic compounds in solution by measuring T_1 of labeled small molecules. Also, Nanny and Maza (2001) used deuterium (^2H) NMR T_1 relaxation to investigate noncovalent interactions between monoaromatic compounds and dissolved HAs.

Besides liquid-state relaxation studies, solid-state NMR has also been employed in relaxation study of HS. Olk et al. (1995) determined ^{13}C T_1 at various stages of humification and suggested that younger mobile HA had longer T_1 s than the older HA bound to calcium. According to ^{13}C spin-spin relaxation time (T_2) measurement of a HA and a FA using solid-state ^{13}C ramp CP/MAS, Cook and Langford (1998) concluded that aliphatic moieties were the least mobile and probably largest while the carbohydrate and aromatic moieties were more mobile and significantly smaller. Here, T_2 determines how rapidly the transverse magnetization components in x-y plane decay.

The proton T_1 is a well-established NMR parameter for structural, configurational and conformational analysis of organic molecules in solution. Because T_1 is sensitive to the nuclear spins' environmental conditions, changes in concentration, temperature, or solvent pH can affect this parameter. So far, the proton spin-lattice relaxation has been widely used in organic chemistry, biochemistry and pharmaceutical chemistry to determine the mobility and structural features of carbohydrate, protein, pharmaceutical products, or other organic compounds (Kotowycya et al., 1980; Early et al., 1981; Dais and Perlin, 1987; Ohta et al., 1991; Keller and Carper, 1993; Effemey et al., 2000; Duncan and Whitten, 2000). However, systematic studies of proton spin-lattice relaxation of humic substances in solutions are rare in the literature, and the T_1 values of proton from different functional groups of humic substances in solution remain largely unknown.

Individual parts of a large molecule often move at different rates, which can be reflected by relaxation rates ($1/T_1$) of different segments. As mentioned above, spin-lattice relaxation is induced by fluctuation of local magnetic fields associated with the dipoles in molecules. These fluctuations are generated by the random molecular motions in solution. Both T_1 and T_2 are functions of molecular correlation time (τ_r), which can directly describe molecular motions. According to the Solomon equation, the spin-lattice relaxation time (T_1) due to the dipolar interaction is a function of molecular correlation time (τ_r), and is given as (Kotowycz et al., 1980; Fung et al., 1976):

$$\frac{1}{T_1} = \frac{C^6}{r^6} \left(\frac{\tau_r}{1 + \omega^2 \tau_r^2} + \frac{4\tau_r}{1 + 4\omega^2 \tau_r^2} \right) \quad (1.1)$$

where, C is a product of physical constants [$C^6 = (3/10)(\hbar^2 \gamma_H^4 / 10^{-48})$, $\hbar = h/2\pi$, h is the Planck's constant, γ_H is proton gyromagnetic ratio], r is the inter-proton distance in Å, ω

is the proton resonance frequency in rad s^{-1} . Correlation time is defined as the average time for a molecule to rotate by 1 radian for molecules undergoing rotational motion. Therefore, τ_r can be used to characterize molecular mobility or segmental mobility within molecules (Stockmayer et al., 1997; Carper and Nantsis, 1998). It is obvious that the shorter the correlation time, the more mobile the molecule (Harris, 1983): The actual correlation time of a molecule in solution depends on the molecular size and symmetry of the molecule. In general, large molecules have longer correlation times (Peng and Wagner, 1994; Steelink, et al., 1989).

Theoretically, any factor that slows molecular motion shortens the T_1 for molecules in the fast motion range ($\omega\tau_r \ll 1$), and most small organic molecules are in this category. A T_1 minimum is reached near the condition for intermediate molecular tumbling rate where $\omega\tau_r \approx 1$. In this T_1 minimum range, relaxation is most efficient. Most medium-size molecules in non-viscous solutions near room temperature fall close to the T_1 minimum (Levitt, 2001). For molecules in the slow motion regime ($\omega\tau_r \gg 1$), motion slowing increases the relaxation time (T_1). Molecular tumbling of large macromolecules falls in this category (Freeman, 1997). Thus, as one can see, T_1 has different relationships with molecular mobility depending on what motional regime the molecule is in. Therefore, it is certainly not appropriate to deduce mobility information from T_1 magnitude unless we know the value of τ_r .

The correlation time can be estimated by measuring relaxation times (T_1 , T_2 or both). Fung et al. (1976) assessed the correlation time by measuring the ratio of spin-lattice relaxation rates at two frequencies, and used those values to interpret the segmental mobility and conformation of enzyme-bound propionyl coenzyme A. This

approach has also been utilized by Kotowycz et al. (1980) to evaluate the dipolar correlation times for the different type of protons in the molecule. Moreover, Carper and Keller (1997) directly determined the correlation times of ^{13}C , ^1H , ^{113}Cd , and ^{31}P from T_1 and T_2 over the dipolar R_2/R_1 [R_1 and R_2 stand for spin-lattice relaxation rate ($1/T_1$) and spin-spin relaxation rate ($1/T_2$), respectively] for range 1.1-1200 at several field strengths. The calculated correlation times of ^{15}N and ^{19}F was used to characterize the rotational mobility of 5-fluorouracil-substituted *Escherichia coli* tRNA (Carper and Nantsi, 1998). However, correlation times of humic substances have not been determined, they are usually expected to be of an order of 10^{-9} s or greater because molecular weights are high (>500) (Steelink et al., 1989).

The characterization of the relative mobility of different HS components is of importance to the understanding of HS functions in the environment. Sorption and spectroscopic studies suggested that HS consists of an expanded, rubbery (mobile) domain and a condensed, glassy (rigid) domain (Xing and Pignatello, 1997; Xing and Chen, 1999; Gunasekara et al., 2001). In recent years, solid-state relaxation study has been used to identify the segmental mobility of HS. Kogel-knabner and Hatcher (1989) estimated the mobility of alkyl carbon in forest soils by using cross polarization magic angle spinning (CP-MAS) ^{13}C NMR technique. They concluded that the peak at 30 ppm showed both a rapidly decaying component and a slowly decaying component, indicative of the fact that two types of alkyl carbon contribute to this resonance. Moreover, Cook and Langford (1998) measured ^{13}C T_2 of a HA and a FA using solid-state ramp CP-MAS ^{13}C NMR. Based on the thought that shorter relaxation time reflected reduced mobility, they concluded that aliphatic moieties are the least mobile

and probably largest while the carbohydrate and aromatic moieties are more mobile and smaller. Smernik and Oades (2000) determined proton spin-lattice relaxation time (T_1) of an Aldrich humic acid sample from inversion-recovery experiments and reported that more rapid relaxation was observed for the aromatic and carbonyl protons than for the aliphatic protons.

In the case of liquid-state NMR, Newman and Tate (1984) inferred from ^{13}C T_1 measurement that aromatic carbons had similar mobility with aliphatic carbons. Because T_1 is sensitive to the nuclear spins' environmental changes, such relaxation studies can let us assess the internal dynamics of locations within the molecule (Smernik and Oades, 2000), which can then provide additional insight for macromolecular structures such as protein (Peng and Wagner, 1994). However, little work has been done for relaxation and segmental mobility of HS in solvents.

1.4 Structural and sorption characteristics of adsorbed humic acid on clay minerals

The heterogeneous nature of HS and their colloidal aggregates give rise to numerous potential binding sites for a wide range of materials of diverse chemistry. HS can bind metal ions, pollutants, and biocides used for agricultural purposes, thus playing a major role in the transport and fate of such materials in the environment. HS can also interact with clay particles, and eventually enhance the colloidal stability of the clay particles and the structural stability of soils (Jones and Bryan, 1998). One important consequence of those interactions is to stabilize organic matter in soils as clay-humic complexes (Laird, et al., 2001).

In soil and sedimentary environments, organic substances may be strongly adsorbed on clay mineral grains (Ransom et al., 1997). Consequently, most of organic matter is associated with the clay fractions as clay-organic complexes (Schnitzer and Khan, 1972; Mckeague et al., 1986), commonly in forms of coating on solid surfaces (Maurice and Namjesnik-Dejanovic, 1999). For, example, in surface soil containing as little as 1% organic matter, the surface chemistry was found to be controlled by organic components coated on the phyllosilicate clay minerals and Al and iron oxides (Bertsch and Seaman, 1999). Moreover, Mayer and Xing (2001) reported that soil samples with lower pH, especially from upper soil horizons, have most mineral surface covered by organic matter. In addition, organic matter may react with minerals quickly. Day et al. (1994) reported that the sorption of natural organic matter (NOM) on goethite occurred very rapidly at very low NOM concentrations, implying that mineral surfaces in the natural aquatic environment would not remain uncoated by NOM for long.

The manner in which organic matter is associated with mineral surfaces has important implications for understanding the interactions of these combined SOM with HOCs in soils and sediments. Several mechanisms of interactions between SOM and clay minerals include: anion and ligand exchange to crystal edges, cation or water bridges to basal surface, H-bonding, van der Waals forces, trapping in intradomain regions, and adsorption in interlayer spaces (Varadachari et al. 1991). These mechanisms may operate separately or collectively depending on types of minerals and humic macromolecules (Stevenson, 1994; Varadachari et al. 1997, 2000). Kubicki et al. (1997) suggested that adsorption of organic matter occurs primarily at the edge sites of clay minerals. However, Varadachari et al. (1995) found that basal surfaces provide the

major bonding sites for HA in both montmorillonite and illite, while bonding through a cation bridge at crystal edges is important in kaolinite. As to the interaction of HA with oxides in soils, Varadachari et al. (2000) suggested that cation-bridged bonds as well as coordination bonds might be major modes in forming both goethite-HA and hematite-HA complexes, while cation bridge bonding is the only dominant mode in gibbsite-HA complexes and coordinate linkage is the only dominant mode in boehmite-HA complexes. In addition, Zhou et al. (1994) suggested that humic acid coatings were formed by a chemisorption mechanism while fulvic and hydrophilic macromolecular acid coatings were the result of a physical adsorption mechanism, and this implied that humic acids would contribute far more significantly than fulvic acids in the formation of humic coatings. Laird (2001) identified two distinct types of clay-associated humic substances: diffuse filamentous films covering basal surfaces of 2:1 phyllosilicates in the medium and fine clay fractions, and discrete particles of high density metal-humic substance complexes in the coarse clay fraction.

Not every component of HS has the same affinity to mineral surfaces. It has been suggested that high-molecular weight, more hydrophobic fractions are preferentially adsorbed on clay surface (Specht et al., 2000). NMR spectroscopy has recently demonstrated that fractions larger than 100000 Daltons of a HA are primarily aliphatic in nature, while fractions smaller than 30000 Daltons are predominantly aromatic (Khalaf et al., 2003). Hence, it is reasonable to infer that aliphatic fractions would have higher affinity to clay surface. However, contrary findings were reported. Namjesnik-Dejanovic et al. (2000) observed that more aromatic fractions of a muck fulvic acid were preferentially adsorbed on clay surface; and Balcke et al. (2002) also

suggested that the sorption affinity to clay surface was related directly with the aromaticity of HS. Moreover, Gu et al. (1995) reported that carboxyl and hydroxyl groups of HS were actively involved in the adsorption by iron oxide, while Specht et al. (2000) concluded that fractions with a small molecular size and a high content of carboxylic groups made up the non-adsorption portion of HS using kaolinite and montmorillonite. All these conclusions were inferred from the structural changes of the HS remaining in solution before and after adsorption by clays, the direct characterization of the bound HS by clay minerals has not been reported.

Sorption of hydrophobic organic compounds (HOCs) in soils and geologic materials is of growing concern due to their strong affinity to soil colloids, and the potential risks associated with their long-term persistence in the environment. It is well accepted that SOM is the predominant sorbent of HOCs. Mineral fractions play only minor roles except that these mineral fractions influence the spatial distribution and configurations of associated natural organic matter (NOM). Although sorption mechanisms are still poorly understood and are currently a focus of environmental research, sorption of HOCs is normally regarded as a partition process because linear sorption isotherms are frequently observed and there is a good relationship between the carbon-normalized coefficient (K_{oc}) and the octanol/water partition coefficient of chemicals (K_{ow}). In this respect, SOM is treated as a homogeneous partitioning medium, similar to organic liquids such as octanol. However, SOM is heterogeneous in nature. Evidence inconsistent with partitioning is abundant including nonlinear isotherms, which have been observed for numerous apolar organic compounds (Weber and Huang, 1996; Xing and Pignatello, 1997, 1998; Xing and Chen, 1999), competitive sorption

(McGineley et al., 1993; Xing and Pignatello, 1998), and non-uniform sorption site energy (Yuan and Xing, 1999). These led to a new model describing sorption to SOM, dual-mode model (Xing and Pignatello, 1997, 1998). In this model, SOM consists of condensed (relatively rigid or glassy) and expanded (flexible or rubbery) domains. Glassy and rubbery states of an amorphous polymer are offered as analogous systems for which nonlinear, competitive adsorption and general partitioning mechanisms dominate, respectively. Partitioning occurs in both domains, while only the condensed domains are responsible for the nonlinearity of adsorption isotherms common for HOC uptake by many soils and sediments. Recent evidence has indicated the existence of both flexible and condensed segments in both solid SOM and dissolved colloidal humics (Aochi and Farmer, 1997; Chien and Bleam, 1998; Xing and Chen, 1999). In dual-mode model, sorption in the rubbery domain is anticipated to exhibit a linear (partitioning) isotherm contribution and sorption in glassy domain is expected to represent the nonlinear (Langmuir) contribution. Thus the total sorption is the sum of partition and Langmuir terms:

$$q_e = K_p C_e + \sum_{i=1}^n \frac{q_i^0 b_i C_e}{1 + b_i C_e} \quad (1.2)$$

Where q_e is the total sorbed concentration ($\mu\text{g/g}$), C_e is the solution concentration ($\mu\text{g/ml}$), K_p is a constant that characterizes the true partitioning occurring in the system (ml/g), and b_i ($\text{ml}/\mu\text{g}$) and q_i^0 ($\mu\text{g/g}$) are the Langmuir affinity and the ultimate adsorption capacity, respectively, for each of n different kinds of sites. Because it is impossible to determine the type and number of Langmuir sites in SOM at present, the empirical Freundlich equation is often used as a substitute. Freundlich model has been

used successfully to describe sorption phenomena for a wide range of natural geosorbents and synthetic polymers. Therefore, the total sorption becomes:

$$q_e = K_F C_e^N \quad (1.3)$$

Where K_F ($\text{mL}^N \text{g}^{1-N} \text{g}^{-1}$) and N (dimensionless) are parameters relating to the amount sorbed and the linearity of the sorption process, respectively. The value of N is referred to as the index of isotherm nonlinearity (Weber et al., 1992), i.e., the smaller the N , the more nonlinear the isotherm. It is generally believed that whether sorption isotherms of organic solutes by SOM are linear or nonlinear might give insight into whether such sorptions through partitioning or surface adsorption mechanisms (Weber et al., 2001).

It has been well documented HS or SOM may undergo structural changes chemically or physically upon adsorption by clay minerals and such modification may consequently impact their sorption behavior with HOCs (Salloum et al., 2001b; Gunasekara and Xing, 2003). Therefore, it may not be appropriate to extrapolate the sorption behavior of HS coated on mineral surfaces from that of extracted HS. Few studies included sorption of HOCs by constructed clay-humic complexes in the past. Murphy et al. (1990, 1994) observed nonlinearity and competitive sorption of three organic compounds by humic-coated minerals as compared to linear and noncompetitive sorption of extracted HAs, indicative of the involvement of adsorption process. It has also been reported that partitioning is the process in high organic carbon content systems while both partitioning and adsorption mechanisms are involved in very low carbon content systems using reagent CaCO_3 , calcite, and montmorillonite as sorbents (Onken and Traina, 1997). Moreover, the organic carbon normalized sorption coefficients (K_{OC}) are found to be lower for mineral-bound humic materials compared

with dissolved humic materials, inferring HA fractionation during its sorption onto clays (Jones and Tiller, 1999), but not investigated.

Understanding the mechanisms of hydrophobic pollutant interactions with humic substances that are associated with minerals is important for better predicting the transport and bioavailability of those organic contaminants. However, a significant research effort has focused on sorption of HOCs either by extracted, nearly 100% organic materials (Chefetz et al., 2000; Xing, 2001a 2001b; Gunasekara and Xing 2003) or by clean minerals (Boyd et al., 2001; Hundal et al., 2001). Information on sorption by humic-clay complexes is rather limited.

1.5 Effect of different chemical extractions on soil humin structure and phenanthrene sorption

Humin is usually defined as the fraction of humic substances that is insoluble in an aqueous solution at any pH condition. It has a significant aliphatic nature and appears to be the oldest of the three humic fractions (Rice, 2001). Being the major component of soil organic matter, typically represents more than 50% of organic carbon in soils (Stevenson, 1994), humin may dominate the sorption and transport of organic contaminants in soils and sediments. It has been reported that more than 50%, typically 70-80% of bound polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are associated with humin in soils (Kohl and Rice, 1998). However, research on compositions, structures, and sorption properties of humin has been hampered by its limited solubility and high ash content.

The application of solid-state nuclear magnetic resonance spectroscopy (NMR) in humic substances has revealed that humin is enriched with aliphatic structure (Kang and Xing, 2005). A NMR relaxation study (Preston and Newman, 1992) has proposed that aliphatic and aromatic/carbohydrate carbons exist as spatially distinct domains in a HF/HCl deashed humin sample: aliphatic domains are relatively rigid whereas aromatic and carbohydrate domains are somewhat mobile. But recent sorption (Perminova et al., 2001) and spectroscopic studies (Xing and Chen, 1999; Johnson et al., 2001) have shown that the condensed domains are mainly attributed to aromatic components. Among aliphatic carbon components, both mobile and rigid components have also been identified in NMR investigations (Kogel-Knabner et al., 1992; Hu et al., 2000). Moreover, Cuypers and co-workers (2002) investigated the composition of mobile and rigid domains of one soil sample and four sediment samples using thermogravimetric analysis, pyrolysis-GC-MS, and CP-MAS ^{13}C NMR, they reported that rigid domains in relatively undecomposed SOM were enriched in aliphatic carbons, whereas rigid domains in relatively weathered SOM were enriched in aromatic carbons. The heterogeneous domains in SOM have been used to explain nonlinear sorption isotherms (Huang and Weber, 1997).

Sorption of hydrophobic organic compounds (HOCs), e.g., phenanthrene, is greatly affected by chemical structure and composition of humic substances (Kang and Xing, 2005). It has also been documented that the capacity and affinity of SOM to retain HOCs vary significantly among samples of different origins and fractions (Johnson et al., 2001; Kile et al., 1999; Salloum et al., 2001b). Kohl et al. (2000) employed ^{19}F solid-state NMR to demonstrate the existence of multiple sorption

domains. It has been considered that organic contaminants are bound to humin via both covalent (Guthrie et al., 1999) and non-covalent (Hatcher et al., 2000) interactions. Humin is reported to have higher sorption affinity (K) and more nonlinear sorption isotherms than HA and its source soil (Salloum et al., 2001a; Xing, 2001a). Using dual-mode model, it has been proposed that soil organic matter in humin is more condensed, thus having more “hole-filling” sites than HA and its source soil. This condensed nature of SOM in humin may result from the interaction between minerals and humic molecules, as suggested by Murphy et al. (1990). However, Chiou et al. (2000) attributed such sorption nonlinearity of nonpolar organic contaminants to high-surface-area carbonaceous material (HSACM), an extrinsic component of soil humic materials.

Based on the notion that expanded organic matter (HA and FA) would be easily extracted as compared with insoluble condensed organic matter (humin) (Xing and Pignatello, 1997), Gunasekara and Xing (2003) proposed a novel structural conformation of humin to explain its high sorption affinity and nonlinearity. They suggested that SOM macromolecules, especially the first several molecular layers of the amorphous region in close contact with soil mineral surfaces, might rearrange to take a more condensed conformation, which consequently altered its sorption affinity and isotherm nonlinearity. In this respect, humin samples with different content of minerals may differ in the distribution of mobile/condensed domains of SOM; in other words, extraction and enrichment/purification processes are likely to impact the structural conformation of soil humins and their sorption properties.

Humin samples used in the literature were often extracted by different methods. The residues after extraction of HA and FA are usually defined as crude humin. The

crude humin, in most cases, contain only a small amount of organic matter dispersed in a large volume of inorganic matrices. In practice, crude humin is often collected after soil is extracted in 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ three times (Ding et al., 2002), in 0.1 M NaOH once, twice or three times (Dec et al., 1997; Nam and Kim, 2002; Tremblay et al., 2005; Guignard et al., 2005), or in 0.5 M NaOH once for 24 hours followed by washing five times with 0.5 M NaOH (Kohl and Rice, 1998). It should be noted that not all HA and FA fractions are extracted out due to the limited number of extractions (Xing et al., 2005). Some studies used the humins derived from exhaustive extraction to determine the variation of HOCs sorption among SOM fractions (Chiou et al., 2000; Salloum et al., 2001a). The main point of the exhaustive method is to repeat 0.1 M NaOH extraction many times to further remove HA and FA from soil, hence the organic matter left in the soil residue is not extractable by 0.1 M NaOH, which matches the definition of humin.

To get organic C enriched humin samples, the soil residues are usually treated with HF to reduce mineral content. One approach is to treat the soil residues (crude humin) with 1.0 M or 2% (v/v) HF solution (Ding et al., 2002; Smernik and Oades, 2003). Another is to digest the residues with a hot concentrated HF/HCl (Song et al., 2002); this procedure is more chemically aggressive than 1.0 M HF, leading to a variety of possible modifications (Rice, 2001). It is anticipated that different extraction methods may result in structural changes; as a consequence, humins from various extractions may have different sorption behaviors. However, the impact of extraction methods on humin structure and its sorption behavior has not been reported, yet.

CHAPTER 2

STRUCTURAL AND SORPTION CHARACTERISTICS OF ADSORBED HUMIC ACID ON CLAY MINERALS¹

2.1 Abstract

Clay-humic complexes are commonly distributed in natural environments. They play very important roles in regulating the transport and retention of hydrophobic organic contaminants in soils and sediments. This study examined the structural changes of HA after adsorption by clay minerals and determined phenanthrene sorption by clay-humic complexes. Solid-state and liquid-state ¹³C NMR, for the first time, provided direct evidence for HA fractionation during adsorption on mineral surfaces, i.e., aliphatic fractions were preferentially adsorbed by clay minerals while aromatic fractions were left in the solution. The ratio of UV absorbance of HA at 465 and 665 nm (E_4/E_6), which is related to aromaticity, corroborated with the NMR results. For both montmorillonite and kaolinite, adsorbed HA fractions had higher sorption linearity (N) and affinity (K_{OC}) than the source HA. The K_{OC} of adsorbed HA for the clay-humic complexes could be up to several times higher than that of the source HA. This large increase may be contributed by the low polarity of the bound HA. Moreover, for each mineral, the N values of adsorbed HA increased with increasing HA loading. It is believed that HA may develop a more condensed structure on mineral surfaces at lower HA loading level due to the stronger interactions between HA and the mineral surface

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as a result of close contacts. Considering the high aliphaticity and sorption capacity of the adsorbed HA, the clay-humic complexes in this study, to some extent, resemble the humin fractions in natural environments.

2.2 Introduction

Sorption of HOCs in soils and sediments is a very important research area because of their high affinity for soil colloids and the potential risks associated with their long-term persistence in the environment. It is well accepted that SOM is the predominant sorbent for HOCs, and mineral fractions play only a minor role due to the strong suppression of HOCs by water on the polar mineral surface (Kile et al., 1995). Thus, sorption of HOCs by HS extracted from soils and sediments or by other organic samples such as peat has been extensively studied and it is generally believed that partitioning and adsorption-like mechanisms are involved in the sorption process (LeBoeuf and Webber, 2000; Xing and Pignatello, 1997; Zhao et al., 2001).

Clay-humic complexes are widely distributed in natural environments, commonly in the form of coatings on solid surfaces (Maurice and Namjesnik-Dejaaovic, 1999). It has been reported that soil of low pH, especially from upper soil horizons, have most of their mineral surface covered by organic matter (Mayer and Xing, 2001). In this respect, soil organic matter may exert significant impact on the sorption capacity of mineral particles (Zhou et al., 1995) and play a critical role in regulating the retention and transport of contaminants in soils. Even in surface soils containing as little as 1% organic matter, organic components coated on phyllosilicate

and oxide minerals were found to dominate the surface chemistry (Bertsch and Seaman, 1999).

On the other hand, HS may undergo chemical or physical changes upon adsorption by clay minerals. It has been suggested that high-molecular weight, hydrophobic fractions are preferentially adsorbed on clay surface (Specht et al., 2000). Based on the finding that HA fractions larger than 100,000 Daltons are primarily aliphatic in nature, while fractions smaller than 30,000 Daltons are predominantly aromatic (Khalaf et al., 2003), it is reasonable to assume that aliphatic fractions would have higher affinity for clay surfaces. This assumption has been confirmed by Specht et al. (2000) that the HA fractions with a small apparent molecular size and a high carboxylic group content made up the non-adsorbing portion of HS. However, contrary results have been reported. For example, Namjesnik-Dejanovic et al. (2000) observed that more aromatic fractions of a muck fulvic acid were preferentially adsorbed onto clay surfaces; Balcke et al. (2002) also reported that the sorption affinity to the clay surface was related directly with the aromaticity of HS. No matter what the conclusion is, all these results were inferred from the structural changes of the HS remaining in solution before and after adsorption by clays; the direct characterization of the bound HS by clay minerals has never been reported.

Due to the potential fractionation of HS upon adsorption by minerals, it may not be appropriate to extrapolate the sorption behavior of HS coated on mineral surfaces from that of extracted HS. Nonlinearity and competitive sorption by humic-coated minerals has been reported (Murphy et al., 1990, 1994). The K_{OC} has been found to be lower for mineral-bound humic materials compared with dissolved humic materials

(Jones and Tiller, 1999). Nevertheless, information on sorption by humic-clay complexes is very limited. The objectives of this study were to investigate the structural features of the HA fractions bound to clay minerals and to determine the sorption behavior of those bound HA fractions.

2.3 Materials and Methods

2.3.1 Clays and HA

Kaolinite and montmorillonite, two commonly found clay minerals in soils, were purchased from Fluka for use in this study. Kaolinite represents a non-expanding 1:1 layered silicate mineral, while montmorillonite represents an expanding 2:1 layered silicate mineral. The organic carbon (OC) contents of the two minerals were analyzed using the modified Pregl method on a 240XA Elemental Analyzer (Ma and Rittner, 1979). Both minerals had OC content below the detection limit ($< 0.01\%$). Thus, no H_2O_2 treatment was made to avoid any potential structural change of the clays. Because divalent cation-saturated clays usually have a higher sorption affinity of humic substances than monovalent cation-saturated clays (Theng, 1982; Varadachari et al., 1997; Oste et al., 2002), we used Ca^{2+} -saturated clays to construct clay-humic complexes to obtain high HA loadings. In addition, Ca^{2+} is one of the most common cations in soil solution, thus preparing clay-humic complexes with Ca^{2+} -saturated clays may better simulate natural soil conditions. Briefly, Ca^{2+} -saturated kaolinite and montmorillonite were prepared by saturating the minerals with 0.5 M CaCl_2 solution 5 times followed by washing with deionized distilled water until a negative test with Cl^- was obtained using AgNO_3 . The Ca^{2+} -saturated clays were freeze-dried, ground and

passed through a 0.15 mm sieve. Amherst humic acid, extracted from Amherst peat soil (Terrestrial Haplosaprist) based on the procedures outlined by Swift (1996), was used for construction of humic-clay complexes. The elemental composition of Amherst HA is 54.6% C, 4.16% H, 38.5% O, 2.46% N, and 0.24% ash, and was free of black carbon, as determined with the method by Song et al. (2002).

2.3.2 Clay-humic Complex Preparation

A known amount of HA was dissolved in a minimum volume of 0.5 M NaOH to make 500 mg/L HA solution after adjusting pH to 5 with 0.1 M NaOH and HCl. Humic-clay complexes were prepared at HA-clay ratios of 1:5, 1:50 and 1:100 (w/w) to obtain different HA loading levels. The HA-clay suspensions were gently shaken on hematology mixers for 24 hours and then centrifuged at 7600 g for 20 minutes. The precipitates were freeze-dried. The freeze-dried precipitates were washed repeatedly with deionized distilled water until there was no color in the supernatant. The washed precipitate was freeze-dried, ground and passed a 0.15 mm sieve, and stored for subsequent uses. This procedure was considered to guarantee the short range reactions between humic substances and clay minerals that would normally have occurred in the soil environment as a result of periodic drying (Varadachari et al., 1991) as well as eliminate the presence of unassociated HA fractions and avoid their interference during structural characterization and sorption experiments. The N₂ BET method with a Beckman Coulter™ SA3100 surface area analyzer was used to measure the specific surface areas of the minerals, HA and clay-humic complexes (Mayer and Xing, 2001).

2.3.3 Carbon-13 NMR Spectroscopy

Solid-state ^{13}C NMR techniques were employed to analyze the structural composition of the source HA and the HA fraction bound on clays. The NMR data were acquired at a frequency of 75.48 MHz for carbon on a Bruker Avance DSX300 spectrometer. The ^{13}C spectrum of Amherst HA was obtained using a total sideband suppression pulse program (CP/MAS-TOSS) in a 7 mm zirconia rotor with a Kel-F cap. The parameters were: spinning speed of 5 KHz, contact time of 1.5 ms, recycle delay of 1 s, 7000 scans, and line broadening of 25 Hz. Only the montmorillonite-humic complex sample prepared at the HA to clay ratio of 1:5 was used for the NMR determination because the carbon contents of other complex samples were too low (Table 2.1). The ^{13}C spectrum of this complex was acquired using a ramp-cross-polarization pulse program (RAMP-CP/MAS) (Cook and Langford, 1998) with the following parameters: spinning speed 8 KHz, contact time 2.5 ms, recycle delay 1 s, and 114000 scans. The RAMP-CP/MAS technique provides higher signal-to-noise ratio than CP/MAS at a given OC content, thus better-resolved spectra can be obtained for low carbon content samples in reasonable experimental time frames. The spectrum of montmorillonite-humic complex sample required 48 hours for acquisition. Meanwhile, the empty rotor was also run with the exactly same parameters as the complex sample to acquire a background spectrum.

Liquid-state NMR spectroscopy was used to characterize the HA fraction left in solution after coating on montmorillonite. The ^{13}C spectrum was acquired using an inverse gated ^1H -decoupling pulse program with a 90 degree pulse of 8.5 μs , recycle delay of 6 s and 8500 scans at a frequency of 150.9 MHz using a 10 mm tube on a

Bruker Avance 600 spectrometer. 200 mL HA solution after coating was concentrated by freeze-drying and then 0.5 M NaOD in D₂O was added to get D₂O/H₂O ratio about 80%/20%. The 2,2-dimethylsilapentane-5-sulfonic acid (DSS) was used as an internal reference.

Because there was enough HA remaining in the solution after coating on kaolinite at 1:50 HA to clay ratio, solid-state ¹³C NMR was used for characterizing the unsorbed fractions, which avoided the concentration limitation and long acquisition time experienced in liquid-state NMR. To ensure the detection of any structural change, the coating procedure was repeated two more times at the same HA to clay ratio. Then, the HA left in the solution was acid-precipitated, centrifuged, washed with deionized distilled water, and freeze-dried. The spectrum of the freeze-dried HA was obtained using CP/MAS-TOSS pulse program as for the source HA. The structural carbon assignments from both solid-state and liquid-state NMR were aliphatic carbons (0-50 ppm), methoxyl carbons (50-60 ppm), carbohydrate carbons (60-96 ppm), anomeric carbons (96-108 ppm), aromatic carbons (106-145 ppm), phenolic carbons (145-163 ppm), carboxyl and carbonyl carbons (163-190 ppm), and ketone carbons (190-220 ppm) (Mao et al., 2000; Litvina et al., 2003).

2.3.4 UV-visible Spectroscopy

The ratio of absorbance of HA at 465 and 665 nm (E_4/E_6) was determined in a sequential coating process to explore the fractionation of HA due to adsorption by clay minerals. This ratio has been used to relate to aromaticity of HA (Stevenson, 1994). In this experiment, montmorillonite or kaolinite was first mixed with 500 mg/L HA solution at pH 5.0 with a HA to clay ratio of 1 to 25. Solids were then separated from

solution by centrifuging at 7600 g for 20 minutes after 24 hour mixing at room temperature and supernatants were collected. After that, the clean mineral was added to the HA supernatant at approximately the same HA to clay ratio to repeat the coating procedure. Such sequential coating was repeated 4 times for kaolinite, but only 3 times for montmorillonite because almost all HA was adsorbed at the fourth coating step. HA solution (or supernatant) was sampled at each coating step and the solution pH was adjusted to 5.0 with diluted HCl and NaOH before measurement. The E_4/E_6 ratio of HA was measured with an Agilent 8453 UV-visible spectrometer. Because the E_4/E_6 ratio is independent of HA concentration (Chen et al., 1977), dilution or other adjustment on HA concentration was unnecessary in this investigation.

2.3.5 Sorption Experiments

The [Ring-UL- ^{14}C] and unlabeled phenanthrene (>98% purity) were purchased from Sigma-Aldrich chemical company (St. Louis, MO) and used without further purification. Sorption experiments were conducted using a batch equilibration method at 20 ± 1 °C. The sorption isotherms were obtained using 2-mL glass GC-vials with Teflon-lined septa cap. The background solution was 0.01 M CaCl_2 with 200 $\mu\text{g/mL}$ HgCl_2 as a biocide. The ^{14}C -labeled phenanthrene and its non-radioactive stock solutions were mixed with humic-clay complexes at different solid-to-solution ratios. The complex concentrations were adjusted to achieve 30-80% sorption of phenanthrene. After the suspensions were shaken for 3 days on hematology mixers to achieve apparent equilibrium, vials were centrifuged at 1000 g for 30 min, and 1 mL supernatant was sampled for liquid scintillation counting (Beckman LS 3801). The amount of compound sorbed was calculated by the difference between the applied and equilibrium

concentrations. The sorption of phenanthrene by the source HA, kaolinite, and montmorillonite was also performed following the same procedures. Sorption to the GC-vials (probably to the Teflon Septa caps) was found to account for about 6-12% of overall sorption, thus a sorption isotherm was built for GC-vials alone and the partition coefficient was used to calculate the amount of phenanthrene sorbed by GC-vials in sorption experiments under the assumption of linear partitioning and the similarity of all GC-vials. The sorption isotherms for the HA, minerals and complexes were corrected by subtracting the amount of phenanthrene sorbed by GC-vials at each final solution concentration point.

All sorption data were fitted to the logarithmic form of Freundlich equation:

$$\log C_s = \log K_f + N \log C_e$$

where C_s is the total sorbed concentration ($\mu\text{g/g}$), C_e is the final solution phase concentration ($\mu\text{g/g}$), K_f is the Freundlich coefficient ($\mu\text{g}^{1-N} \text{mL}^N \text{g}^{-1}$), and N is the exponent indicating isotherm linearity. Isotherms were plotted by $\log C_s$ vs. $\log C_e$ and $\log K_f$ and N were obtained from the intercept and slope of the plot, respectively.

2.4 Results and Discussion

2.4.1 Humic Acid Loading

The montmorillonite-humic complexes obtained at ratios of 1:5, 1:50, and 1:100 contained 0.97%, 0.61%, and 0.34% OC, respectively. The carbon contents of kaolinite-humic complexes at ratios of 1:5 and 1:50 were 0.47% and 0.26%, respectively. The kaolinite-humic complex prepared at the ratio of 1:100 was not used due to low HA sorption resulting in a large uncertainty in the carbon content determination. Therefore,

the two kaolinite-humic complexes prepared at ratios of 1:5 and 1:50 were used to perform sorption experiments. Montmorillonite adsorbed more than twice as much HA as kaolinite at a same HA to clay ratio (Table 2.1).

2.4.2 Solid and Solution NMR Spectroscopy

The solid-state ^{13}C RAMP-CP/MAS spectrum of the montmorillonite-humic complex showed a strong peak at 0-50 ppm in the aliphatic region and a large round hump centered between 100-200 ppm (Figure 2.1A). It has been reported that Kel-F rotor caps and NMR probe components may generate significant background signals in aromatic region when samples contain very low OC contents (Preston, 2001). In our case, the carbon content of the complex sample was less than 1%, thus the spectrometer background signals may overlap. Therefore, we ran the empty rotor to acquire background signals at the identical conditions as for the montmorillonite-humic complex. Figure 2.1B is the spectrum of the empty zirconia rotor with Kel-F cap. Obviously, the round hump in aromatic region in Figure 2.1A was mainly contributed from the spectrometer background signals. The true structural information of the HA fractions adsorbed by montmorillonite (Figure 2.1C) was then obtained by FID (free induction decay) subtraction of background signals (Figure 2.1B) from the complex sample (Figure 2.1A). Hence, the structure of the HA fractions bound to a clay mineral, for the first time, was characterized directly using NMR technique. It is apparent that the adsorbed HA fractions were primarily aliphatic as compared to the ^{13}C CP/MAS-TOSS spectrum of the source HA (Figure 2.1F). In addition, Figure 1D is the solution ^{13}C NMR spectrum of the HA remaining in the solution after coating on montmorillonite. It provided complementary evidence that aromatic and

carboxyl/carbonyl components were dominant fractions of the unsorbed HA. Therefore, we concluded from NMR spectra that aliphatic fractions were preferentially adsorbed on montmorillonite. However, Namjesnik-Dejanovic et al. (2000) reported that aromatic fractions were preferentially adsorbed to mineral surfaces as inferred from absorptivities at the wavelength of 280 nm of a muck fulvic acid in the solution before and after adsorption. This is probably due to the fact that two different humic materials were used in the two studies.

Similarly, solid-state NMR spectrum of the HA left in the solution after three-time sequential coatings on kaolinite also revealed a 47% increase on the content of aromatic carbons (108-162 ppm) and 42% decrease on aliphatic components (0-108 ppm) as compared to the source HA (Table 2.2). These results indicate a similar fractionation pattern of HA on kaolinite as occurred on montmorillonite. Furthermore, carboxyl/carbonyl groups appeared to have a higher percentage in the unsorbed HA than the source HA (Table 2.2), suggesting that both carboxyl and phenolic groups tend to stay in solution after coating, probably due to their hydrophilic and polar nature. We did not perform quantitative analysis and comparison for the montmorillonite-HA complex because of the different NMR techniques used.

2.4.3 UV-visible Spectroscopy

UV-visible measurements showed a gradual decrease of HA E_4/E_6 ratio with sequential coatings on both montmorillonite and kaolinite (Table 2.3). Based on the notion that lower E_4/E_6 ratios may be related to higher aromaticity for a given humic material (Stevenson, 1994), our data suggest that the HA fractions left in solution after coating had a higher aromaticity than the source HA. In other words, the HA fractions

coated on the mineral surfaces had relatively more aliphatic structures. Therefore, UV-visible measurements provided additional evidence to the fractionation of HA during adsorption on mineral surface, consistent with our NMR results.

2.4.4 Phenanthrene Sorption

Although soil organic matter (SOM) is the predominant sorbent for HOCs, clay minerals do contribute to HOC sorption, especially when SOM content is very low in soils. All the sorption data were fitted well by the empirical Freundlich equation. Montmorillonite retained a larger amount of phenanthrene than kaolinite; e.g., at $C_e = 0.1 \mu\text{g/mL}$, K_d of montmorillonite was 14.5 while kaolinite's K_d was 6.1 (Table 2.4). Approximately linear sorption isotherms were observed for both minerals considering the experimental uncertainty (Figure 2.2B and 2.2C, Table 2.4). Similar results were reported by others (Mader et al., 1997; Hundal et al., 2001). Amherst HA had a nonlinear isotherm with the Freundlich exponent (N) of 0.874, even though it contained no black carbon; its K_d value was up to three orders of magnitude higher than those of the clay minerals (Table 2.4).

Figure 2.2B and 2.2C illustrate the sorption isotherms of phenanthrene on clay-humic complexes. Montmorillonite and kaolinite exhibited much higher sorption affinity for phenanthrene after coating with HA. For example, after coating with the HA at a ratio of 1 to 5, the K_d values for montmorillonite at $C_e = 0.1 \mu\text{g/mL}$ were more than 18 times greater than the pure mineral; kaolinite had even a greater increase, nearly 35 times (Table 2.4 and 2.5). Thus, coated SOM did significantly modify the surface chemistry of clay minerals. More interestingly, more linear isotherms and higher K_{OC}

values were observed for the humic-clay complexes as compared with the source HA particles.

It is anticipated that both HA and minerals could contribute to the sorption of phenanthrene by mineral-HA complexes, and that the higher isotherm linearity might be partially attributed to the mineral phase. In order to separate the contribution of the bound HA from the overall sorption in the complexes, the sorption contribution from the clay minerals was approximated by assuming that phenanthrene sorption to clays is proportional to surface area and by using the changes in mineral surface area after HA coating (Table 2.1). Mineral results were subtracted from the total sorption by the complexes to differentiate between mineral and HA coating contribution to sorption. Because it is difficult to determine the conformation and the exact surface area of the sorbed HA on minerals, the surface area of solid HA was used to approximate the HA coverage on minerals. This approximation is reasonable because the surface area of HA of different sources seems not to vary much (around $1 \text{ m}^2/\text{g}$) (Chiou et al., 1990; Xing and Pignatello, 1997, this study). In addition, though the surface area of HA may depend on its physical size and shape, based on fact that the organic carbon content of clay-HA complexes was so low, a little change on surface area estimation will not affect sorption adjustment.

The adjusted Freundlich parameters of the clay-humic complexes were slightly lower than the unadjusted ones as illustrated in Table 2.5. Nevertheless, the N values were still higher than the source HA particles. Statistical analysis (two sample t-test) revealed that the N values of montmorillonite-HA complexes prepared at 1:5 and 1:50 and kaolinite-HA complex at 1:5 were significantly different from that of the source

HA, while the N values of montmorillonite-HA complex at 1:100 and kaolinite-HA complex at 1:50 were not. According to the dual mode sorption model (Xing, 2001a), these results suggest that the HA bound on the mineral surface might have a less condensed conformation than the source HA particles. The pH at which the complexes were prepared could be one possible reason for the more expanded conformation of the adsorbed HA. In our experiment, the source HA particles were obtained by acidic precipitation (pH 1) while the complexes were prepared at pH 5. Because most functional groups were neutralized at pH 1, HA may have developed a more compacted structure due to intra and inter molecular attractions. Huang and Weber (1997) also reported that the isotherm N value of a HA was higher at pH 7 than pH 2.7. Another possible explanation is the fractionation of the HA due to adsorption by mineral surfaces. Previous studies have suggested that the condensed domains and lower N values are often related to aromatic moieties of SOM (Gunasekara et al., 2003; Xing, 2001a; Johnson et al., 2001). Our NMR spectra indicate that a majority of aliphatic components of HA fractions were on mineral surfaces (Figure 2.1C), thus, generating more linear isotherms than the source HA.

Carbon normalized distribution coefficients (K_{OC}) were significantly greater with clay-humic complexes as compared with the source HA (Table 2.4 and 2.5). The K_{OC} values of montmorillonite-humic complexes were 60% higher than that of the source HA, while the kaolinite-humic complexes had up to 150% increase in K_{OC} . Again, this increase might be associated with the fractionation of HA on clay mineral surfaces. According to the ^{13}C NMR (Figure 2.1C and 2.1D), aromatic and carboxyl/carbonyl carbons accounted for the majority of HA left in the solution while

the organic carbons on montmorillonite were mainly the paraffinic type of aliphatic carbons (0-50 ppm). Thus, the O/C ratio of the HA fractions on montmorillonite would be lower than that of the HA fractions remaining in the solution and the source HA due to the lack of O-containing functional groups. As a consequence, the adsorbed HA would be more hydrophobic and less polar (Litvina et al., 2003), which favored phenanthrene sorption, resulting in a higher sorption affinity than the source HA. Our recent results and other's (Kang et al., 2003; Li et al., 2003) demonstrated that aliphatic HA fractions had lower O/C ratios than HAs with more aromatic structures. A similar HA fractionation trend was observed for kaolinite-HA complexes; the HA remaining in the solution after sequential coatings on kaolinite had increased carboxylic/carbonyl carbons and decreased aliphatic carbons (Figure 2.1E and Table 2.2). High sorption affinity with the bound HA fractions tallied with other studies reporting that aliphatic-rich organic samples had high sorption affinity for HOCs (Chefetz et al., 2000; Chefetz, 2003).

The loading level of HA on the mineral surface also impacted sorption linearity and affinity. For both types of clay-humic complexes, N values decreased with decreasing HA loadings suggesting a relatively more condensed character of HA at lower loading levels. Previous study has proposed that the conformation of HA may be rearranged once adsorbed on a mineral surface and the first several molecular layers of amorphous components may take a more condensed form (Gunasekara and Xing, 2003). The K_{OC} values of kaolinite-bound HA appeared to be lower at lower loading levels at both selected equilibrium concentrations (Table 2.5) while montmorillonite-bound HA exhibited a reversed trend at these two concentrations.

For our experiments, the mineral surface not covered by HA, as estimated by surface area changes before and after coating, was over 70% for montmorillonite and over 75% for kaolinite (Table 2.1). We believe that HA existed in discrete spots on the mineral surface as suggested by Arnarson and Keil (2001). Because different HA loading levels had a similar surface coverage or surface area (Table 2.1), the HA coating must have increased in thickness while retaining practically the same surface coverage at a higher loading. In such multilayer arrangement, the first several molecular layers close to the mineral surface may take a more compacted form due to the attractive forces of the mineral surface. This compacted region could enhance nonlinear sorption. Any HA layers beyond this compacted region may be relatively more expanded as the attractive force becomes weaker with distance away from the mineral surface. Therefore, the proportion of the bound HA in the condensed phase (or form) would be relatively high at lower loading, producing isotherms with higher nonlinearity; *vice versa* for the complexes with higher HA loadings. This may also explain why the montmorillonite-HA complex at 1:100 and kaolinite-HA complex at 1:50 did not have significantly higher N values than the source HA. Therefore, both fractionation and conformation changes contributed to the phenanthrene sorption by the HA fractions associated with minerals.

Although montmorillonite and kaolinite exhibited a similar preferential adsorption for aliphatic HA fractions as demonstrated by NMR spectra, kaolinite-humic complexes had greater K_{OC} values than montmorillonite-humic complexes obtained at the same HA to clay ratio (Table 2.5). For instance, K_{OC} of the kaolinite-humic complex prepared at 1:5 HA to clay ratio was 80% higher than that for montmorillonite-humic

complex at $C_e = 0.01 \mu\text{g/mL}$, and 70% higher at $0.1 \mu\text{g/mL}$. Due to the preferential sorption of hydrophobic, aliphatic HA fractions by the clays, the adsorbed HA may have more aliphatic features in component at lower coating levels at a given HA concentration. Therefore, since montmorillonite adsorbed more than twice as much HA as kaolinite at the same clay to HA ratio, the HA fractions adsorbed on kaolinite would be more aliphatic than that on montmorillonite. Consequently, the HA fractions on kaolinite were relatively more hydrophobic and less polar, resulting in higher phenanthrene sorption on an OC basis.

2.5 Conclusion

NMR data clearly demonstrated that the HA was fractionated during adsorption on mineral surfaces; aliphatic fractions were preferentially adsorbed and aromatic and polar HA fractions were apparently left in the solution. The E_4/E_6 ratios of the HA remaining in the solution decreased with further coatings on montmorillonite and kaolinite, indicating a higher aromaticity of HA in the solution, consistent with NMR experiments. For both montmorillonite and kaolinite, complex samples had substantially more linear sorption isotherms and higher sorption affinities than the source HA. The fractionation and conformation changes of HA after adsorptions by clays were the main reasons for the elevated sorption (i.e., higher K_{OC}). In addition, the sorption linearity of clay-humic complexes increased with HA loading levels, which may have resulted from a more expanded structure of the bound HA developed at higher loadings. Also, HS and their clay complexes can cause isotherm nonlinearity in soils in addition to black carbon.

Table 2.1. Organic carbon content and surface area of clay-humic complexes, Amherst HA, and clay minerals.

Sample	OC (%)	Surface Area (m ² /g)	Percent of Surface Covered by HA [†] (%)
Montmorillonite-HA			
Clay:HA 1:5	0.97	233	29
Clay:HA 1:50	0.61	241	27
Clay:HA 1:100	0.34	243	26
Montmorillonite	< 0.01	329	
Kaolinite-HA			
Clay:HA 1:5	0.47	13.4	22
Clay:HA 1:50	0.26	14.1	15
Kaolinite	< 0.01	15.9	
Amherst HA	54.6	1.29	

[†]Under the assumption that the source HA and the bound HA fractions have a similar surface area, the surface coverage of clays by HA was calculated by the following equation (S stands for surface area):

$$\text{Surface coverage (\%)} = 100 \times [1 - (S_{\text{complex}} - S_{\text{HA}} \times \% \text{HA}) / S_{\text{clay}}]$$

where %HA = 100 × (%OC of complex) / (%OC of HA)

Table 2.2. Structural carbon distributions (%) of Amherst HA and the HA fractions left in the solution after coating on kaolinite as obtained from the ^{13}C NMR spectra integration.

	Paraffinic				
	Aliphatic C	O-alkyl C	Aromatic C	Phenolic C	Carboxyl + Carbonyl C
Amherst HA	19.2	24.6	26.4	7.7	22.1
Unsorbed HA	13.3	12.1	38.3	11.8	24.5

Table 2.3. E_4/E_6 ratios of HA after sequential mineral sorption.

Minerals	Original	Step 1	Step 2	Step 3	Step 4
Montmorillonite	7.79 (0.08) [†]	5.15 (0.04)	4.64 (0.05)	4.46 (0.03)	--
Kaolinite	7.79 (0.07)	6.43 (0.05)	5.87 (0.02)	5.49 (0.04)	5.24 (0.04)

[†]Standard deviations calculated from five replicates are shown in the parentheses.

Table 2.4. Phenanthrene sorption parameters of HA and minerals.

Sorbents	N	$\log K_f$	K_d (mL/g) [†]		K_{OC} (mL/g) [†]	
			$C_e = 0.01 \mu\text{g/mL}$	$C_e = 0.1 \mu\text{g/mL}$	$C_e = 0.01 \mu\text{g/mL}$	$C_e = 0.1 \mu\text{g/mL}$
Amherst HA	0.874 ± 0.010	3.853 ± 0.021	12,740	9,528	22,320	17,450
Montmorillonite	1.08 ± 0.05	1.242 ± 0.023	12.1	14.5	-	-
Kaolinite	1.08 ± 0.06	0.862 ± 0.029	5.1	6.1	-	-

[†] K_d is the distribution coefficient at a given final solution concentration (C_e) calculated from the fitted Freundlich equations; K_{OC} is the organic carbon normalized distribution coefficient calculated from K_d and organic carbon content. K_f unit is $\mu\text{g}^{1-N} \text{mL}^N \text{g}^{-1}$. K_f values of different isotherms cannot be directly compared unless the isotherm N values are the same because K_f unit depends on the values of N . Therefore, calculated K_d and K_{OC} at two selected final solution concentrations were used for comparison, which applies for Table 5.

Table 2.5. Phenanthrene sorption parameters of clay-humic complexes before and after removing sorption contribution from mineral phase (i.e., calibration).

Sorbents	Before Calibration		After Calibration [†]		K_{clay} (ml/g)	
	N^{\dagger}	$\log K_f$	N^{\dagger}	$\log K_f$		
Montmorillonite-HA						
1:5	0.969 ± 0.006	2.392 ± 0.007	0.964 ± 0.006	2.370 ± 0.008	28,530	26,260
1:50	0.940 ± 0.008	2.182 ± 0.009	0.931 ± 0.008	2.145 ± 0.009	31,450	26,830
1:100	0.911 ± 0.006	1.943 ± 0.007	0.889 ± 0.006	1.863 ± 0.007	35,770	27,700
Kaolinite-HA						
1:5	0.938 ± 0.005	2.266 ± 0.008	0.935 ± 0.005	2.253 ± 0.008	51,390	44,250
1:50	0.908 ± 0.008	1.855 ± 0.013	0.898 ± 0.008	1.816 ± 0.014	40,270	31,840

[†]The phenanthrene sorption by the clay-humic complexes was calibrated at each concentration point in order to calculate the sorbed amount by the bound HA alone. The calibration is as follows:

1). Estimate the mineral surface not covered by HA:

$$\%non\text{-coverage} = 100 \times (S_{\text{complex}} - S_{\text{HA}} \times \%HA) / S_{\text{clay}}$$

$$\text{where } \%HA = 100 \times (\%OC \text{ of complex}) / (\%OC \text{ of HA})$$

2). Calculate the sorbed amount by clays under the assumption that the sorption amount of clay minerals is proportional to their surface area:

$$\text{sorbed amount by clay } (\mu\text{g/g}) = \text{Sorption}_{\text{clay}} (\mu\text{g/g}) \times \%non\text{-coverage}$$

Sorption_{clay} is the sorbed amount at a given concentration, derived from the isotherm of clay alone.

3). Calculate the sorbed amount by the bound HA alone by subtracting the sorbed amount by clay from the overall amount by the clay-humic complex.

[‡]All N values were significantly deviated from 1 ($p < 0.05$) (one sample t-test), indicating nonlinear isotherms.

$$K_f \text{ unit is } \mu\text{g}^{1-N} \text{ mL}^N \text{ g}^{-1}$$

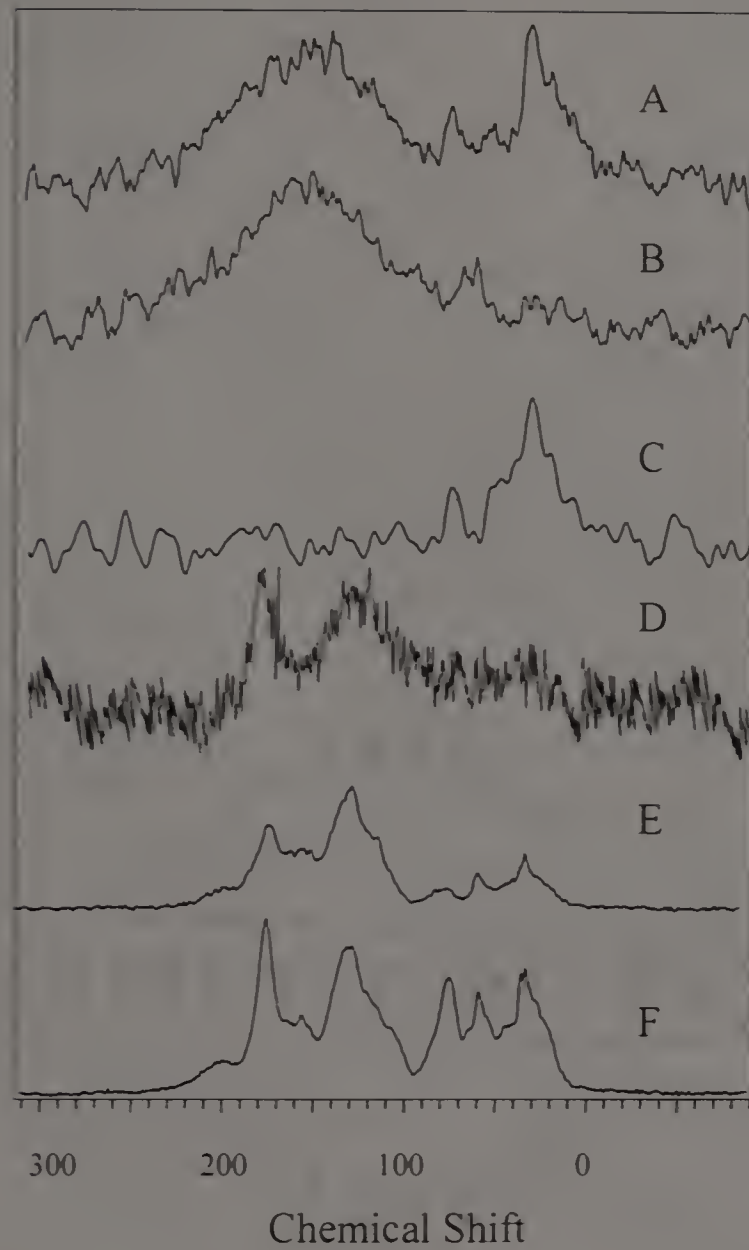


Figure 2.1. Carbon-13 NMR spectra: solid-state NMR spectra of montmorillonite-HA complex (A); rotor background spectrum (B); differential spectrum between A and B (i.e. difference of free induction decay signals) (C); liquid-state NMR spectrum of the HA left in the solution after coating on montmorillonite (D); solid-state NMR spectra of the HA left in solution after coating on kaolinite and freeze-drying (E), and solid-state NMR of the source HA (F).

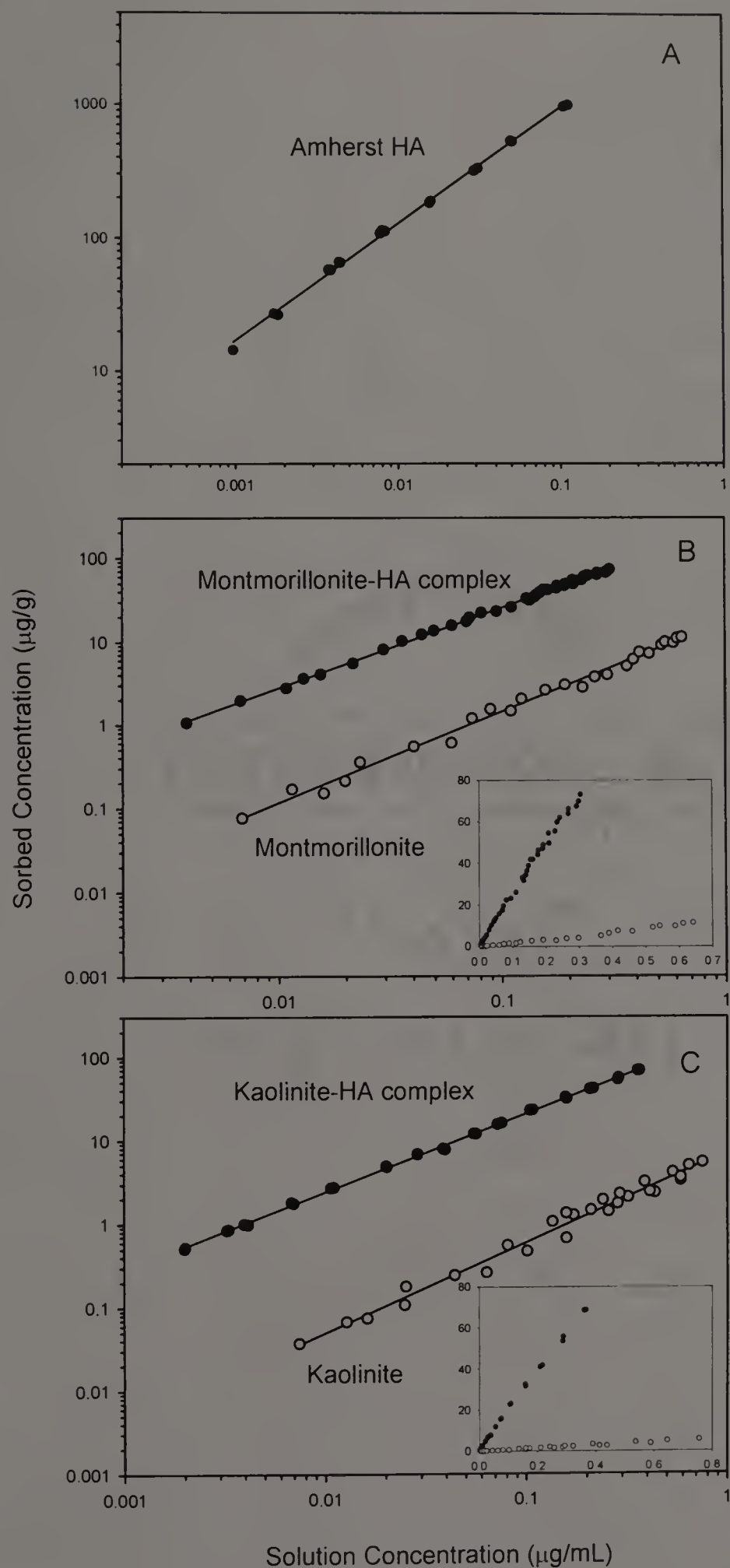


Figure 2.2. Phenanthrene sorption isotherms: the source HA (A); montmorillonite and montmorillonite-HA complex at a HA to clay ratio of 1 to 5 (B); kaolinite and kaolinite-HA complex at a HA to clay ratio of 1 to 5 (C). The inserted figures in B and C are the isotherms in linear scale of B and C, respectively.

CHAPTER 3

EFFECT OF DIFFERENT CHEMICAL EXTRACTION ON SOIL HUMIN STRUCTURE AND PHENANTHRENE SORPTION ¹

3.1 Abstract

Humins is a major fraction of soil organic matter and strongly affects sorption behavior and the fate of organic contaminants in soils and sediments. This study characterized the humin samples extracted from two peat soils using different extraction procedures and examined their consequent effects on phenanthrene sorption. Solid-state ¹³C NMR demonstrated that 0.1 M NaOH exhaustively extracted humin and 6 M HF/HCl at 60 °C extracted humin had a relatively higher amount of aliphatic components as compared with 1 M HF extracted humin. The treatment of 6 M HF/HCl at 60 °C greatly reduced carbohydrate components (50-108 ppm) from humin samples, i.e., more than 50% reduction. In addition, the humin from this 6 M HF/HCl treatment contained relatively more amorphous poly(methylene) domains than the humins extracted by other methods. With the respect to phenanthrene sorption, the linearity of sorption isotherm (*N*) and sorption affinity (*K_{OC}*) varied markedly among the humin samples extracted by different methods. The NaOH exhaustively extracted humin had the most nonlinear sorption isotherm and the HF extracted humin had the lowest *K_{OC}*. It is concluded that humin samples from different extraction procedures exhibited

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substantial differences in structure and sorption characteristics even though they were from the same soil. Therefore, one should be cautious in comparing the structural and sorption features of soil humins, especially when they are extracted differently. The 6 M HCl/HF extraction at elevated temperature is not encouraged due to the modifications of chemical structure and physical conformation of organic matter.

3.2 Introduction

Humin is the major component of SOM and enriched with aliphatic structure (Rice, 2001; Kang and Xing, 2005). A majority of bound polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) have been reported to be associated with humin in soils (Kohl and Rice, 1998). Studies have suggested that both mobile and rigid domains exist spatially in humin (Perminova et al., 2001) and domains of different mobility have also been identified among the same components, i.e. aliphatic carbon components (Kogel-Knabner et al., 1992; Hu et al., 2000). The concept of heterogeneous domains in SOM has been commonly adopted to explain nonlinear sorption isotherms (Huang and Weber, 1997).

Humin has been reported to have higher sorption affinity (K) and more nonlinear sorption isotherms than HA and its source soil (Salloum et al., 2001b; Xing, 2001b). It has been believed that SOM macromolecules might rearrange to take a more condensed conformation in close contact with soil mineral surfaces, which could alter its sorption affinity and isotherm nonlinearity (Gunasekara and Xing, 2003). In this respect, humin samples with different content of minerals as a consequence of extraction and

enrichment/purification processes may differ in structural conformation of SOM and their sorption properties.

Several methods have been adopted in the literature for humin extraction. For example, humin samples are often collected after soil is extracted in 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ three times (Ding et al., 2002), in 0.1 M NaOH once, twice or three times (Dec et al., 1997; Nam and Kim, 2002; Tremblay et al., 2005; Guignard et al., 2005), or in 0.5 M NaOH once for 24 hours followed by washing five times with 0.5 M NaOH (Kohl and Rice, 1998). Some studies also derived humin from exhaustive extraction by repeating 0.1 M NaOH extraction many times to remove any further HA and FA fractions from soil (Chiou et al., 2000; Salloum et al., 2001a). Usually, most of humin samples contain only a small amount of organic matter dispersed in a large volume of inorganic matrices. So in practice, these samples are often treated further to reduce mineral content, either with 1.0 M or 2% (v/v) HF solution (Ding et al., 2002; Smernik and Oades, 2003), or with a hot concentrated HF/HCl solution (Song et al., 2002).

It is anticipated that different extraction methods may alter the structure of organic matter physically or chemically in humin; as a consequence, humin from various extractions may have different sorption behaviors. However, the impact of extraction methods on humin structure and its sorption behavior has not been reported, yet. Accordingly, the objectives of this study were to investigate the structural changes of soil humin as extracted by different methods and to determine their variation in sorption affinity and isotherm nonlinearity.

3.3 Materials and Methods

3.3.1 Humin Extraction

Two peat soils were used in this study: Amherst peat soil (Terric Haplosaprist) and IHSS (International Humic Substances Society) Florida peat soil. Soil residues were collected after 6 extractions of FA and HA with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ at a ratio of 1 to 10. The methods adopted for obtaining different humins were: (1) treating the soil residues with 1.0 M HF at a ratio of 1 to 10 for 24 hours for 4 times; (2) treating the residues with 6 M HF/HCl at 60 ± 2 °C at a ratio of 1 to 10 for 24 hours; (3) repeatedly extracting the residues with 0.1 M NaOH at a ratio of 1 to 10 until no color in the supernatant, this approach is defined as the exhaustive extraction; and (4) deashing the residue with 1.0 M HF at a ratio of 1 to 10 for 24 hours for 4 times after the 0.1 M NaOH exhaustive extraction. We labeled these four extraction methods as HF, HF/HCl, NaOH, and NaOH-HF, respectively. Humin samples obtained from the four different extraction procedures were washed with de-ionized water, freeze-dried and ground to pass 0.15 mm sieve.

3.3.2 Solid-State ^{13}C NMR Spectroscopy

Solid-state ^{13}C NMR experiments were performed using a Bruker Avance DSX300 spectrometer at a ^{13}C frequency of 75.48 MHz in a 7 mm zirconia rotor with a Kel-F cap. The ^{13}C spectra were obtained using a total sideband suppression pulse program (CP/MAS-TOSS). The 90° ^1H pulse length was 5 μs , the spinning speed was 5 kHz, the contact time was 1.5 ms, the acquisition time was 25 ms and the recycle delay was 1 s. The total number of scans was around 7000, and 25 Hz line broadening was applied to the spectra. The structural carbons were interpreted as aliphatic carbons (0-50

ppm), carbohydrate carbons (50-108 ppm), aromatic carbons (108-162 ppm), carboxylic/carbonyl carbons (162-220 ppm) (Mao et al., 2000).

Proton spin-spin relaxation time (T_2) was measured using the method described by Gunasekara et al. (2003). Briefly, the pulse program used was Carr-Purcell pulse sequence followed by Gill-Meibohm phase cycling. Humin samples were packed into 7 mm zirconia rotors with Kel-F cap and analyzed in a static mode because spinning-induced sidebands complicated the T_2 analysis. The ^1H T_2 decay curve contained many data points for decreasing proton signal intensity with increasing time. Relaxation data were first fitted by an exponential decay function, which relates protons in relatively mobile region:

$$I_E(t) = I_E(0) \exp\left(-\frac{t}{T_{2E}}\right)$$

where $I_E(t)$ is the intensity for the exponential portion of the T_2 decay curve at time t , $I_E(0)$ is the initial intensity of the exponential portion at time=0, t is time in μs and T_{2E} is T_2 (μs) value for the mobile domains. A Gaussian decay function, which relates the protons in relatively rigid regions, was used to fit data after subtraction of the exponential portion from the overall intensity data:

$$I_G(t) = I_G(0) \exp\left[-\frac{1}{2} \left(\frac{t}{T_{2G}}\right)^2\right]$$

where $I_G(t)$, $I_G(0)$, t , and T_{2G} are the parameters analogous to the exponential function, but for the protons in the rigid domains. More detailed description and explanation on the methodology and data processing may be found in the paper by Gunasekara et al. (2003).

3.3.3 Sorption Experiments

Phenanthrene was used as a model HOC. The [Ring-UL-¹⁴C] and unlabeled phenanthrene (>98% purity) were purchased from Sigma-Aldrich chemical company (St. Louis, MO) and used without further purification. Sorption experiments were conducted using a batch equilibration method at 20 ± 1 °C. The sorption isotherms were obtained using 100-ml glass bottles with Teflon-lined septa cap. The background solution was 0.01 M CaCl₂ with 200 µg/ml HgCl₂ as a biocide. The ¹⁴C-labeled phenanthrene and its non-radioactive stock solutions were mixed with humin samples at different solid to solution ratios. The humin concentrations in the glass bottles were adjusted to achieve 30-70% sorption of phenanthrene. The suspensions were shaken for 3 days on hematology mixers to achieve apparent equilibrium. Then, bottles were centrifuged at 1000 g for 30 min, and 1 ml supernatant was sampled for liquid scintillation counting (Beckman LS 3801). The amount of phenanthrene sorbed was calculated by the mass difference. Sorption to the glass bottles was found to be negligible.

All sorption data were fitted to the logarithmic form of Freundlich equation:

$$\log C_s = \log K_f + N \log C_e$$

where C_s is the total sorbed concentration (µg/g), C_e is the final solution phase concentration (µg/g), K_f is the Freundlich coefficient (µg^{1-N} mL^N g⁻¹), and N is the exponent indicating isotherm linearity. Isotherms were plotted by $\log C_s$ vs. $\log C_e$ and $\log K_f$ and N were obtained from the intercept and slope of the plot, respectively.

3.4 Results and Discussion

The organic carbon and ash contents of all the humin samples are listed in Table 3.1. It is obvious that humin samples differed greatly in organic carbon and ash contents. The NaOH extracted humin had significantly higher ash contents and lower carbon content than other extraction methods. Treating the soil residues (i.e., after 6 times of 0.1 M Na₄P₂O₇ extraction) with 6 M HF/HCl at 60 °C for 24 hours was much more effective in reducing ash content than with 1 M HF for 24 hours 4 times at room temperature. Thus, the HF/HCl extracted humin had the highest carbon content and lowest ash content among all the humin samples.

3.4.1 NMR Spectroscopy

The solid-state ¹³C NMR spectra (Figure 3.1) and their integrations (Table 3.2) demonstrated that Florida humins had fewer aliphatic carbons, but more aromatic carbons than Amherst humins for each extraction method, suggesting a quite different structural composition of humin between the two soils. For each soil, both NaOH and NaOH-HF extracted humins had relatively higher aliphatic carbon contents but lower aromatic carbon contents than the HF treated humin. It has been known that humin displays more aliphatic nature than HA or FA from the same source (Lichtfouse et al., 1998; Kang and Xing, 2005). Because we collected the soil residues after only 6 extractions with 0.1 M Na₄P₂O₇ at a ratio of 1 to 10, some HA and FA would still remain in the residues. The NaOH exhaustive extraction was able to remove HA and FA more completely, thus, it is not surprising that the humins from the exhaustive extraction was enriched in aliphatic carbons, consistent with our previous studies (Kang and Xing, 2005; Xing et al., 2005).

The 6 M HF/HCl treatment at 60 °C substantially reduced carbohydrate carbon (50-108 ppm) content of the humins. As seen in Table 3.2, the HF/HCl extracted humin contained only half of the carbohydrate carbon content of those as extracted by other methods. Moreover, compared with the HF extracted humin, the HF/HCl extracted humin had more aliphatic and aromatic components. It should be noted that although both the NaOH extracted humin and the HF/HCl extracted humin had enriched aliphatic carbon content as compared with the HF humin, the causes were different. NaOH extraction removed most (if not all) of the remaining HA and FA from the samples, resulting in higher aliphatic carbon contents. On the contrary, the HF/HCl extraction did not remove the remaining HA and FA fractions; instead, this procedure eliminated a large portion of carbohydrate components. As a result, aliphatic and aromatic carbon contents in the HF/HCl extracted humin were proportionally increased due to the preferential removal of carbohydrate carbons. Also, the HF/HCl extracted humin sample had relatively higher aromatic carbon content than the NaOH extracted humins (Table 3.2).

The polarity of SOM has been reported to be closely related to the sorption of organic contaminants (Xing et al., 1994; Xing, 1997; Kile et al., 1999; Kang and Xing, 2005). In our study, a polarity index was calculated by combining organic carbon fractions of 50-108 ppm, 145-162 ppm, and 162-220 ppm regions of the ¹³C spectra. The calculation is modified from that used by Kile et al. (1999) because we included the phenolic groups (145-162 ppm) as polar carbons. The index scores suggest that the HF extracted humin had highest polarity while the HF/HCl extracted humin (at 60 °C) had

the lowest polarity (Table 3.2). In addition, for a given extraction procedure, Florida humin had a slightly higher polarity than Amherst humin.

In all the spectra (Figure 3.1), the characteristic doublets of relatively narrow and high poly(methylene) signals can be clearly distinguished. The peaks centered at 32.8 ppm and 31 ppm are assigned to crystalline poly(methylene) and amorphous poly(methylene) carbons, respectively (Hu et al., 2000). Although the amorphous and crystalline poly(methylene) carbons have very similar chemical structures, they exhibit slightly different chemical shifts due to their different conformation. Figure 3.3 shows expanded views of the nonpolar aliphatic carbon NMR regions (0-50 ppm) of Amherst and Florida humins. The intensity changes of the doublets imply that amorphous poly(methylene) components were augmented in the HF/HCl extracted humin while crystalline components were the major fraction of poly(methylene) carbons in the humin samples by other extraction methods. Therefore, the 6 M HF/HCl treatment at 60 °C not only reduced carbohydrates dramatically, but also altered the structural conformation of nonpolar aliphatic components by increasing the amount of amorphous domains. The amorphous nonpolar aliphatic domains were reported to exhibit rubbery-like mobility, which is favorable for sorption of nonpolar organic chemicals (Mao et al., 2002).

It is well understood that ^1H spin-spin relaxation (T_2) is related to molecular motion: protons in rigid regions relax rapidly, represented by short T_2 values; while the protons in mobile regions relax slowly, corresponding to long T_2 values. In our study, proton T_2 relaxation of the HF extracted humin and HF/HCl extracted humin were measured and the relaxation data were processed based on the method described by

Gunasekara et al. (2003). The only difference in data processing is that we fitted exponential function sequentially for two times instead of once considering that there was no sharp boundary between rigid and mobile domains in reality due to the heterogeneity of humic substances. We then defined the portion of the T_2 decay obtained by the second exponential fit as semi-mobile domains, which describe the phase between rigid and mobile states. Table 3.3 summarized the T_2 values and percent domain distribution. Apparently, the HF/HCl extracted Florida humin contained relatively more mobile domains than HF extracted humin, which is in agreement with our ^{13}C NMR results that HF/HCl extracted humin had more amorphous poly(methylene) domains. Amherst humin also had similar trend, but with smaller difference in percent distribution. The increased mobile domains might be the consequence of deashing and reducing carbohydrate components. The treatment of 6 M HF/HCl at 60 °C was more effective than 1 M HF at room temperature to remove ash content (Table 3.1). The released humic materials due to removal of minerals were more likely to have higher mobility than those associated with minerals (Cuypers et al., 2002; Gunasekara and Xing, 2003). On the other hand, reducing carbohydrate components might also loose the humin structure as a result of reduction in bond linkages (e.g., H-bonds) between different SOM structural moieties. Protons in mobile domains had longer T_2 in Amherst humin than in Florida humin, but Florida humin had higher percentage of mobile domains than Amherst humin, reflecting the structural and conformational differences between two humins. However, the proton T_2 method seems not a very sensitive measure here for monitoring distribution changes of mobile/condensed domains, especially for Amherst humin samples. This may be due to

the fact that domains in Amherst humin are already very mobile as indicated by high sorption linearity described in the sorption section below and the large T_2 (58 μ s) in the mobile domain (Table 3.3).

To discriminate between the effects of temperature and 6 M HF/HCl, we treated the soil residues with 6 M HF/HCl at the room temperature for 24 hours, and with deionized water at 60 °C for 24 hours. The NMR spectra of Amherst humin are shown as an example in Figure 3.3. Higher temperature alone could increase the composition of amorphous poly(methylene), consistent with the data by Hu et al. (2000), but the change was not as much as in the humin extracted by 6 M HF/HCl at 60 °C (Figure 3.3). The integration suggests that neither 6 M HF/HCl nor high temperature could reduce carbohydrate carbon content dramatically (results not shown), indicating that the increased amount of amorphous poly(methylene) and reduced carbohydrate content were the consequence of the combined action of 6 M HF/HCl and elevated temperature.

3.4.2 Phenanthrene Sorption

The alteration of structural composition and conformation of humins impacted their sorption behaviors. For Florida humin, the NaOH exhaustively extracted humin had the lowest N value and the HF/HCl extracted humin had the highest (Figures 3.4 and 3.5, Table 3.4). Deashing humin with HF after NaOH exhaustive extraction (NaOH-HF method) led to a higher N than without deashing (NaOH method). The N values of humin samples using different extraction methods were well correlated to their ash contents. It has been considered that interaction of aliphatic components of organic matter with mineral surface might lead to rearrangement of amorphous domains into a condensed configuration, hence, resulting in more nonlinear isotherms

(Gunasekara and Xing, 2003). Deashing humin with HF or HF/HCl could release the mineral-associated humin components and reduce the contact between minerals and SOM; thus, the *N* value would be increased. Compared to 1 M HF, 6 M HF/HCl at 60 °C was more efficient in deashing, and more importantly, this extraction procedure yielded humin with an increased amount of amorphous nonpolar aliphatic components, which would explain the relatively higher *N* value of the HF/HCl-extracted humin than the HF extracted humin.

A similar trend was observed for Amherst humin, but the variation of *N* values was not as great as that for Florida humin. According to the mobile/rigid concept of SOM and its relationship with sorption nonlinearity, Amherst humin was more mobile in structure than Florida humin, as represented by higher *N* values for each extraction method. This can also be confirmed by ¹³C NMR integration results (Table 3.2) and proton T₂ values (Table 3.3). Proton T₂ value of Amherst humin was much larger than that of Florida humin in the mobile domain. Florida humin had fewer aliphatic carbons and more aromatic carbons than Amherst humin where aromatic components are usually considered to be more rigid than aliphatic moieties (Xing and Chen, 1999; Johnson et al., 2001). However, Amherst humin contained similar or even more ash content than Florida humin; for instance, the HF- and NaOH-extracted Amherst humins contained 41% and 59% ash, respectively while the HF- and NaOH-extracted Florida humin had 10% and 50% ash content, respectively. Thus, sorption nonlinearity and its variations cannot be simply interpreted by ash content alone. It is anticipated that organic matter in Amherst humin was not associated with minerals as strongly as that in

Florida humin; or some minerals might exist separately with organic matter instead of complexes. In this regard, deashing would have a less impact on N value.

Large differences in K_{OC} were observed for the humin samples extracted by different methods (Table 3.4). For Amherst humin, the K_{OC} value of the HF/HCl-extracted humin was nearly twice as high as those of humins extracted by other methods. The sorption affinity order in terms of K_{OC} value by extraction methods was HF < NaOH \approx NaOH-HF < HF/HCl at both equilibrium concentrations of 0.01 and 0.1 $\mu\text{g mL}^{-1}$. The same trend was observed for Florida humin at $C_e = 0.1 \mu\text{g mL}^{-1}$. However, at the concentration of 0.01 $\mu\text{g mL}^{-1}$, the HF/HCl-extracted humin had slightly lower K_{OC} than the NaOH and NaOH-HF extracted humins. Regardless of extraction methods, both Amherst and Florida humins had substantially higher K_{OC} than their corresponding HAs (Table 3.4). Because the HF-extracted humin contained a certain amount of HA, removal of such HA fraction by NaOH exhaustive extraction corresponded to the increased amount of aliphatic components, resulting in increased K_{OC} values. As for the HF/HCl extracted humin, the enhancement of phenanthrene sorption might be attributed to an increased amount of amorphous poly(methylene) components and reduced polarity due to the significant reduction of carbohydrate components. It has been reported that the fraction of amorphous poly(methylene) has a strong positive correlation with the sorption of nonpolar molecules (Mao et al., 2002), and low polarity of SOM is favorable for HOCs sorption (Kile et al., 1999; Kang and Xing, 2005).

3.5 Conclusion

In summary, humin samples obtained from various extraction methods differed greatly in their chemical and structural composition, ash content, and sorption properties of HOCs. The HF extraction is the simplest method to prepare humin sample, however, the sample from this approach is really a mixture of humin, HA, and FA. As compared to HF extraction, the 0.1 NaOH exhaustive extraction successfully removed HA from soil samples, resulting in more aliphatic components (0-50 ppm) and consequently higher K_{OC} values. We believe that samples by NaOH exhaustive extraction represent the characteristics of humin. The deashing with HF after NaOH exhaustive extraction effectively lowered ash contents of humin samples with only slight changes of their chemical structure and sorption properties. Therefore, both NaOH and NaOH-HF would be appropriate approaches for humin extraction. The extraction with 6 M HF/HCl at 60 °C not only removed ash content efficiently, but also substantially modified the chemical structure of humin as indicated by dramatically reduced carbohydrate components (50-108 ppm). Further, this 6 M HF/HCl extraction also enhanced the amount of amorphous poly(methylene) domains. Therefore, this extraction method would be discouraged for use due to structural modifications of humin both chemically and physically.

Table 3.1. Organic Carbon and Ash Contents of Humin Samples Obtained after Different Extraction Methods ^a

Humin	Organic Carbon (%)	Ash (%)
Amherst Humin		
HF	33.6	41.1
HF/HCl	62.9	1.4
NaOH	23.7	59.2
NaOH-HF	57.0	5.8
Florida Humin		
HF	54.7	9.5
HF/HCl	56.6	3.9
NaOH	21.1	50.0
NaOH-HF	48.4	27.9

^a HF = treating the soil residue (crude humin) with 1.0 M HF for 24 hours 4 times; HF/HCl = treating the soil residue with 6 M HF/HCl at 60 ± 2 °C for 24 hours; NaOH = repeatedly extracting the soil residue with 0.1 M NaOH until no color in the supernatant (i.e., exhaustive extraction); and NaOH-HF = after the 0.1 M NaOH exhaustive extraction, deashing the soil residue with 1.0 M HF for 24 hours for 4 times.

Table 3.2. Organic Carbon characteristics of Humin Samples as Determined by Solid-State ^{13}C NMR ^a

Sample	Aliphatic (0-50 ppm)	Carbohydrate (50-108 ppm)	Aromatic (108-162 ppm)	Carboxylic (162-220 ppm)	Polarity index
Amherst Humin					
HF	35	32	24	9	0.47
HF/HCl	43	15	30	12	0.35
NaOH	39	29	22	10	0.45
NaOH-HF	44	30	18	8	0.43
Florida Humin					
HF	19	33	35	13	0.54
HF/HCl	30	15	39	16	0.41
NaOH	26	30	30	14	0.52
NaOH-HF	27	34	31	8	0.49

^a HF = treating the soil residue with 1.0 M HF for 24 hours 4 times; HF/HCl = treating the soil residue with 6 M HF/HCl at 60 ± 2 °C for 24 hours; NaOH = repeatedly extracting the soil residue with 0.1 M NaOH until no color in the supernatant (i.e., exhaustive extraction); and NaOH-HF = after the 0.1 M NaOH exhaustive extraction, deashing the soil residue with 1.0 M HF for 24 hours for 4 times.

Table 3.3. Spin-Spin Relaxation Times (T_2) and Distribution of Rigid and Mobile Domains

Humin	Domain	T_2 (μ s)	% Domain distribution
Florida			
HF Extracted Humin	Mobile	37.0	33
	Semi-mobile	9.0	58
	Rigid	3.8	9
HF/HCl Extracted Humin	Mobile	37.6	40
	Semi-mobile	9.0	54
	Rigid	3.7	6
Amherst			
HF Extracted Humin	Mobile	57.8	15
	Semi-mobile	9.0	75
	Rigid	3.3	11
HF/HCl Extracted Humin	Mobile	60.2	17
	Semi-mobile	11.0	73
	Rigid	3.4	11

Table 3.4. Isotherm Parameters for Phenanthrene Sorption by Soil Humins Extracted by Different Methods ^a

	<i>N</i>	log <i>K_F</i> $\mu\text{g}^{1-N} \text{mL}^N \text{g}^{-1}$	<i>K_{oc}</i>	
			$C_e = 0.01 \mu\text{g mL}^{-1}$ -----mL g ⁻¹ -----	$C_e = 0.1 \mu\text{g mL}^{-1}$
Amherst Humin				
HF	0.882 ± 0.007	3.982 ± 0.013	49200	37500
HF/HCl	0.894 ± 0.014	4.592 ± 0.030	101000	79100
NaOH	0.868 ± 0.009	3.841 ± 0.016	53700	39600
NaOH-HF	0.910 ± 0.007	4.301 ± 0.013	53000	43100
Amherst Humic Acid				
	0.883 ± 0.011	3.868 ± 0.023	23200	17700
Florida Humin				
HF	0.648 ± 0.010	3.945 ± 0.021	81500	36200
HF/HCl	0.743 ± 0.009	4.236 ± 0.019	99400	55000
NaOH	0.553 ± 0.010	3.527 ± 0.018	125000	44700
NaOH-HF	0.598 ± 0.008	3.947 ± 0.017	116000	46100
Florida Humic Acid				
	0.893 ± 0.009	3.869 ± 0.009	22000	17200

^a HF = treating the soil residue with 1.0 M HF for 24 hours 4 times; HF/HCl = treating the soil residue with 6 M HF/HCl at 60 ± 2 °C for 24 hours; NaOH = repeatedly extracting the soil residue with 0.1 M NaOH until no color in the supernatant (i.e., exhaustive extraction); and NaOH-HF = after the 0.1 M NaOH exhaustive extraction, deashing the soil residue with 1.0 M HF for 24 hours for 4 times.

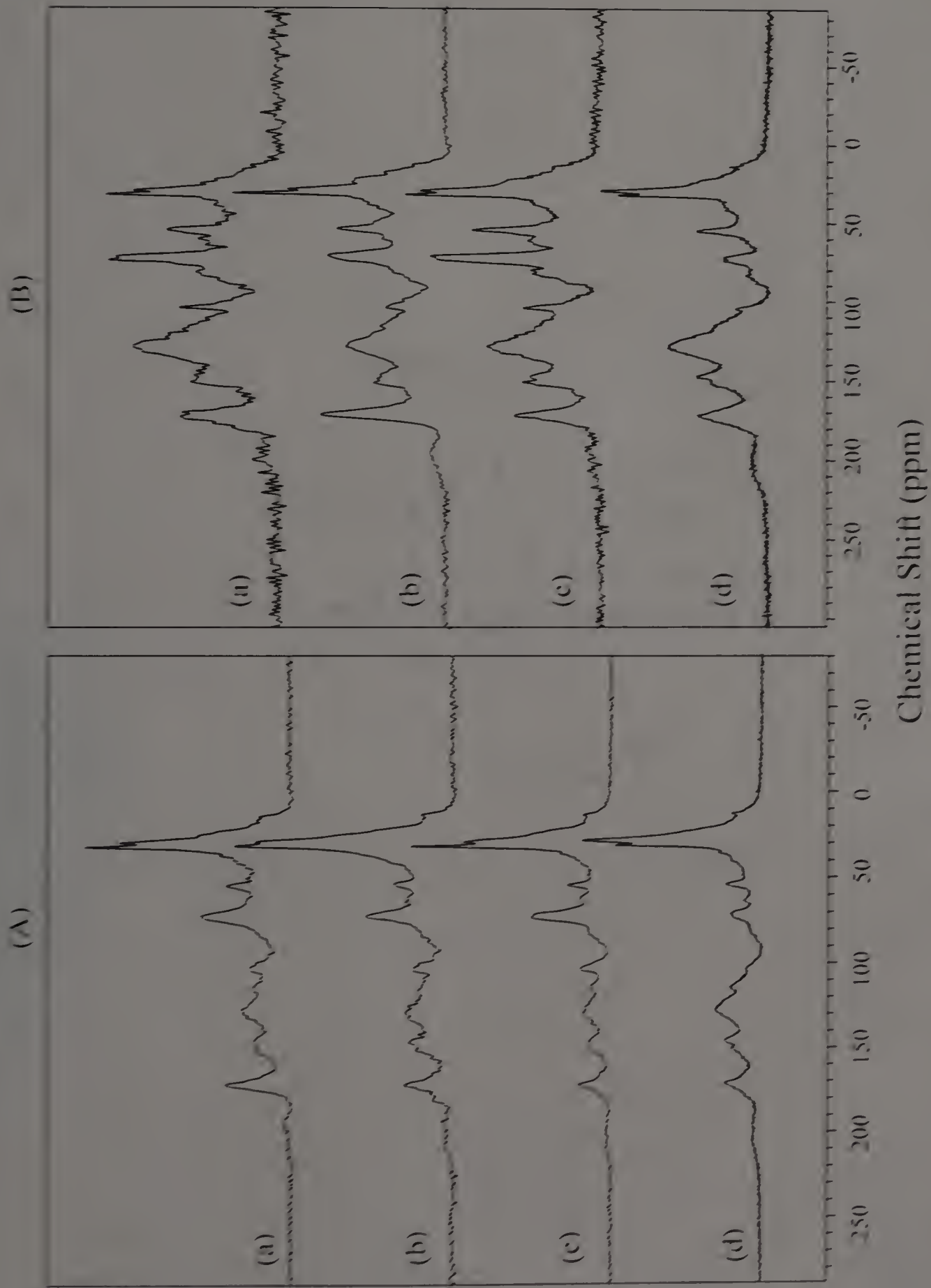


Figure 3.1. Solid-state ^{13}C spectra of humin samples extracted from Amherst peat soil (A) and Florida peat soil (B) by different methods: (a) HF; (b) NaOH; (c) NaOH-HF; and (d) HF/HCl.

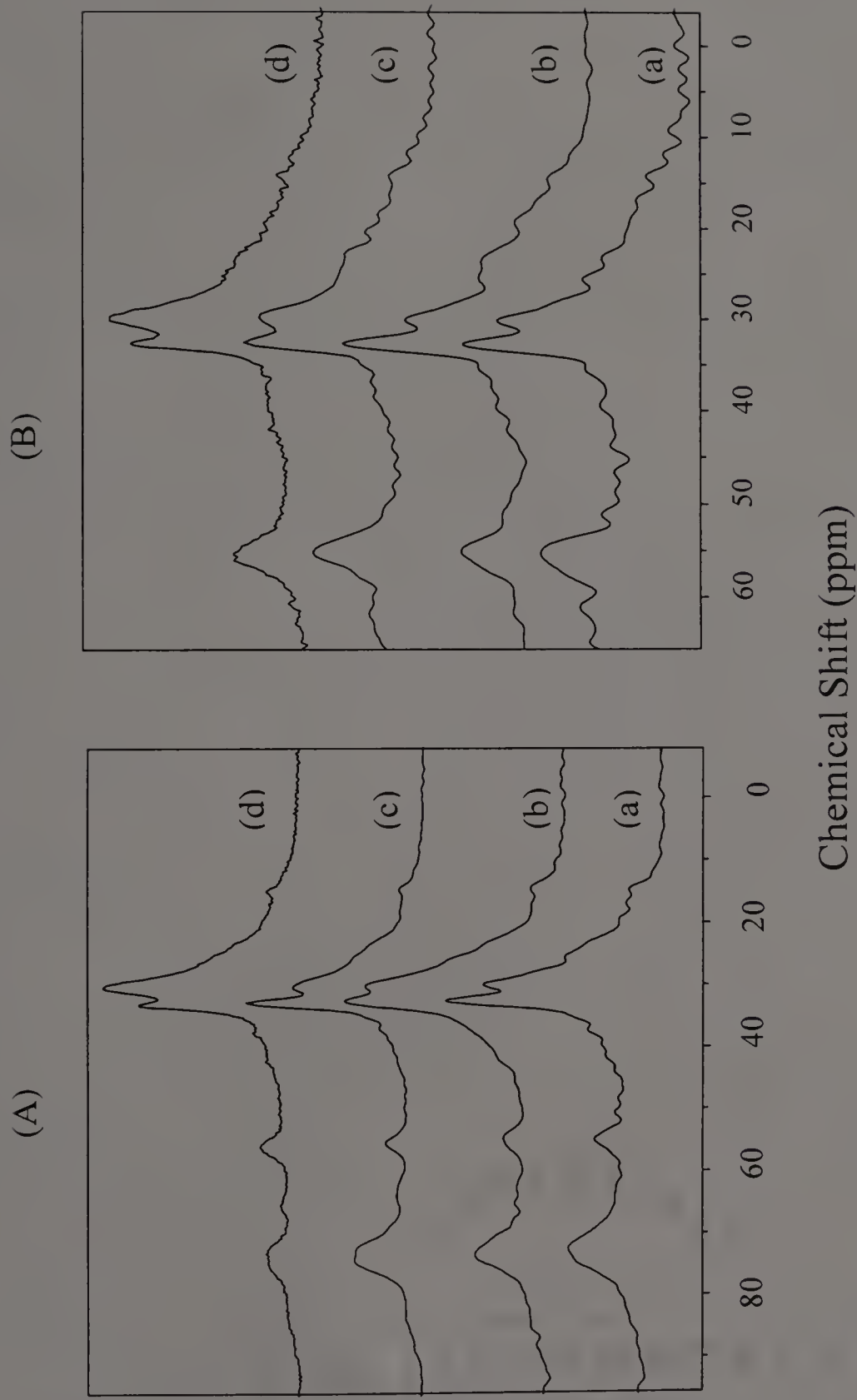


Figure 3.2. Expanded solid-state ^{13}C spectra of humins in the nonpolar aliphatic regions for (A) Amherst humin samples, and (B) Florida humin samples. Humin was extracted by four methods: (a) HF; (b) NaOH; (c) NaOH-HF; and (d) HF/HCl.

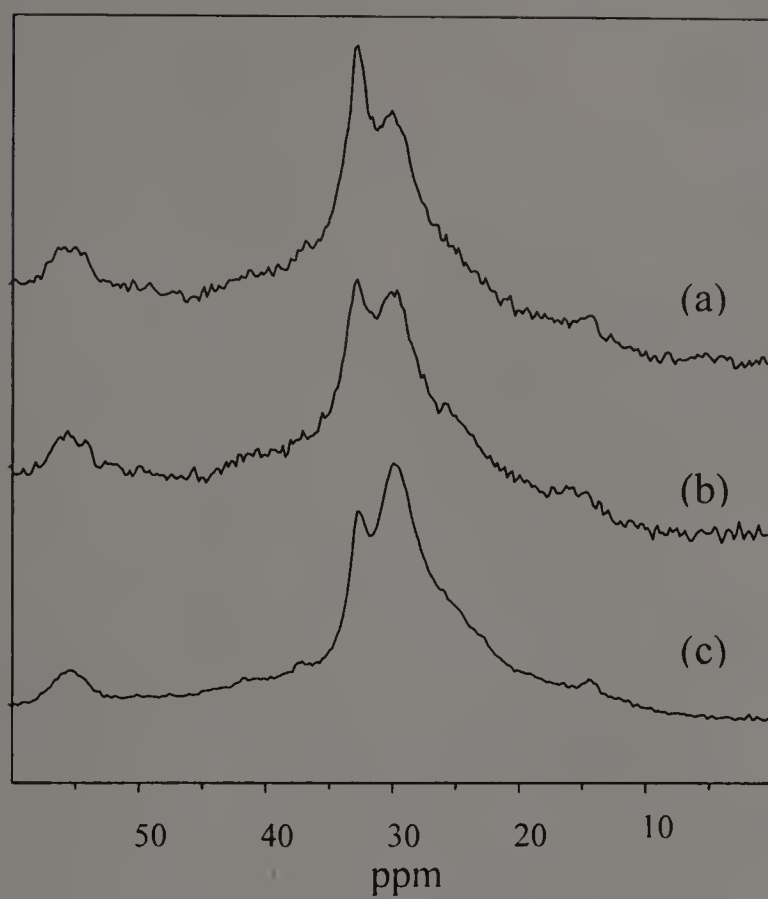


Figure 3.3. Comparison of expanded ^{13}C NMR line shapes in nonpolar aliphatic regions for Amherst humin samples with different treatments: (a) 6 M HF/HCl at room temperature; (b) deionized water at 60 °C; and (c) 6 M HF/HCl at 60 °C.

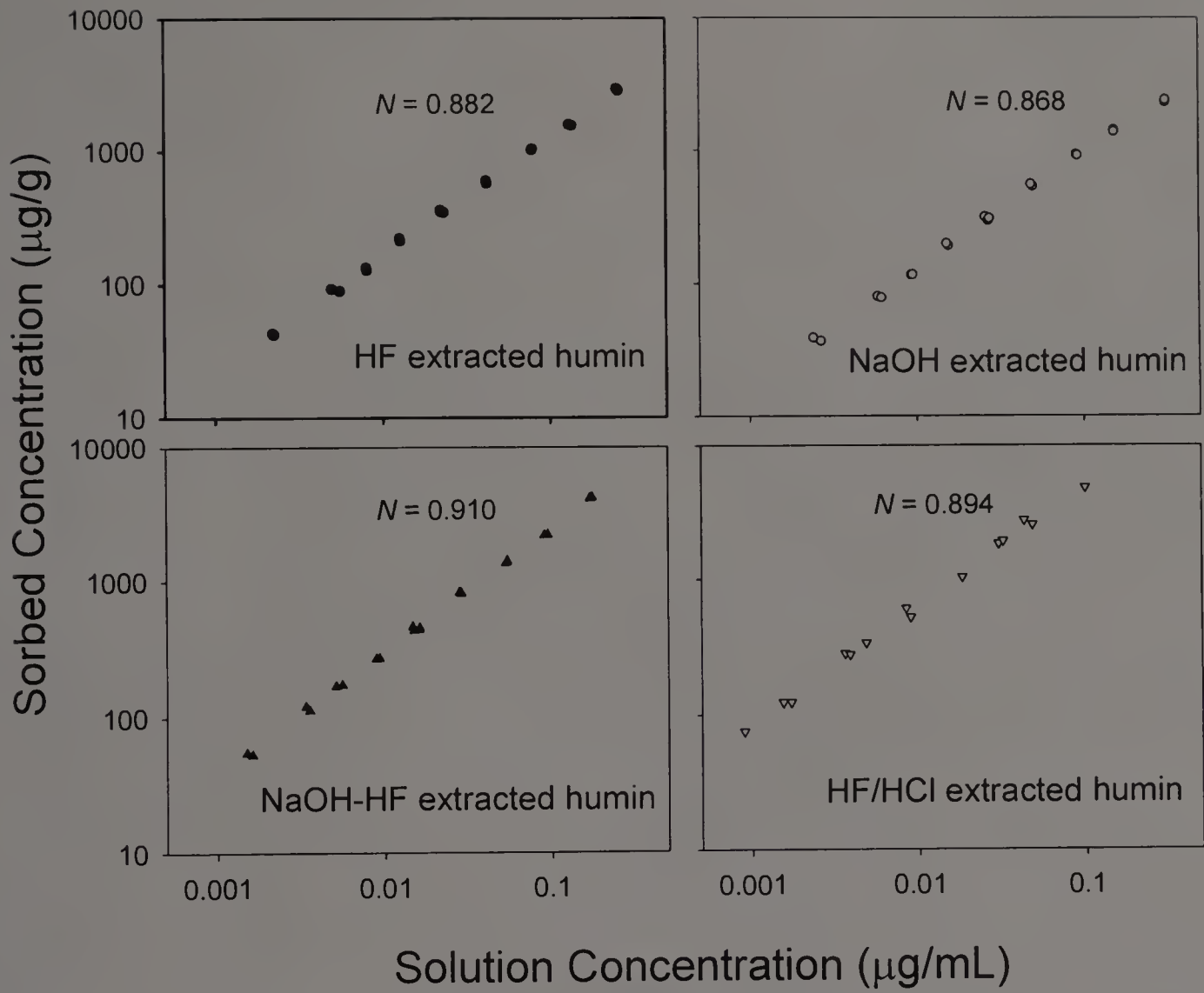


Figure 3.4. Phenanthrene sorption isotherms of humin samples extracted by different methods from Amherst peat soil.

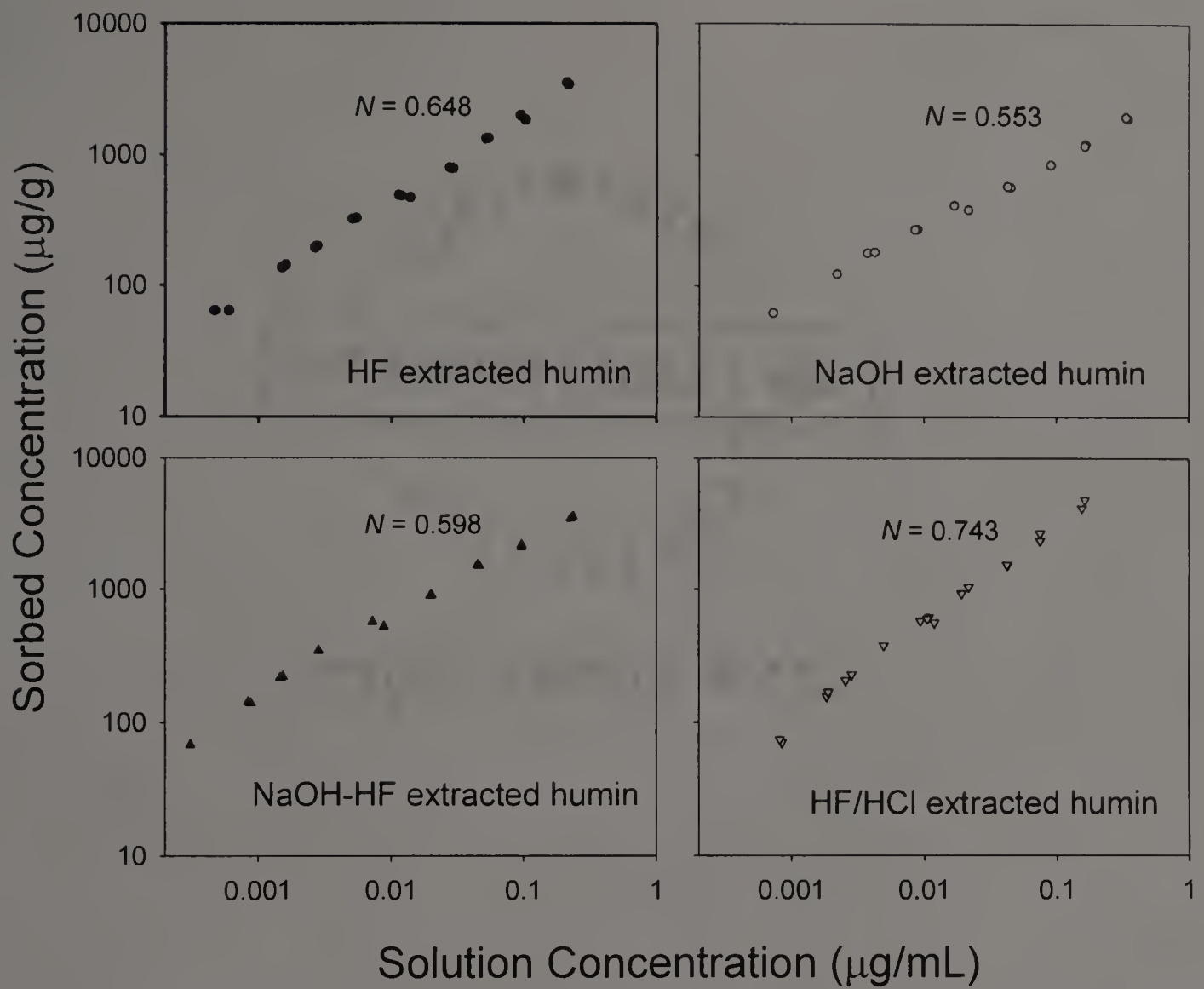


Figure 3.5. Phenanthrene sorption isotherms of humin samples extracted by different methods from Florida peat soil.

CHAPTER 4

PROTON SPIN-LATTICE RELAXATION TIMES OF HUMIC ACIDS AS DETERMINED BY SOLUTION NMR ¹

4.1 Abstract

Nuclear magnetic resonance spectroscopy is used frequently for structural characterization of HS. However, the study of relaxation phenomena of HS, including spin-lattice and spin-spin relaxation, has received little attention. The present study investigated proton T_1 of HA in deuterated 0.5 M sodium hydroxide (NaOD) and dimethylsulfoxide (d_6 -DMSO) at various concentrations, temperatures and pHs. Two HAs were used, which were extracted from an Amherst peat soil and a New Hampshire peat soil. Overall, proton T_1 s of humic acids used in this study ranged from 0.3 to 0.8 s in d_6 -DMSO, and from 0.26 to 2.3 s in 0.5 M NaOD/D₂O. Aromatic protons had much longer T_1 than aliphatic protons in 0.5 M NaOD, while both types of protons exhibited similar T_1 values in d_6 -DMSO. These results indicate that humic acids might have different conformations in the two solvents. Proton T_1 generally decreased with increasing HA concentration. Only protons from methyl and methylene/methine groups exhibited a slight T_1 increase with a temperature increase from 20 °C to 60 °C, while protons from other functional groups displayed a reduced T_1 except for aromatic protons of New Hampshire HA in 0.5 M NaOD. Moreover, both HAs demonstrated

¹ A version of this chapter has been published.

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distinct T_1 changes in alkaline solution as pH decreased. The proton T_1 values and their changes with environmental conditions can assist the interpretation of segmental mobility and molecular conformation of dissolved HAs in different solvents.

4.2 Introduction

The structural and functional properties of HS are of significant importance for understanding their sorption characteristics and roles in environmental processes.

Nuclear magnetic resonance spectroscopy (NMR) is a powerful approach and has been commonly used for structural characterization of HS. Spin-lattice relaxation time is an important NMR parameter. It is essential to know nuclear relaxation rates ($1/T_1$) in order to obtain reliable spectra. More importantly, T_1 is a sensitive and powerful probe of molecular structures and dynamics. Most nuclear relaxation studies relate relaxation parameters to the shape, size, mobility, and dynamic processes of molecules or molecular fragments (Veeman, 1997; Cook and Langford, 1998; Duncan and Whitten, 2000).

The proton T_1 is a well-established NMR parameter for structural, configurational and conformational analysis of organic molecules in solution. It has been widely used in organic chemistry, biochemistry and pharmaceutical chemistry to determine the mobility and structural features of carbohydrates, proteins, pharmaceutical products and other organic compounds (Kotowycz et al., 1980; Keller and Carper, 1993; Duncan and Whitten, 2000). However, systematic studies of proton T_1 s of HS in solutions are rare in the literature, especially for relaxation behaviors of protons or/and carbon centers from different fragments in HA.

Because T_1 is sensitive to the nuclear spins' environmental conditions, changes in concentration, temperature, or solvent pH can affect this parameter. Therefore, the objective of this work was to investigate the proton spin-lattice relaxation of HA in different solvents and their changes with temperature, concentration, and pH. A better knowledge of proton T_1 s and their changes with environmental conditions will help interpret molecular conformation and segmental mobility of HS and understand HS chemistry in the environment.

4.3 Materials and Methods

4.3.1 Materials

Two humic acids were used in this study: Amherst peat HA and New Hampshire peat HA. They were extracted using the standard procedures outlined by the International Humic Substances Society (IHSS) (Swift, 1996). Elemental compositions of HA were determined by the Microanalytical Laboratory at the University of Massachusetts, Amherst. The composition of Amherst HA is: 54.6% C, 4.16% H, 38.5% O, 2.46% N, and 0.24% ash; New Hampshire HA contains 52.9% C, 5.40% H, 39.7% O, 2.00% N, and 0.25% ash. The functional group composition of Amherst HA as determined by solid-state NMR is 17.3% aliphatic carbon, 25.0% carbohydrate carbon, 40.8% aromatic carbon, and 16.9% carboxylic carbon; while the corresponding values for New Hampshire HA are 17.0%, 31.6%, 39.0% and 12.4% (Mao et al., 2000). Deuterium oxide (D_2O), deuterated sodium hydroxide solution (30% in D_2O), deuterated dimethylsulfoxide (d_6 -DMSO) and deuterated hydrochloric acid (20% DCl

in D₂O) were purchased from Cambridge Isotope Laboratories, Inc.. All these reagents were used as received.

4.3.2 Methodology

Three HA concentration levels (1%, 3%, and 5% w/w) were prepared to study any concentration effect. Relatively high HA concentrations were used in order to obtain better signal to noise ratios. 0.5 M deuterated sodium hydroxide (NaOD) in D₂O and deuterated dimethylsulfoxide (d₆-DMSO) were used as solvents. The pH experiments were conducted at a HA concentration of 3% (w/w). Deuterated hydrochloric acid (DCl) was used to achieve three different pH levels for each HA (10.8, 8.4, and 5.3 for Amherst HA; 10, 8.3, and 6.2 for New Hampshire HA). Samples at a concentration of 5% (w/w) were run at 20 °C, 40 °C and 60 °C to evaluate the effect of temperature on proton relaxation.

The T₁ was measured by the commonly used inversion recovery pulse sequence (PD - 180° - τ - 90° - AT)_n. The delay time, τ, between two pulses was the variable parameter, which was set at a different fixed value for each spectrum. Except for the temperature variation experiment, which was conducted on a Bruker Avance600 (600 MHz) spectrometer, all the other T₁ measurements were carried out on a Bruker DPX300 spectrometer at room temperature. The ¹H 90° and 180° pulse lengths were 9.25 μs and 18.5μs, respectively, and the recycle delay was 10 seconds. Proton spectra were recorded at 10 different τ: 10μs, 500μs, 10ms, 100ms, 250ms, 500ms, 1s, 2s, 5s, and 10s. A preliminary test showed that this time range was suitable for proton T₁ measurements. Tetramethylsilane (TMS) was used as an internal chemical shift

standard for samples dissolved in d_6 -DMSO and an external reference for those dissolved in 0.5 M NaOD.

4.4 Results and Discussion

4.4.1 Proton T_1 measurement and the HA concentration effect

The proton spectra for T_1 measurement using the inversion recovery method consisted of a series of one-dimensional spectra recorded at different τ (Fig. 4.1). The intensities of peaks changed from a negative minimum at very short τ (10 μ s) to a positive maximum at $\tau = 10$ s for most samples. It is convenient to divide the spectrum into three chemical shift regions: 0.8-3.0 ppm, representing aliphatic protons; 3.0-5.5 ppm, representing the protons associated with oxygen-containing functionalities; and 6.0-8.5 ppm for aromatic protons. The zero-crossing points (the τ at which the signal intensity reached zero) varied with protons from different functional moieties. For most aliphatic protons, the zero-crossing points were at about 0.25 s, while for aromatic protons the points were around 0.5 s. To simplify interpretation of the relaxation data 5 peaks were selected from different regions to elucidate the T_1 changes for each T_1 measurement. They were at 0.8 ppm, 1.2 ppm, 4.2 ppm, 7.2 ppm and 8.1 ppm for HAs in d_6 -DMSO and at 0.7 ppm, 1.1 ppm, 3.6 ppm, 6.4 ppm and 7.1 ppm for HAs in 0.5 M NaOD. The chemical shifts of these peaks in some proton NMR spectra may have slightly different values.

Figure 4.2 illustrates the intensity variation of the three selected signals (0.83, 4.24, and 7.21 ppm in d_6 -DMSO; 0.73, 3.63, and 7.14 ppm in 0.5 M NaOD) with the delay time in proton T_1 measurement of Amherst HA. For each proton signal

determined in this study, the relaxation curve (intensity vs. delay time) was approximately exponential with $R^2 > 0.995$ in both solvents. The uncertainties in T_1 measurement were less than $\pm 5\%$ in most cases.

Overall, the proton T_1 was on the order of a second, with its value ranging from 0.35 s to 0.8 s for HAs in DMSO (Figs. 4.3A and B) and from 0.26 to 2.3 s for HAs in 0.5 M NaOD (Figs. 4.3C and D) for all concentration levels studied at a magnetic field strength of 300 MHz. For HAs in DMSO, it is clear that the T_1 values of protons from different regions decreased with increasing HA concentration. This result is consistent with the common notion that molecular mobility is relatively higher in more dilute solutions. Theoretically, T_1 is a reflection of the rotation or reorientation of a molecule in solution. In the extreme narrowing limit ($\omega\tau_c \ll 1$, ω is Larmor frequency and τ_c is rotational correlation time), relative larger molecules generally tumble more slowly in solution and have more time to interact with the lattice; thus, they require a shorter time for their nuclear spins to return to the equilibrium state. The opposite is true with small molecules, which tend to tumble quickly and spend less time interacting with the lattice, resulting in longer relaxation times (Bortiatynski et al., 1997). Our measurement on proton correlation time demonstrated that $\omega\tau_c$ was less than 1 for most protons in HAs (data shown next Chapter). Hence, it is reasonable to speculate that HA tends to aggregate as the concentration increases, leading to a slow rotational diffusion. Consequently, the rotational correlation time becomes longer and T_1 gets shorter. Another possible explanation is T_1 reduction by paramagnetic ions in the solutions, even though this is unlikely to be the main reason for T_1 decrease in this study. Paramagnetic elements can shorten relaxation times dramatically (Wehrli et al., 1988).

However, the ash contents of our HA samples were very low ($< 0.25\%$) and the molarity of paramagnetic iron in humic acids should be less than 1.6×10^{-5} assuming all ash content was iron oxide. On the other hand, proton T_1 did not show substantial reduction as the HA concentration increased from 1% to 5%. Newman and Tate (1984) also found that natural concentrations of co-extracted transition metals were too low to be effective as relaxation agents.

A similar trend of T_1 decrease with concentration was also observed for the two HAs in 0.5 M NaOD ($\text{pH} \approx 12.5$). However, under these conditions aliphatic protons appeared to be less concentration-dependent than aromatic protons (Fig. 4.3C & D). A greater T_1 decline for New Hampshire HA occurred for aromatic protons with increasing concentration, while aliphatic protons exhibited only slight changes. These results may be caused by differences in overall negative charges and different functional group proportions of the two HAs. It is well known that HAs are pH-dependent polyelectrolytes. According to the structure of HA proposed by Schulten and Schnitzer (1997), the majority of carboxyl groups are connected to aromatic rings. More negative charges may be developed on aromatic moieties by deprotonation of carboxyl and phenol groups in alkaline conditions than for aliphatic functionalities. Thus, it is plausible to suggest that such negatively charged aromatic regions may repel one another more strongly and become more expanded than aliphatic moieties that are efficiently packed and have lower negative charge density. Thus the aliphatic moieties may experience less of a concentration-dependent effect on T_1 , while aromatic protons are more sensitive to HA concentration changes.

4.4.2 Solvent effects

Aromatic protons had much longer T_1 in 0.5 M NaOD than in DMSO, but similar values were observed for aliphatic protons in both the solvents (Fig. 4.4). These results indicate that HA likely has different conformations in the two solvents. Because spin-lattice relaxation involves magnetization transfer between nuclear spins and the lattice, the alteration of proton T_1 usually reflects changes of the environment around nuclear spins, often in terms of fluctuations of interproton distance (Bax and Davis, 1985). As discussed above, in 0.5 M NaOD the interproton distance for aromatic protons might be much larger than that for aliphatic protons due to the charge repulsion on deprotonated aromatic components. Accordingly, aromatic protons had much longer T_1 , inferring that aromatic moieties may have higher mobility than aliphatic components in alkaline solution. Because DMSO is a dipolar solvent, its polar face can associate with the phenolic and carboxyl groups of HA (mostly aromatic fragments) while its non-polar face can combine with the hydrophobic moieties of HA (aliphatic components) (Hayes, 1997). Consequently, aromatic and aliphatic segments can be linked together through DMSO molecules. In this case, protons from different functional groups may have similar mobility, as evidenced by similar T_1 values in d_6 -DMSO.

4.4.3 Temperature effect

In theory, the dipolar relaxation rate ($1/T_1$) decreases exponentially with increasing temperature in extreme narrowing limit region (Wehrli et al., 1988). Our results demonstrate that protons of methyl or methylene/methine groups (chemical shifts at 0.8 ppm and 1.2 ppm, respectively) had slightly longer T_1 at higher temperature

in both DMSO and 0.5 M NaOD (Fig. 4.5), indicative of increased mobility of such moieties at elevated temperatures. These results are consistent with the theory: elevated temperatures increase molecular mobility. However, for protons associated with oxygen-substituted aliphatic and aromatic groups, T_1 values decreased with temperature in both solvents, except for aromatic protons of New Hampshire HA in 0.5 M NaOD.

Free radicals may contribute to the T_1 decline in aromatic and oxygen-substituted aliphatic regions. Radicals are very active and can speed up spin-lattice relaxation process (Wehrli, et al., 1988; Steelink, et al., 1989), resulting in a large decrease of T_1 . It is well documented that humic substances contain significant quantities of free radicals (Senesi and Schnitzer, 1977; Cheshire and McPhail, 1996). A temperature increase can release more free radicals by breaking relatively weak bonds in a molecule (Perkins, 1994). It has been reported that elevated temperature led to increased densities of radicals in a solid humic acid (Steelink and Tollin, 1962; Senesi and Steelink, 1989). Therefore, more radicals could be formed on aromatic moieties as the temperature was increased from 20 to 60 °C, resulting in reduced T_1 values for protons on these fragments. The exception that New Hampshire HA showed an increased T_1 in the aromatic region in 0.5 M NaOD may be related to lower carboxylic contents than Amherst HA (Mao et al., 2000). A previous study found a positive relationship between radical concentrations and carboxylic content in peat HA (Shinozuka et al., 2001). Fewer radicals would exert less impact on proton T_1 of the aromatic region of New Hampshire HA. On the other hand, as suggested above, aromatic moieties became more flexible in 0.5 M NaOD than in d_6 -DMSO due to the development of negative charge, which would lead to T_1 increase. Both radical and

negative charge mechanisms might be involved in the reaction but eventually the effect of negative charge took over that of radicals. Similarly, as the temperature increased, oxygen-containing aliphatic groups might produce radicals or be easily accessed by small free radicals resulting in the decrease of T_1 . Nevertheless, the exact mechanism is unknown. It is noted that T_1 values in the temperature dependence experiments can not be compared with those in the other experiments at room temperature because they were performed at different magnetic field strengths.

4.4.4 pH influence

Amherst HA and New Hampshire HA demonstrated dissimilar proton relaxation patterns in deuterated alkaline solution as the pH was decreased (Fig. 4.6). For New Hampshire HA, all proton T_1 at each chemical shift decreased with pH. This is not surprising because molecules tend to aggregate due to neutralization of negative charge at lower pH, and consequently the tumbling rate slows down as the aggregates grow in size, which causes decreased T_1 . On the contrary, T_1 for aliphatic and oxygen-containing aliphatic protons of Amherst HA increased as the pH decreased, while T_1 for aromatic protons decreased with pH. Because Amherst HA has higher carboxylic content than New Hampshire HA, its aromatic fragments might carry higher negative charge density in alkaline solution. Therefore, aromatic moieties in Amherst HA might have a more expanded conformation. Correspondingly, the aliphatic and oxygen-containing aliphatic groups of Amherst HA might be in a more compacted state. As negative charges are gradually neutralized by decreasing pH, the intensity of repulsion decreases. Then, aromatic moieties could be closer to one another, resulting in a T_1

decrease. Meanwhile, relatively compacted aliphatic groups might become more expanded at lower pH, as illustrated by the T_1 increase.

4.5 Conclusion

Spin-lattice relaxation time is an important NMR parameter and a useful approach for molecular dynamics studies. For the two humic acids examined, proton T_1 ranged from 0.3 to 0.8 s in d_6 -DMSO, and from 0.26 to 2.3 s in 0.5 M NaOD at 300 MHz. Aliphatic protons had similar T_1 values in both solvents, but aromatic protons exhibited much longer T_1 in 0.5 M NaOD than in d_6 -DMSO, indicative of different conformations of humic acid molecules in the two solvents. All three environmental conditions: HA concentration, temperature, and pH, affected the proton T_1 values of HAs. The variation of proton T_1 values was a result of molecular mobility changes and the production of radicals at higher temperatures. Different HA might have dissimilar T_1 variations probably due to different functional group compositions. The proton T_1 ranges of HAs in solvents and their changes with environmental conditions provide a useful reference for NMR spectroscopy of HAs, and may facilitate the interpretation of the configuration and segmental mobility of HAs, leading to better understanding of their behavior in natural environment.

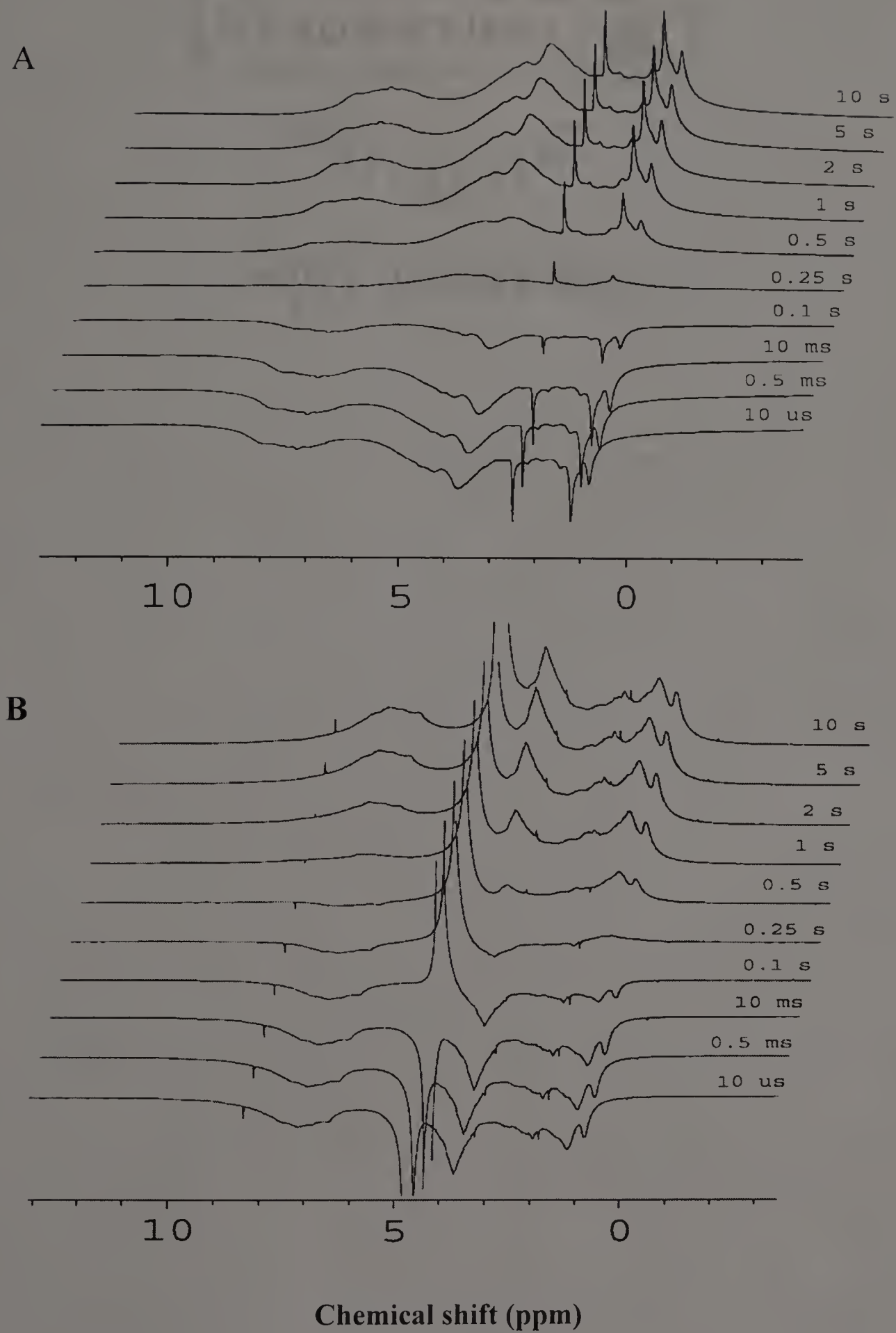


Figure 4.1. Proton stack NMR spectra of Amherst humic acid in d_6 -DMSO (A) and in 0.5 M NaOD (B) as a function of the delay time (τ).

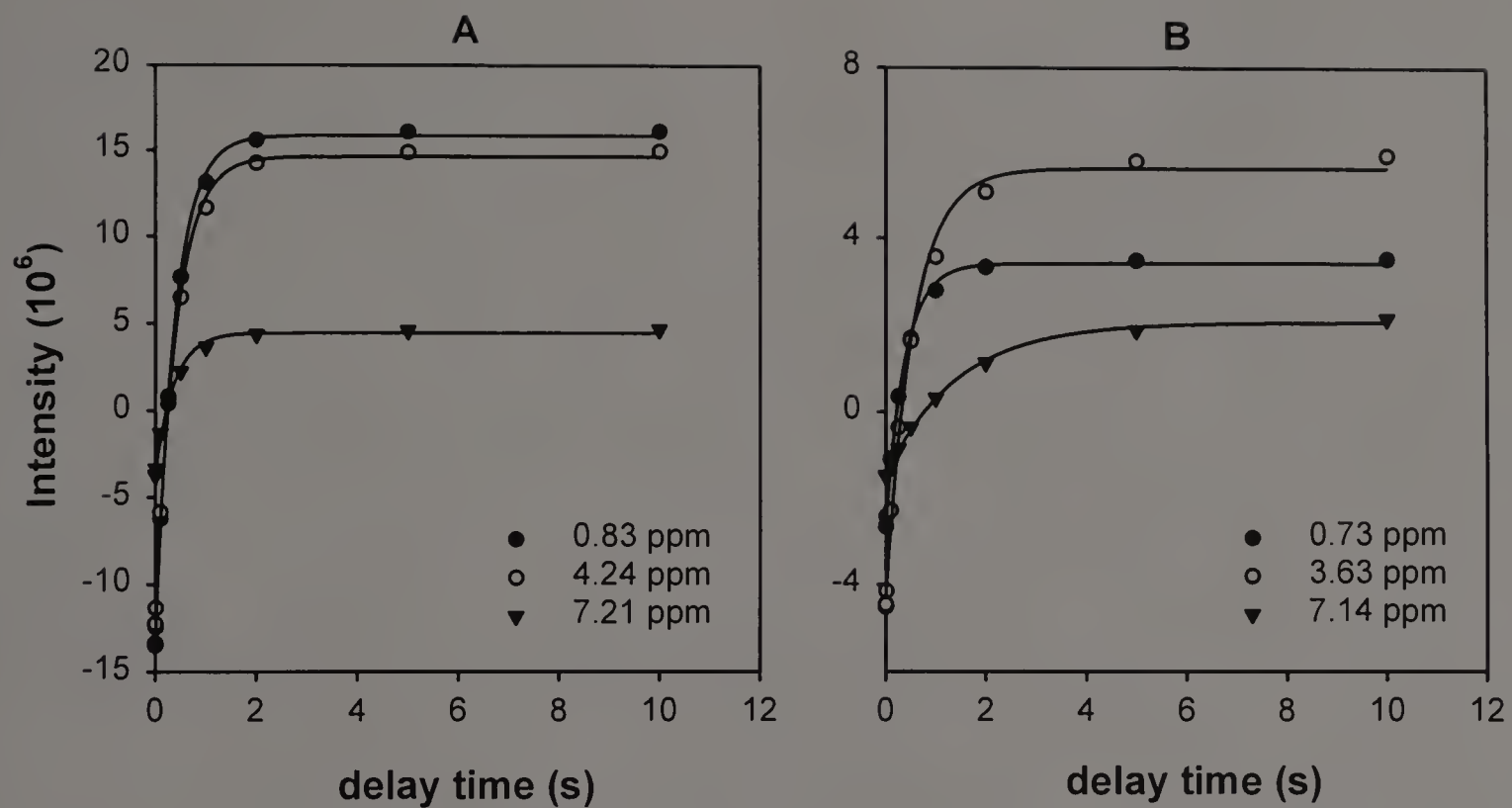


Figure 4.2. Signal intensity recorded at different delay times at different chemical shifts for Amherst humic acid in d_6 -DMSO (A) and in 0.5 M NaOD (B).

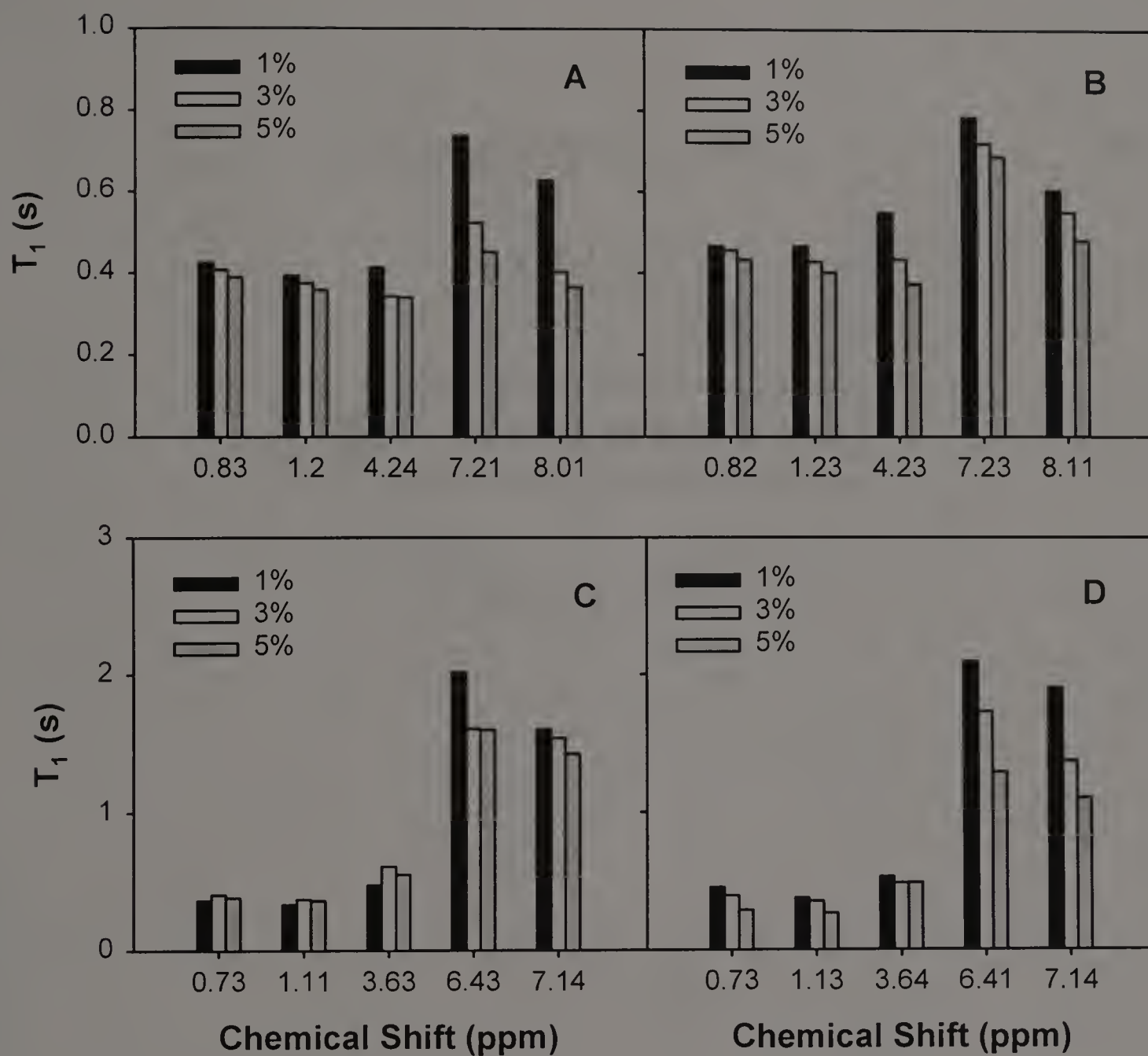


Figure 4.3. Changes of proton T_1 with HA concentration at different chemical shifts: Amherst HA in d_6 -DMSO (A); New Hampshire HA in d_6 -DMSO (B); Amherst HA in 0.5 M NaOD (C); New Hampshire HA 0.5 M NaOD (D).

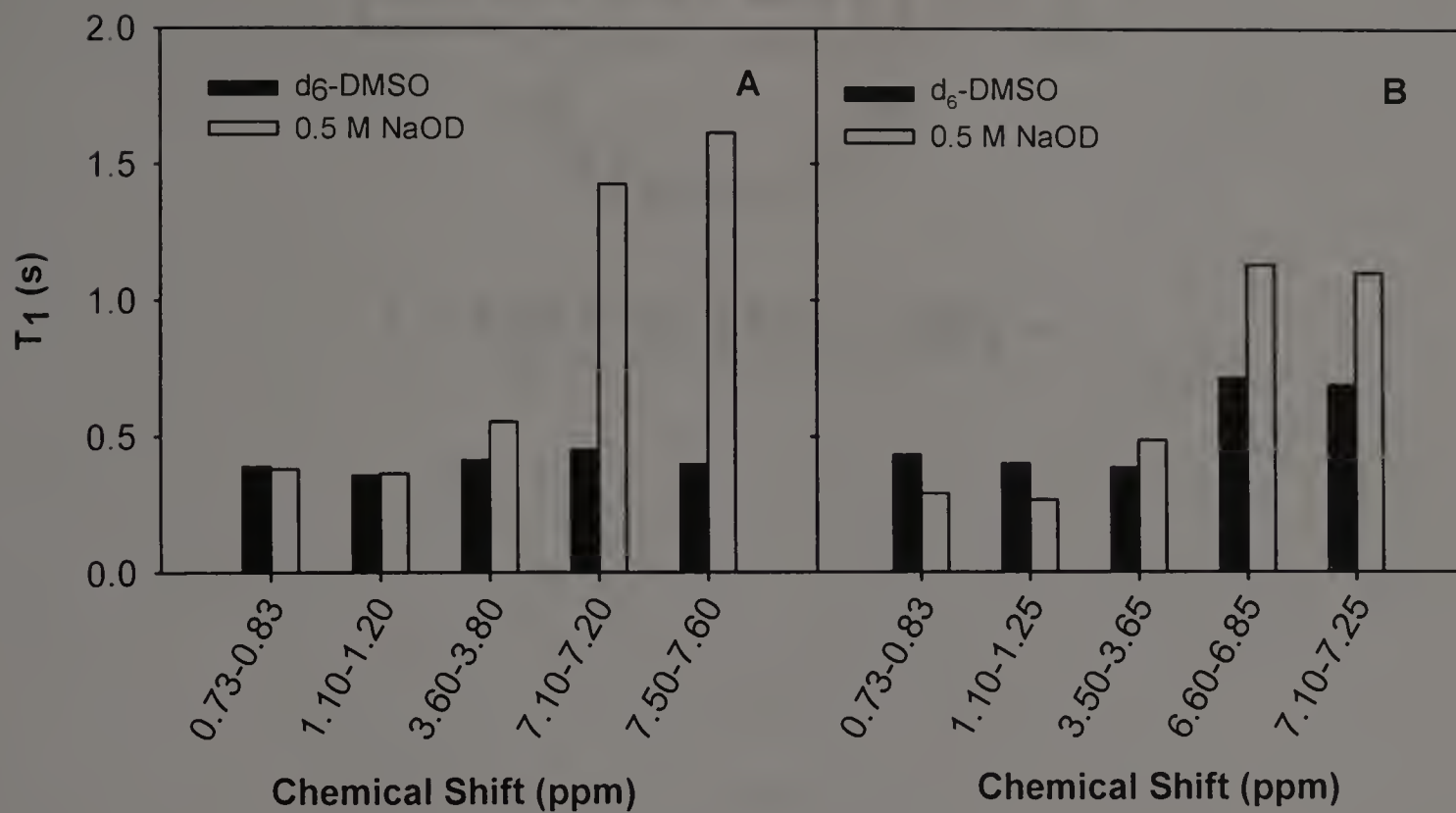


Figure 4.4. Proton T₁ of Amherst HA (A) and New Hampshire HA (B) at a concentration of 5% (w/w) in d₆-DMSO and 0.5 M NaOD at different chemical shifts.

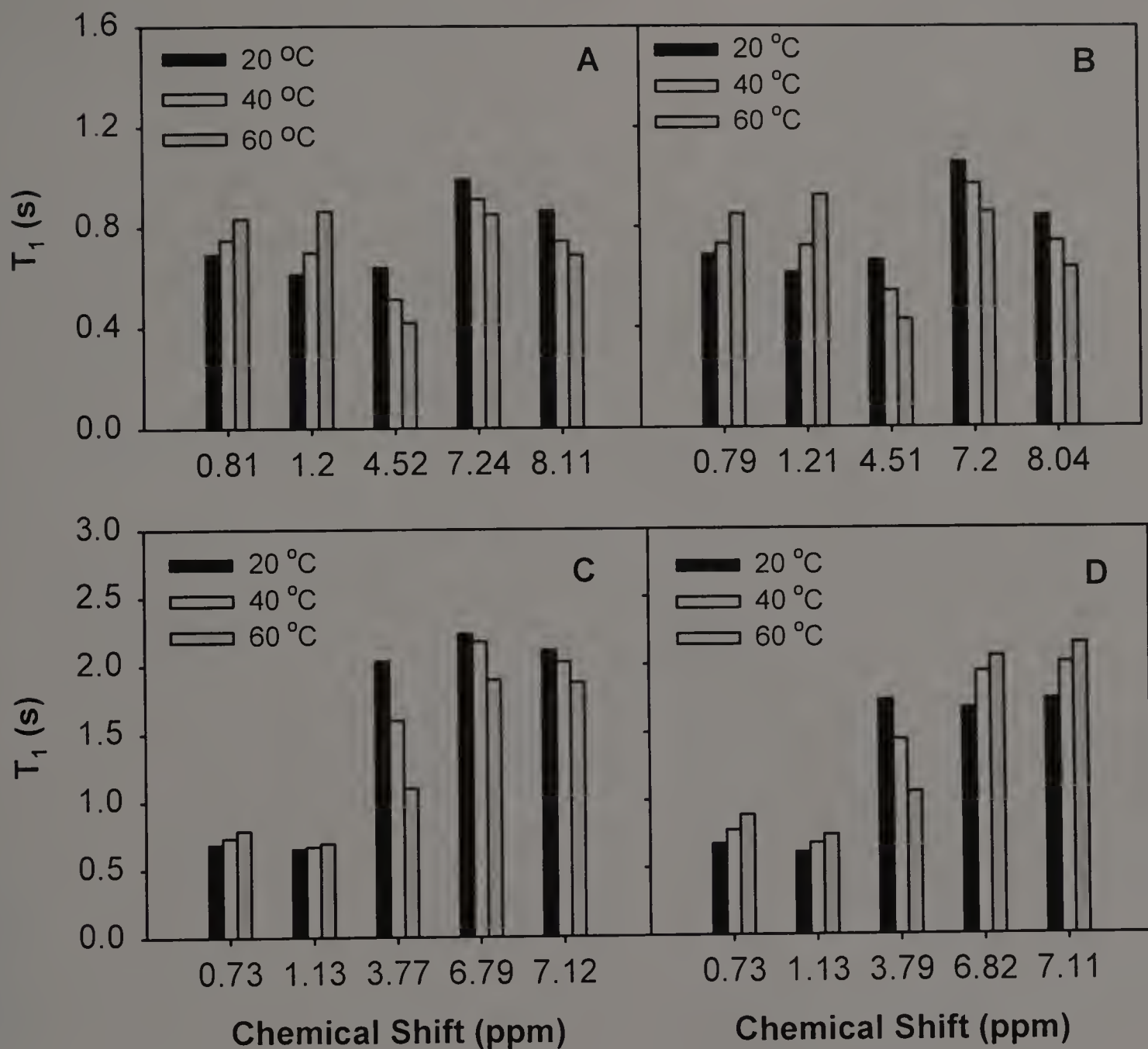


Figure 4.5. Changes of proton T_1 of humic acids with temperature at different chemical shifts: Amherst HA in d_6 -DMSO (A); New Hampshire HA in d_6 -DMSO (B); Amherst HA in 0.5 M NaOD (C); New Hampshire HA 0.5 M NaOD (D).

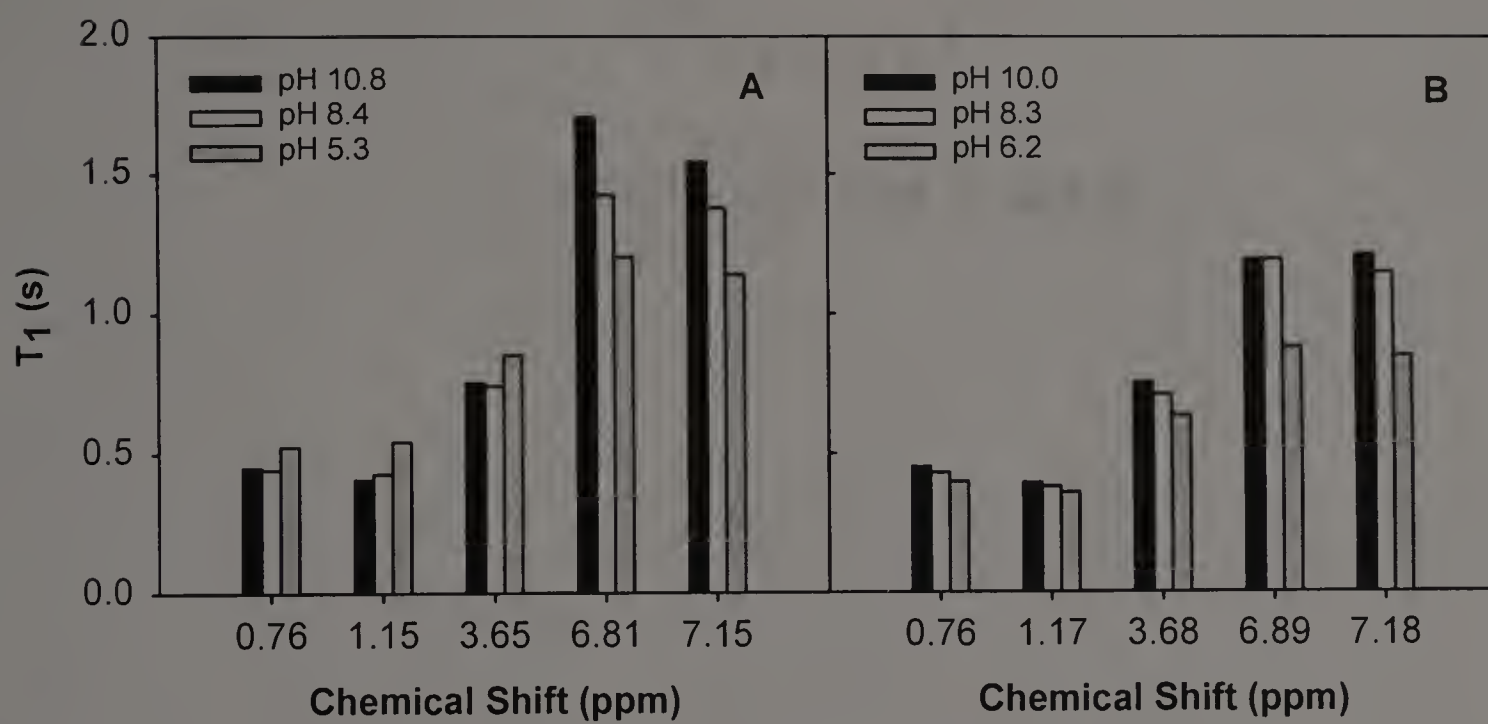


Figure 4.6. Proton T_1 of Amherst HA (A) and New Hampshire HA (B) at different pH and chemical shifts in deuterated alkaline solution.

CHAPTER 5

PROTON CORRELATION TIMES AND SEGMENTAL MOBILITY OF HUMIC ACIDS IN TWO SOLVENTS ¹

5.1 Abstract

Molecular dynamics of humic acid in solution are rarely investigated. Thus, the objective of this work is to determine proton correlation time and segmental mobility of humic acids in solution. Proton spin-lattice relaxation time (T_1) was determined for HAs in DMSO- d_6 and 0.5 M NaOD at NMR frequencies of 300 MHz and 600 MHz, then the correlation times were estimated for aliphatic protons, protons associated with oxygen-containing functionalities, and aromatic protons. Results suggest that proton correlation times of humic acids in solvents were on the order of 10^{-10} second, which were near the T_1 minimum at 600 MHz. Carbohydrate was identified as the largest and the most immobile components in humic acids, while aliphatic and aromatic fragments were relatively smaller and more mobile. The relaxation approach is helpful for investigating the segmental mobility of humic acids in aqueous solution.

5.2 Introduction

Understanding the relative mobility of different components of HS is necessary to discern their functions in the environment. Conventionally, HS are believed to be macromolecules and their aromatic moieties are relatively more rigid than aliphatic

¹ A version of this chapter has been published.
Kaijun Wang, and Baoshan Xing. 2004. Soil Sci. 168: 128-136.

groups in HS (Chien and Bleam, 1998; Xing, 2001b). It has also reported that HS consist of domains with different mobility (Hu et al., 2000; Xing and Chen, 1999).

NMR relaxation studies, i.e. molecular correlation time, have emerged as a useful approach for investigating molecular motions in solution. But, only a few studies have used these techniques to explore the segmental mobility of HS. In the case of solid-state NMR, it has been suggested that aliphatic moieties are the least mobile and probably largest while the carbohydrate and aromatic moieties are more mobile and smaller (Cook and Langford, 1998). Smernik and Oades (2000) also reported that more rapid relaxation was observed for the aromatic and carbonyl protons than for the aliphatic protons. In the case of liquid-state NMR, aromatic carbons were found to have similar mobility with aliphatic carbons because these two types of carbon had similar T_1 values (Newman and Tate, 1984).

Because HS are conventionally thought to be macromolecules, they are expected to be in the slow motion regime ($\omega\tau_r \gg 1$) and their carbon correlation times are predicted to be on the order of 10^{-9} s (Steelink et al., 1989). However, the proton or carbon correlation times of HS in solution have not been determined and their slow motional regime has not been confirmed. Thus, the objectives of this study were to investigate the proton correlation times of HAs and to estimate their segmental mobility in different solvents.

5.3 Materials and Method

5.3.1 Materials

Three humic acids were used in this study. Amherst HA was extracted from Amherst peat soil, IHSS Florida HA from Florida peat soil, and New Hampshire HA was from New Hampshire peat soil. Extractions followed the standard procedures as outlined by International Humic Substances Society (IHSS) (Swift, 1996). Elemental compositions of humic acids were determined by the Microanalytical Laboratory at the University of Massachusetts, Amherst. Amherst HA contains 54.6% C, 4.16% H, 38.5% O, 2.46% N, and 0.24% ash; IHSS Florida HA: 55.5% C, 3.83% H, 37.1% O, 3.61% N, and 0.09% ash; and New Hampshire HA: 52.9% C, 5.40% H, 39.7% O, 2.00% N, and 0.25% ash. The functional group composition of Amherst HA as determined by solid-state NMR is 17.3% aliphatic carbon, 25.0% carbohydrate carbon, 40.8% aromatic carbon, and 16.9% carboxylic carbon; while the corresponding values for New Hampshire HA and IHSS Florida HA are 17.0%, 31.6%, 39.0%, 12.4%; and 16.9%, 21.4%, 40.3%, 21.4%, respectively (Mao et al., 2000). Deuterium oxide (D_2O), deuterated sodium hydroxide solution (NaOD, 30% in D_2O) and dimethylsulfoxide (d_6 -DMSO) were purchased from Cambridge Isotope Laboratories, Inc. (Massachusetts). All reagents were used without further purification.

5.3.2 NMR Spectroscopy

Humic acid samples were prepared at a concentration of 5% (w/w) in 0.5 M NaOD/ D_2O and d_6 -DMSO. Because no precipitate was observed, we believed that the HAs were fully dissolved in the solvents. Relatively high HA concentration was used to obtain higher signal to noise ratios in order to shorten experiment time. The proton

correlation time is usually estimated by measuring relaxation times (T_1 , T_2 or both) (Fung et al., 1976; Carper and Keller, 1997). In this study, correlation time was estimated by measuring the ratio of spin-lattice relaxation rates ($1/T_1$) at two frequencies, 300 MHz and 600 MHz.

The T_1 was measured by the commonly used inversion recovery pulse sequence (PD - 180° - τ - 90° - AT)_n. The delay time, τ , between the two pulses was the variable parameter, which was set at a different fixed value for each spectrum. The ^1H 90° and 180° pulse lengths were 9.25 μs and 18.5 μs , respectively, the number of scans was 64, and the recycle delay was 10 seconds. Proton spectra were recorded automatically from short delay to long delay at 10 different τ : 10 μs , 500 μs , 10 ms, 100 ms, 250 ms, 500 ms, 1 s, 2 s, 5 s, and 10 s. A preliminary test showed that this time range was suitable for proton T_1 measurement. Tetramethylsilane (TMS) was used as an internal chemical shift standard for samples dissolved in d_6 -DMSO and as an external reference for those dissolved in 0.5 M NaOD/D₂O. A Quad-Nucleus probe was used on Bruker DPX300 and TXI (triple nucleus) probe was used on Bruker Avance 600. Experiments were performed on undegassed samples at 20 °C because the proton relaxation times of humic acids are less than 3 s according to our previous work, and in such cases the presence of dissolved oxygen is not expected to contribute significantly to relaxation (Ravindranathan, 1996).

5.3.3 Correlation Time Calculation

By taking the ratio of Solomon equation at two frequencies, namely 300 and 600 MHz in the present study, and rearranging, we can obtain the following equation:

$$\frac{T_1(600)}{T_1(300)} = \frac{\left(\frac{5 + 8\omega^2 \tau_r^2}{1 + 5\omega^2 \tau_r^2 + 4\omega^4 \tau_r^4} \right)_{300 \text{ MHz}}}{\left(\frac{5 + 8\omega^2 \tau_r^2}{1 + 5\omega^2 \tau_r^2 + 4\omega^4 \tau_r^4} \right)_{600 \text{ MHz}}} \quad (5.1)$$

Hence, from knowledge of the T_1 values at 300 MHz, $T_1(300)$, and the corresponding values at 600 MHz, $T_1(600)$, τ_r for any given proton in one-dimensional ^1H spectra can be evaluated. Such approach has been adopted by Kotowycz et al. (1980) who evaluated the dipolar correlation times for the different type of protons in vitamin D derivatives, and by Fung et al. (1976) who assessed the segmental mobility and conformation of enzyme-bound propionyl coenzyme A. Because proton T_1 is always greater at higher Larmor frequency unless magnetic strength has no effect on T_1 (for example, small molecules in extremely narrow limit range have same T_1 at different Larmor frequencies), the minimum value of $T_1(600)/T_1(300)$ is 1. Equation 5.1 yields the scattered plot of Figure 5.1 by numerically plugging in a series of T_1 ratios between 1 and 3. Regressing on these data points gave a simpler equation of τ_r as a function of $T_1(600)/T_1(300)$. Then all proton τ_r were directly estimated from this standard curve.

5.4 Results and discussion

One-dimensional ^1H NMR spectra are shown in Figure 5.2. It is conventional to partition the spectra into three regions (Chien and Bleam, 1998): 0.8-3.0 ppm, representing aliphatic protons; 3.0-5.5 ppm, protons associated with oxygen-containing functionalities, mostly carbohydrate protons (Hatcher et al., 1980); and 6.0-8.5 ppm, aromatic protons. The spectra of New Hampshire HA and Amherst HA were calibrated

by the first peak's chemical shift in the spectrum of IHSS HA because the chemical shifts of the first peak for the three HAs were almost identical. Figure 5.2 incorporated the spectra of New Hampshire HA and Amherst HA without chemical shift reference.

For each chemical shift tested in this study, relaxation curves (peak intensity vs. delay time) were approximately exponential with $R^2 > 0.995$ in both solvents as illustrated in Figure 5.3 with IHSS Florida HA as an example. Three resonances each from the three chemical shift regions were picked to show the variation of intensity with delay time. The fitted exponential equation is:

$$I[t] = I[0] + P \times e^{-\frac{t}{T_1}} \quad (5.2)$$

where $I[t]$ is the intensity at $\tau = t$, $I[0]$ is the intensity at $\tau = 0$, and P is a constant. The T_1 measurement software automatically calculated T_1 value based on equation 5.2. The uncertainties in T_1 measurement, as indicated by the standard deviation of estimated T_1 derived from the regression, were less than 5% in most cases.

Overall, the proton T_1 was in the order of a second for all three humic acids at a concentration of 5% (w/w). At a Larmor frequency of 300 MHz, the magnitude of proton T_1 ranged from 0.3 s to 0.9 s in DMSO- d_6 and from 0.26 s to 2.1 s in 0.5 M NaOD (Figure 5.4, IHSS Florida HA was used as an example). These proton T_1 values were consistent with results in Chapter 4. However, proton T_1 of humic acids in both solvents exhibited a strong dependence on NMR frequency. The magnitudes of proton T_1 at 600 MHz were from 0.6 s to 1.5 s in DMSO- d_6 and from 0.6 s to 3.5 s in 0.5 M NaOD, roughly 1.5 times longer than that at 300 MHz. Theoretically, molecules in fast motion limit ($\omega\tau_r \ll 1$) have little, if any, frequency dependency of T_1 . Our results

suggest that humic acids in DMSO-d₆ or alkaline solution are not in the fast motion limit ($\omega\tau_r \ll 1$).

The calculated values of τ_r (Table 5.1 and 5.2) demonstrate that proton correlation times were in the order of 10^{-10} s within a range between 0.95 and 2.47×10^{-10} s in DMSO-d₆ and between 1.05 and 6.04×10^{-10} s in 0.5 M NaOD. At the NMR frequency of 600 MHz, the magnitude of $\omega\tau_r$ is in the range of 0.3 to 2.3, and in most cases the value is less than 1. More specifically, all values of proton $\omega\tau_r$ were less than 1 in DMSO-d₆. But in 0.5 M NaOD, protons τ_r exhibited relatively larger variation depending on the proton chemical shift range and individual humic acids. Aromatic protons of the three humic acids and aliphatic protons of Amherst HA and IHSS Florida HA had $\omega\tau_r$ value less than 1, while the $\omega\tau_r$ values of aliphatic and carbohydrate protons of New Hampshire HA were greater than 1. For most carbohydrate protons of Amherst HA and IHSS Florida HA, the magnitudes of $\omega\tau_r$ were close to 1 although only a few of them were beyond 1. Nevertheless, all the proton $\omega\tau_r$ fall close to 1, suggesting that the molecular motion of humic acids in both solvents was around T_1 minimum.

Individual components of large molecules in solution may have different mobility. The proton rotational correlation time has shown to be a useful indicator of molecular or segmental mobility (Fung et al., 1976). In this study, three signals from each chemical shift region were selected for the calculation of proton correlation times. The selected chemical shifts correspond to peaks or small bump/shoulders in the spectra. As shown in table 1, protons from carbohydrate components had longer τ_r than aliphatic and aromatic protons in DMSO-d₆. This suggests that the carbohydrate fragments may be relatively less mobile than aliphatic and aromatic components. Since

τ_r is positively related to molecular size (Levitt, 2001), the results suggest that in DMSO- d_6 carbohydrate components might be larger than aliphatic and aromatic segments for the humic acids investigated. Simpson et al. (2001c) also reported that carbohydrate was the biggest moieties in a DOSY study of a fulvic acid in DMSO- d_6 . Protons at 1.4 ppm exhibited longer τ_r than protons at 0.8 ppm and 1.2 ppm. This result is reasonable because protons at 1.4 ppm are from long chain methylene groups while protons at 0.8 ppm and 1.2 ppm are from methyl groups and short chain aliphatic components or terminal methyl groups like $\text{CH}_3\text{-C-O}$, respectively (Simpson et al., 1997). Terminal groups usually have higher mobility than internal groups (Evans, 1995; Friebolin, 1998). Moreover, aliphatic and aromatic components provided mixed information on τ_r . For IHSS Florida HA and Amherst HA, aliphatic species at 0.8 ppm and 1.2 ppm had slightly higher mobility than aromatic moieties. But for New Hampshire HA, aromatic components were more mobile as indicated by the shorter τ_r (Table 5.1).

For humic acids in 0.5 M NaOD, carbohydrates had even longer τ_r than aliphatic and aromatic segments. Meanwhile, all three humic acids exhibited relatively longer τ_r in aliphatic components than in aromatic range. These results indicate that carbohydrates might be the biggest and the least mobile segments in humic acids followed by aliphatic components, and the aromatic moieties are the most mobile and smallest in 0.5 M NaOD. Compared with the corresponding values of proton correlation times in DMSO- d_6 , carbohydrate protons had substantial longer τ_r in 0.5 M NaOD, indicating that carbohydrate moieties might be even more immobile and bigger in

alkaline solution. In addition, aliphatic protons of New Hampshire HA and IHSS Florida HA also had relatively longer τ_r in 0.5 M NaOD than in DMSO-d₆.

Signal intensity loss was observed in T_1 measurement, especially for humic acids in 0.5 M NaOD (Fig. 5.3B). Only about 50% of full intensity (in negative direction) for humic acids in 0.5 M NaOD was obtained at the first delay time (10 μ s). This remarkable intensity loss at such a short delay time indicates that quite a few molecules have very short T_1 in 0.5 M NaOD and they have reached equilibrium after excitation within 10 μ s. Paramagnetic materials, including paramagnetic elements and radicals, have been shown to decrease relaxation time and eventually cause signal loss (Smernik and Oades, 2002). But they were unlikely to be the main reason because little intensity loss, if any, was observed for the samples in DMSO-d₆ (Fig. 5.3A). Another possibility is that 0.5 M NaOD may be less efficient to separate humic acid molecules than DMSO-d₆. As a result, some molecules might still be associated as aggregates and such aggregates might behave as solid and have a very short T_1 . Further study is necessary to unveil this phenomenon.

The determination of correlation time is a useful approach to explore the segmental mobility of humic acids in solutions. However, calculations and interpretations were restricted to the assumption that the relaxation is due to the dipole-dipole mechanism. If other mechanisms, such as scalar coupling, chemical shift anisotropy, or spin-rotation, are involved in the process, Equation 1 can no longer be used. Because the dipole-dipole interaction is the most important mechanism, it is usually the dominant relaxation mechanism for proton spin-lattice relaxation in solutions (Van der Maarel, et al., 1987; Carper and Keller, 1997). Thus, the results of T_1

and τ_r based on the assumption of dipole-dipole interaction would still be meaningful. Another consideration is that some uncertainty is unavoidable in the calculation of τ_r due to the overlap of spectra. Specifically, we are not quite confident that the signal we picked at two frequency levels are from the same function group even though we kept the chemical shift at the exact same value. Thus, the calculated τ_r may not represent exactly the mobility of the given specific function group. Fortunately, similar chemical shifts often have close T_1 values as illustrated in Figure 5.4, which limits the uncertainties in determination. Therefore, the τ_r values are still valid to compare the overall mobility of components from different chemical shift regions.

5.5 Conclusion

Relaxation study can provide useful domain dynamic information of HAs in solution. Our study suggests that proton correlation time is a useful approach to investigate the segmental mobility of HAs in solvents. However, proton T_1 cannot be used as an absolute indicator of segmental mobility as shown in this study that proton $\omega\tau_r$ of structural components of HAs were close to 1 (T_1 minimum). We estimated proton correlation times of HAs by determination of spin-lattice relaxation time at two different NMR frequencies, 300 MHz and 600 MHz. The proton correlation times of HAs were in the order of 10^{-10} s and ranged between 0.95 and 2.47×10^{-10} s in DMSO- d_6 and between 1.05 and 6.04×10^{-10} s in 0.5 M NaOD. Results reveal that the molecular motion of HAs in solution is around T_1 minimum. Carbohydrate was identified as the largest and the least mobile components, while aliphatic and aromatic species were relatively small and mobile. Moreover, aromatic moieties were found to be the most

mobile and the smallest in 0.5 M NaOD, but in DMSO their correlation times were relatively shorter or longer than aliphatic groups depending on individual HAs. Since molecular mobility of HAs may have great influence on their reactivities, a knowledge of segmental mobility will help us better understand their behaviors in natural environments.

Table 5.1. Proton correlation times (τ_r) of humic acids in DMSO-d₆ at a concentration of 5% (w/w)

New Hampshire HA			IHSS Florida HA			Amherst HA		
Chemical shift (ppm)	τ_r ($\times 10^{-10}$ s)	$\omega\tau_r$ at 600 MHz	Chemical shift (ppm)	τ_r ($\times 10^{-10}$ s)	$\omega\tau_r$ at 600 MHz	Chemical shift (ppm)	τ_r ($\times 10^{-10}$ s)	$\omega\tau_r$ at 600 MHz
0.82	1.55	0.58	0.82	1.38	0.52	0.82	1.79	0.67
1.22	1.43	0.54	1.22	1.44	0.54	1.23	1.74	0.66
1.47	1.88	0.71	1.44	1.89	0.71	1.46	2.09	0.79
3.72	1.79	0.67	3.81	2.03	0.76	3.70	2.47	0.93
4.19	1.75	0.66	4.18	2.08	0.78	4.25	2.18	0.82
4.53	1.73	0.65	4.91	2.08	0.78	4.55	2.25	0.85
6.63	0.95	0.36	6.63	1.80	0.68	6.63	2.10	0.79
6.99	0.98	0.37	7.11	1.82	0.68	6.95	1.94	0.73
7.23	1.45	0.55	7.23	1.71	0.65	7.25	2.06	0.78

Table 5.2. Proton correlation times (τ_r) of humic acids in 0.5 M NaOD at a concentration of 5% (w/w)

New Hampshire HA			IHSS Florida HA			Amherst HA		
Chemical shift (ppm)	τ_r ($\times 10^{-10}$ s)	$\omega\tau_r$ at 600 MHz	Chemical shift (ppm)	τ_r ($\times 10^{-10}$ s)	$\omega\tau_r$ at 600 MHz	Chemical shift (ppm)	τ_r ($\times 10^{-10}$ s)	$\omega\tau_r$ at 600 MHz
0.76	3.00	1.13	0.76	1.74	0.66	0.76	1.66	0.63
1.15	2.95	1.11	1.13	1.99	0.75	1.16	1.87	0.70
1.39	2.99	1.13	1.41	2.31	0.87	1.40	1.89	0.71
3.20	4.44	1.67	3.21	2.42	0.91	3.22	5.48	2.07
3.65	4.15	1.56	3.67	2.47	0.93	3.69	2.09	0.79
3.93	6.04	2.28	3.95	2.20	0.83	3.99	2.87	1.08
6.43	1.05	0.40	6.47	1.73	0.65	6.47	1.55	0.59
6.83	1.35	0.51	6.83	1.77	0.67	6.86	1.59	0.60
7.15	1.52	0.57	7.14	1.79	0.67	7.17	1.26	0.47

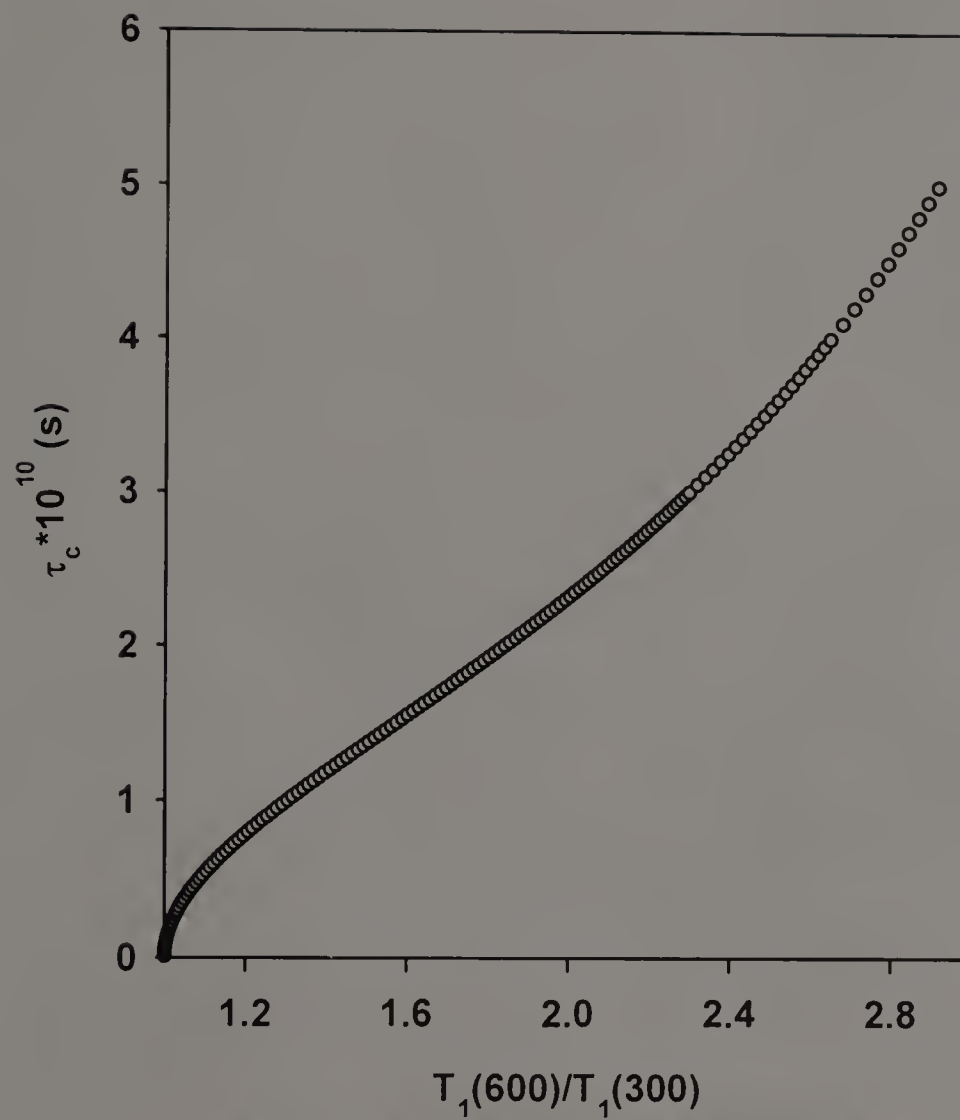


Figure 5.1. The proton rotational correlation time as a function of the ratio of spin-lattice relaxation time at two different frequencies: 600MHz and 300 MHz. The curve is generated from Equation 5.1 in the text.

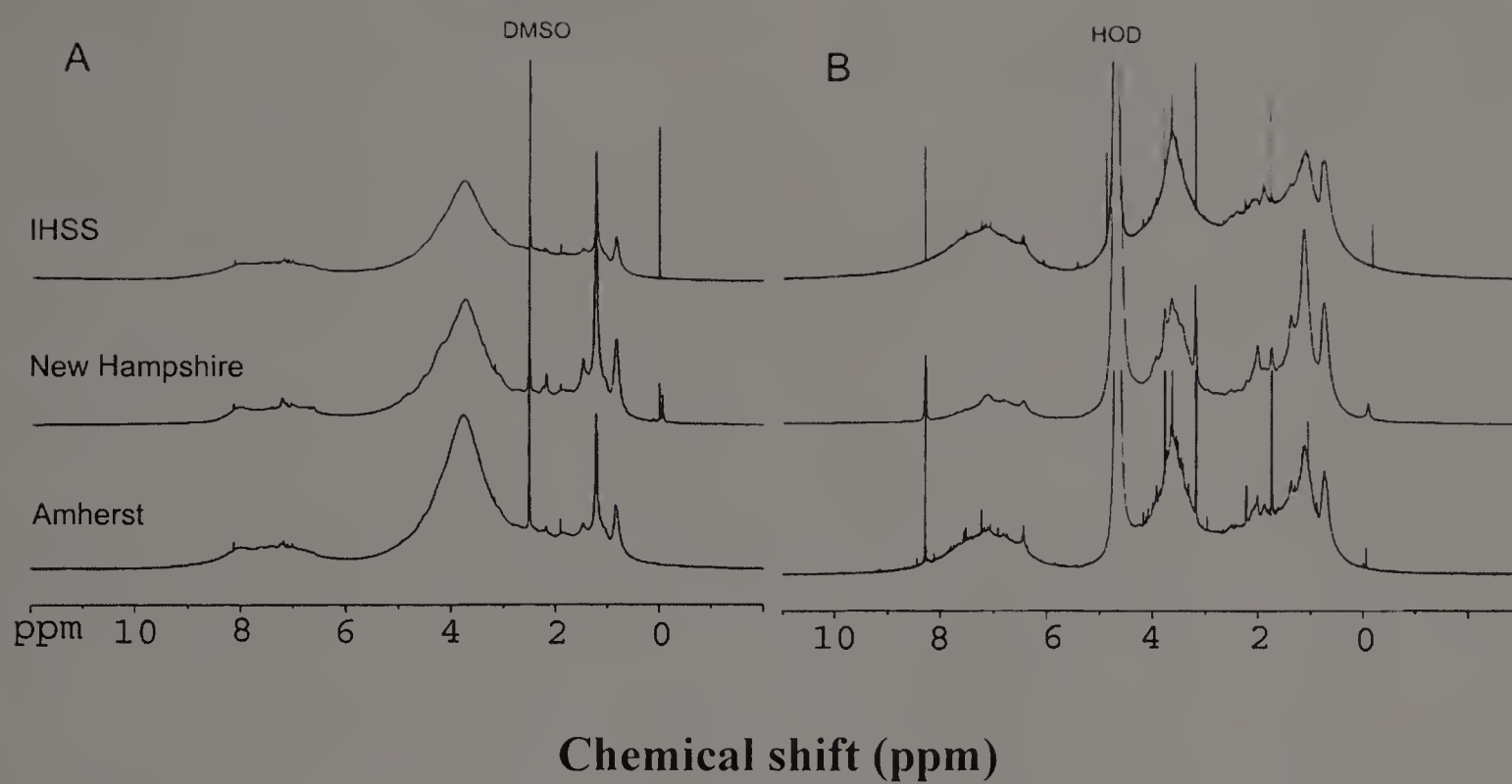


Figure 5.2. One-dimensional ^1H NMR spectra of humic acids in DMSO-d_6 (A) and 0.5 M NaOD (B) at 600 MHz.

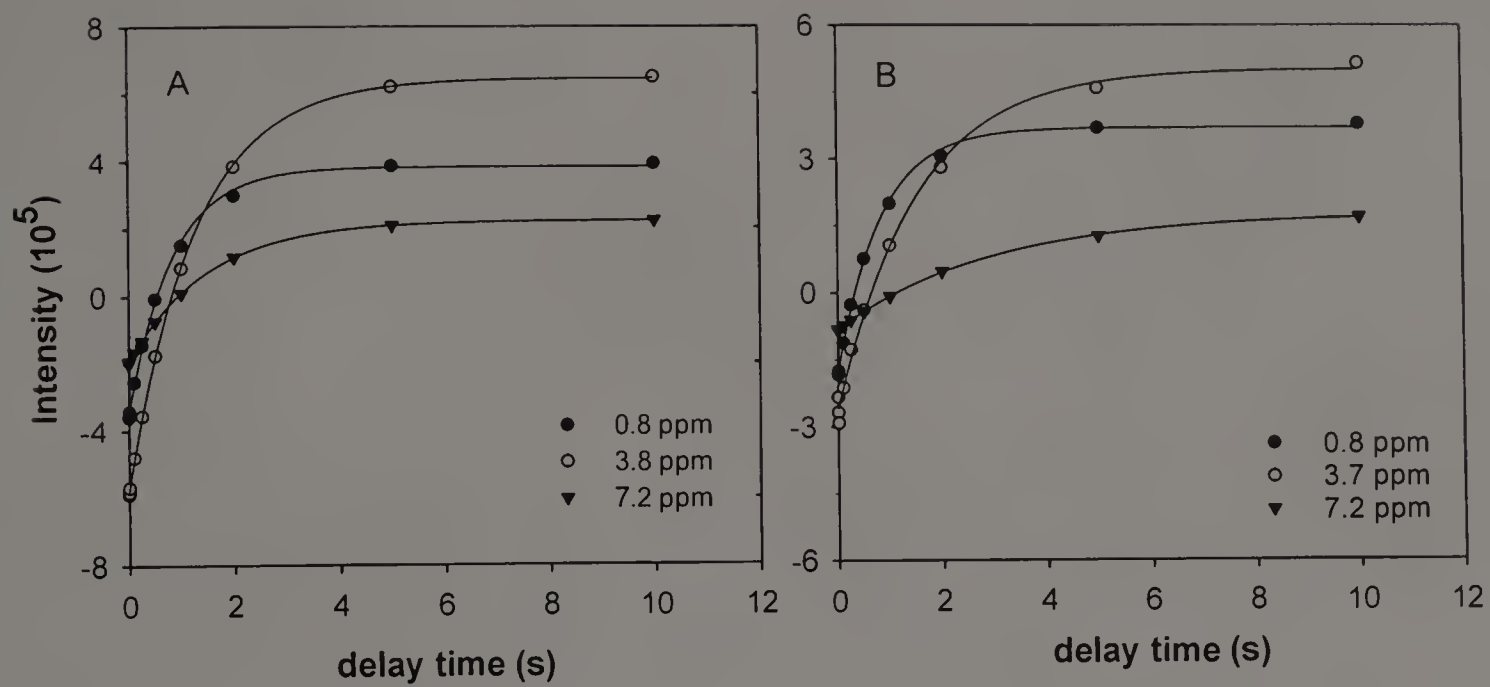


Figure 5.3. Signal intensity at different chemical shifts as a function of delay time at 600 MHz for IHSS Florida humic acid in DMSO-d₆ (A) and 0.5 M NaOD (B).

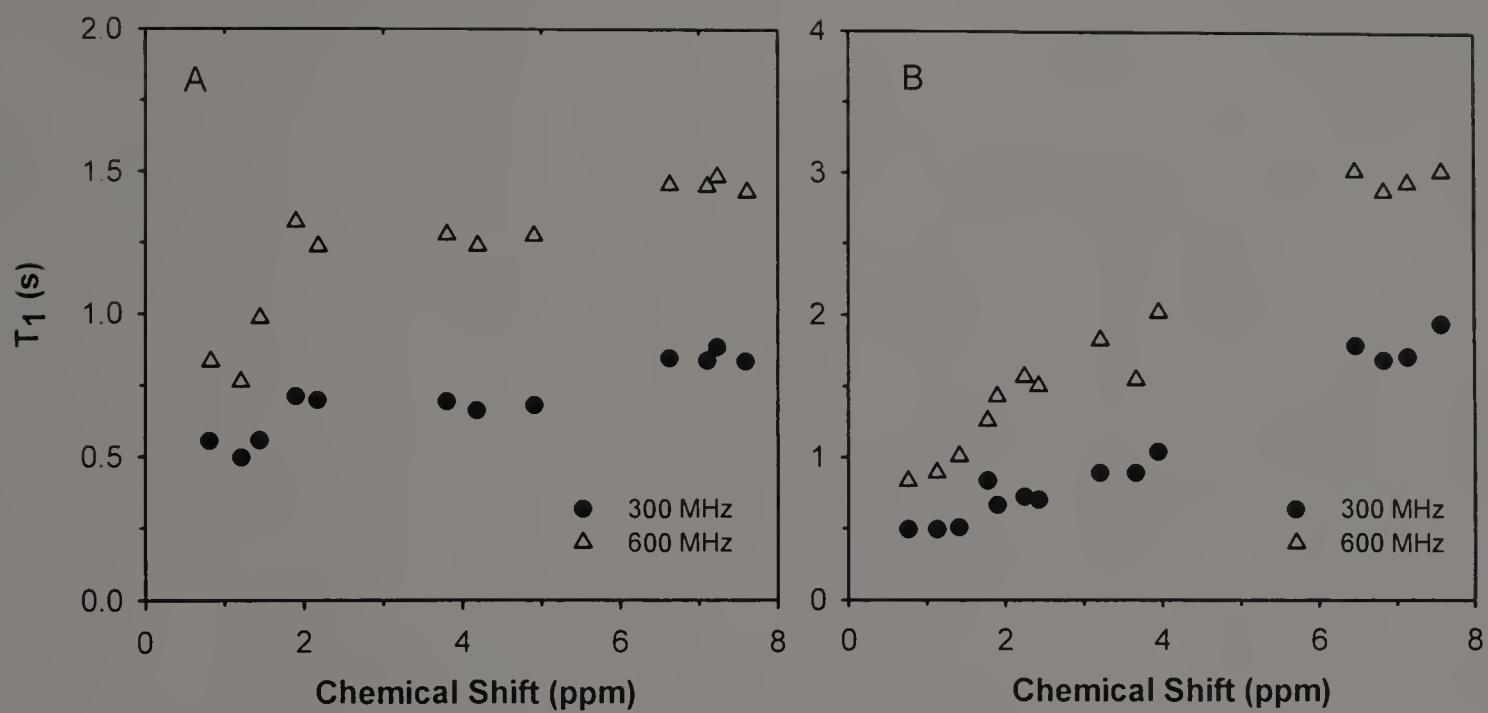


Figure 5.4. Proton spin-lattice relaxation time of IHSS Florida humic acid in DMSO- d_6 (A) and 0.5 M NaOD (B) at 300 MHz and 600 MHz.

CHAPTER 6

SYNTHESIS

The overall objective of my doctoral research was to advance understanding of interactions between organic matter and clay minerals in soils. Specifically, this research investigated the fractionation of humic substances upon adsorption by clay minerals and structural variations of humin samples extracted differently from soils. Moreover, the non-ideal sorption of hydrophobic organic compounds by clay-humic complex and humin was examined and the fragmental mobility of humic acid in solution was also explored using relaxation approach. Humic acids and humin from peat soils and phyllosilicate clay minerals were used in my dissertation. The minerals used in the study are commonly found in soils. Solid-state and liquid-state NMR (^{13}C and ^1H), UV-visible spectroscopy, and batch equilibration with liquid scintillation counting were employed in the experiments.

6.1 Summary

From the clay-humic complex investigation (Chapter 2), two main conclusions were reached. First, HA fractionation during adsorption on clay minerals was confirmed by direct NMR evidence. Aliphatic fractions of HA were preferentially adsorbed while aromatic fractions were major components of HA left in solution. Second, higher sorption linearity (N) and affinity (K_{OC}) for phenanthrene were observed for bound HA fractions as compared to the source HA. For example, the K_{OC} of adsorbed HA on

montmorillonite and kaolinite were up to several times higher than that of the source HA. Such high affinity might be contributed by low polarity of the bound HA as a result of fractionation. Chapter 3 examined the impacts of different extraction procedures on structure and sorption properties of soil humin. It was concluded that extraction procedures exerted substantial modifications of structural and sorption characteristics of humin samples even though they were from the same soil. Specifically, humin from 0.1 M NaOH exhaustive extraction and 6 M HF/HCl extraction at 60 °C had a relatively higher amount of aliphatic components than 1 M HF extracted humin. More than 50% carbohydrate components (50-108 ppm) were reduced from humin samples in 6 M HF/HCl extraction at 60 °C. Moreover, relatively more amorphous poly(methylene) domains were observed in 6 M HF/HCl extracted humin as compared with those extracted by other methods. As a consequence of structural modifications, the sorption linearity (N) and affinity (K_{OC}) for phenanthrene varied markedly among the humin samples. The NaOH exhaustively extracted humin had the most nonlinear sorption isotherms while the HF extracted humin had the lowest K_{OC} . Results suggest that both NaOH and NaOH-HF procedures were appropriate approaches for humin extraction. However, the extraction with 6 M HF/HCl at 60 °C should be discouraged for use due to structural modifications of humin both chemically and physically.

The relaxation investigation (Chapter 4) provided the proton T_1 ranges of HAs in solvents. Proton T_1 ranged from 0.3 to 0.8 s in d_6 -DMSO, and from 0.26 to 2.3 s in 0.5 M NaOD at 300 MHz. Protons from different functional groups differed in T_1 values in different solvents where aliphatic protons had similar T_1 values in both solvents, but aromatic protons exhibited much longer T_1 in 0.5 M NaOD than in d_6 -DMSO. In

addition, proton T_1 varied with HA concentration, temperature, and pH. The exploration of segmental mobility via relaxation (Chapter 5) suggested that proton correlation times of HAs in solvents were in an order of 10^{-10} s, near the T_1 minimum at 600 MHz.

Relaxation results suggest that carbohydrate were the largest and the most immobile components while aliphatic and aromatic fragments were relatively smaller and more mobile in the two solvents tested in this study.

6.2 Progress of Thought through Dissertation Research

Minerals and organic matter are the two solid components of soil. They often interact with each other and co-exist in some forms of complexes. Clay-organic complexes are widely distributed in natural environments, in most cases, organic matter exists as coatings on mineral surfaces (Chapter 1). In this respect, organic coatings on mineral surfaces play a critical role in regulating retention and transport of organic contaminants even in soils and sediments containing as little as 1% organic matter (Chapter 1). Therefore, SOM is usually the dominant sorbent for hydrophobic organic contaminants if organic carbon content is greater than some critical level (Karickhoff, 1984). The sorption of HOCs by clay-humic complex conducted under laboratory condition may represent the real interactions occurring in natural conditions. So far, sorption by extracted HS or clay minerals alone has been extensively studied (Chapter 1); however, sorption by clay-humic complex is rarely reported. HS are structurally complex, heterogeneous materials and not all the fractions have the same affinity to clay minerals. The potential fractionation upon adsorption by minerals has been inferred (Chapter 1), but direct evidence was lacking.

Nuclear magnetic resonance spectroscopy has been accepted as the most powerful approach providing insights for SOM structure. Therefore, the solid-state and liquid-state NMR characterization of HA adsorbed on montmorillonite and kaolinite was performed in Chapter 2. The ^{13}C NMR spectra in Chapter 2, for the first time, provided direct evidence confirming that fractionation of HA occurred during adsorption on clay minerals. UV-visible spectroscopy has been used to estimate aromaticity of HA in solution. The E_4/E_6 ratios of HA in solution after each sequential coating step were determined in Chapter 2, and the conclusions were consistent with NMR results. Due to the fractionation, the sorption behavior of bound HA on clay minerals was greatly altered. Phenanthrene sorption results in Chapter 2 suggest that bound HA fractions had higher sorption linearity and affinity than the source HA.

Fourier transform infrared spectroscopy (FTIR) has routinely been used to qualitatively characterize organic compounds (Davis et al., 1999; Pere et al., 2001). An attempt was made to run DRIFT on clay-humic complex to examine any structure changes of HA upon adsorption on minerals. Unfortunately, this approach was not successful due to low organic carbon content of complex and strong interference from mineral phase.

Humins are defined as the fraction of mineral-bound organic matter that is insoluble in an aqueous solution at any pH and has a significant aliphatic nature (Chapter 1). Due to the similarities of mineral-associated and aliphatic features between humins and clay-humic complex, it is reasonable to treat humins as one class of clay-humic complexes. In this respect, the clay-humic complexation may simulate some processes in the formation of humins and this inspired me to do the work reported in

Chapter 2. The fractionation during clay-humic complexation may provide insights for better understanding the formation of humin in soil (Chapter 2).

The characterization and sorption of humin have frequently been reported in recent years (Chapter 1). Usually most of humin samples contain only a small amount of organic matter dispersed in a large volume of inorganic matrices. It has been reported that organic matter is in condensed conformation due to close contact with minerals and that humin from different origins differ greatly on their sorption of HOCs (Chapter 3). Several approaches have been found in the literatures used to extract and enrich humin from soil (Chapter 3). However, there is no specific study assessing impacts of these methods on chemical and physical structure of organic matter and their corresponding sorption properties. This fact, in addition to the desire to advance understanding interaction between SOM and minerals, give me the motivation to do the work in Chapter 3. Results in Chapter 3 revealed that extraction procedure was critical in modifying structure of humin chemically and/or physically, and the phenanthrene sorption was also differed accordingly.

Due to the complexity and heterogeneity of HS, it is still a great challenge to determine the chemical structure of HS even with substantial improvement in NMR techniques in recent years, especially applications of 2D NMR techniques such as proton homonuclear experiments (COSY, TOCSY, NOESY) and proton-carbon heteronuclear experiments (HMQC, HSQC) (Chapter 1). Chemical shift, coupling constant, and relaxation time are the three NMR parameters often used to investigate composition of organic compounds where relaxation is often used for molecular dynamics interpretation. In HS research, most of studies have focused on chemical shift,

but relaxation study has gained little attention. On the other hand, knowledge of correlation time is critical in determining molecular mobility because relaxation time has different relationships with mobility at different correlation time. However, the correlation time of HA in solution has not ever been estimated although HS is usually taken for grant to be in the slow motion regime and have a longer correlation time than small molecules (Chapter 1). Segmental mobility of HA can be inferred from relaxation time when correlation time is available. Therefore, works in Chapter 4 and 5 explored relaxation of HA in solvents by measuring proton spin-lattice relaxation time and correlation time. Segmental mobility of HA was further assessed based on observed correlation time. An effort was also made to run ROSEY to investigate conformational changes of HA in solution (Bauer et al., 1990). However, this 2D approach was not successful because the spectrum was too blurred to draw any certain information for structure or conformation interpretation probably due to the concentration limit of HA in solvents.

6.3 Advance of Knowledge in the Field

Extracted HS have been examined extensively on their structure and sorption properties. Research on clay-humic complexes is relatively scarce and such investigations either focused on composition of SOM on clay minerals (Spark et al., 1997), or on sorption of organic chemicals by complex. Few studies so far have bridged the two aspects of clay-humic complex research. Moreover, potential structural changes of SOM upon sorption by minerals have been inferred in previous studies (Chapter 1), but no direct evidence has been reported. In Chapter 2, NMR spectra provided strong

direct evidences that the fractionation of HA happened during adsorption on mineral surface. Aliphatic fractions were preferentially adsorbed by montmorillonite and kaolinite while aromatic components were more likely to be left in solution. This conclusion was also confirmed by UV-visible spectroscopic results. The consequent impacts of the structural fractionation on sorption properties of clay-humic complexes were also investigated in Chapter 2. The higher sorption affinity was observed because of the lower polarity of bound HA due to the fractionation. I believe this work advanced our understanding on interactions between HS and minerals and also provided new information for retention of anthropogenic organic containments in soils.

Humin is the major component of SOM with a high aliphatic feature and strong interaction with minerals. Four extraction approaches were thoroughly examined in Chapter 3. These procedures have often been used in literatures to derive and purify humin samples (Chapter 1). I concluded that humin samples from different extraction processes exhibited substantial variations in structural composition, conformation, and sorption behaviors. The NMR spectra and sorption results suggest that the structures of humin samples and their consequent phenanthrene sorption were not merely depended on the source of soil; extraction procedures were also much involved in modifications of chemical/physical structure and sorption behavior. The study draws attentions of researchers in HS field to use cautions in the comparison of humin characteristics especially when they are extracted differently.

Solid-state or liquid-state NMR has been frequently used for structural configuration of SOM. Most of the applications are based on chemical shift parameter. Knowledge of relaxation time is critical in setting up NMR spectrometer parameters

correctly; similarly, knowing correlation time is necessary for molecular dynamic investigation. However, relaxation study has gain little attention so far. In Chapter 4 and 5, proton spin-lattice relaxation times of HA in two solvents were measured and segmental mobility was explored based on observed correlation time. The relaxation study not only yielded a basic spectrum of relaxation times of HA in solvents, but also provided a unique approach for mobility investigation.

6.4 Future Research

Clay-humic complexation is a very promising research area. The results in Chapter 2 revealed the fractionation of HA on mineral surface by examining clay-humic complex samples prepared via one-time coating procedure. The fractionation phenomena can be further investigated by modifying coating procedure. For example, a series of clay-humic complex samples can be prepared by using sequential coating procedures; doing so may uncover a pattern of structural changes. More investigations on new HA from different sources are necessary to check consistency or any deviation on fractionation. Regarding detecting techniques, atomic force microscopy, dynamic light scattering, or size exclusion chromatograph can be employed besides NMR in the future work to investigate the changes of molecular size and weight of HA fractions adsorbed on minerals.

The components of humin are believed to be closely associated with the inorganic minerals. However, little consideration has been given to the formation of humin and the binding mechanisms involved. Based on the similarity between humin and clay-humic complex as found in this dissertation, attentive examine on clay-humic

complexation may provide us fundamentals for understanding the formation of humin. To simulate the actual process of clay-humic complexation in natural environment, the extracted HA solid particles can be mixed with clean minerals and then the mixture can be incubated at a consistent moisture level or in a wetting and drying cycle analogizing natural situations. By monitoring the structural and sorption changes of HA in the mixture, we can collect more details of clay-humic interactions which are more likely to happen in natural soils.

It is well known that global warming arises from increasing concentration of CO₂ in the atmosphere. The carbon emission from soil via microbial transformations of SOM is believed to be 10 times more than the emission from fossil fuels (Hayes and Clapp, 2001). Thus, international concerns on global warming have been focusing on the potential of soils to sequester CO₂ from atmosphere (Lal et al., 1998; Swift, 2001). Close association of SOM with minerals such as complexes in soils has been reported to enhance the resistance of SOM to decomposition (Jenkinson, 1988; Eshwarn et al., 1995). Since humin materials are the major components of SOM, they are considered to be major reservoir of the C sequestered by SOM. Further investigation is much needed on interactions between minerals and HS and their consequent impact on carbon sequestration in soils. Results from these works will help us improving soil management or practice for better carbon sequestration.

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