Interactions of hydrophobic organic solutes with dissolved humic substances
by Shaojin Chen

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University
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Abstract:
Interactions of hydrophobic organic solutes with dissolved humic substances may have a significant effect on the chemistry and fate of contaminants in natural systems. Fluorescence quenching, headspace gas chromatography (GC) and phytotoxicity bioassays were used to assess the complexation of nonionic, hydrophobic organic solutes with humic substances. No increase in quenching at elevated temperature, an increase in quenching ratio at higher viscosity, and no significant shortening of the fluorescence lifetimes of the solutes in the presence of the quencher indicated that the primary fluorescence quenching mechanism was static, resulting from the formation of solute-humic complexes. Fluorescence quenching studies with fluoranthene, 1-naphthol and napropamide, static headspace GC studies with herbicides dichlobenil, triallate and trifluralin showed significant complexation of these solutes with a variety of dissolved humic (HA) and fulvic acids (FA). The ionic strength of the solution adjusted using KCl did not significantly affect the complexation and Henry's law constants, but an increase in pH resulted in more complexation for 1-naphthol. Conditional complexation constants ranged from 9.7 to 91.5 L/g C for these solutes and generally increased with increasing solute hydrophobicity, suggesting a hydrophobic partitioning mechanism. The complexation of 1-naphthol with HA was enhanced in the presence of Cu^{2+} and Zn^{2+} at a pH higher than the pKa of 1-naphthol (9.34). This suggests that cation bridging between functional groups of anionic organic solutes and HA or FA is an additional binding mechanism for the association of organic solutes with soluble humic substances. Generally, HA showed stronger affinity for complexation with these hydrophobic organic solutes than FA. Bioassay studies with oats and tomatoes as indicator species showed that the phytotoxicity of atrazine, picloram and triallate was reduced in the presence of HA, probably resulting from reductions in bioavailability of these herbicides due to complex formation. In summary, a significant portion of these organic solutes may exist as water soluble complexes of dissolved humic substances in aqueous systems. The complexation of organic solutes with humic substances is an important process in determining the behavior and fate of these chemicals in natural systems.

INTERACTIONS OF HYDROPHOBIC ORGANIC SOLUTES WITH DISSOLVED HUMIC SUBSTANCES

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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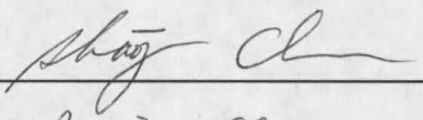
Signature 

Date April 23, 1992

VITA

Shaojin (Sam) Chen was born in Raoping, Guangdong, China on October 26, 1961. He is married to Zihui Zhang.

Because of his parents' frequent relocation, he attended several schools in Chaozhou and Raoping for his elementary and secondary education. In 1982, he obtained his B.S. degree in soil science and agrochemistry from South China Agricultural University (SCAU) in Guangzhou, China. Upon graduation, he was assigned a professional teaching assistant position in SCAU, where he taught about 3 years.

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After graduation from Cornell, he started his Ph.D. program in soil/environmental chemistry at Montana State University under Dr. W. P. Inskeep.

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ABSTRACT

Interactions of hydrophobic organic solutes with dissolved humic substances may have a significant effect on the chemistry and fate of contaminants in natural systems. Fluorescence quenching, headspace gas chromatography (GC) and phytotoxicity bioassays were used to assess the complexation of nonionic, hydrophobic organic solutes with humic substances. No increase in quenching at elevated temperature, an increase in quenching ratio at higher viscosity, and no significant shortening of the fluorescence lifetimes of the solutes in the presence of the quencher indicated that the primary fluorescence quenching mechanism was static, resulting from the formation of solute-humic complexes. Fluorescence quenching studies with fluoranthene, 1-naphthol and napropamide, static headspace GC studies with herbicides dichlobenil, triallate and trifluralin showed significant complexation of these solutes with a variety of dissolved humic (HA) and fulvic acids (FA). The ionic strength of the solution adjusted using KCl did not significantly affect the complexation and Henry's law constants, but an increase in pH resulted in more complexation for 1-naphthol. Conditional complexation constants ranged from 9.7 to 91.5 L/g C for these solutes and generally increased with increasing solute hydrophobicity, suggesting a hydrophobic partitioning mechanism. The complexation of 1-naphthol with HA was enhanced in the presence of Cu^{2+} and Zn^{2+} at a pH higher than the pK_a of 1-naphthol (9.34). This suggests that cation bridging between functional groups of anionic organic solutes and HA or FA is an additional binding mechanism for the association of organic solutes with soluble humic substances. Generally, HA showed stronger affinity for complexation with these hydrophobic organic solutes than FA. Bioassay studies with oats and tomatoes as indicator species showed that the phytotoxicity of atrazine, picloram and triallate was reduced in the presence of HA, probably resulting from reductions in bioavailability of these herbicides due to complex formation. In summary, a significant portion of these organic solutes may exist as water soluble complexes of dissolved humic substances in aqueous systems. The complexation of organic solutes with humic substances is an important process in determining the behavior and fate of these chemicals in natural systems.

CHAPTER 1

INTRODUCTION

The interaction between organic solutes (pesticides and industrial chemicals) and water soluble humic substances can influence the behavior and fate of these solutes in natural systems. Solute properties including bioactivity, persistence, biodegradability, leachability and volatility have been shown to be affected by complexation with soluble humic substances (Ballard, 1971; Bollag, 1983 ; Carter and Suffet, 1983; Landrum et al., 1987; Spencer et al., 1988; West, 1984). For example, according to Ballard (1971), the downward movement of the insecticide DDT in the organic layers of forest soils is caused by binding (complexation) of DDT with water soluble humic substances. Other studies on the interactions between dissolved humic (HA) and fulvic (FA) acids and organic pollutants have shown an increase in the water solubility of these compounds in the presence of humic substances (Chiou et al., 1986). Numerous studies have shown that the adsorption, leaching and bioaccumulation of hydrophobic organic solutes are correlated with their water solubilities (Chiou et al., 1977; Hassett et al., 1981; West, 1984). Consequently, the water solubility of a compound is a key factor influencing its behavior and fate in soils and its tendency to form complexes with soluble humic substances.

Humic substances can be divided into two groups according to their original sources: aquatic and terrestrial (soil). Although these two groups differ slightly in

elemental composition, their basic chemical structure is thought to be similar (Stevenson, 1982). Soil organic matter (SOM) is a generic term used to include all organic compounds found in soils. It is well known that SOM is comprised of a diverse and dynamic group of chemicals. Traditionally, SOM is classified into 2 groups: the non-humic and the humic substances, with the latter playing an important role in the complexation of organic solutes. Humic substances (humus) in soils are operationally defined as those organic materials extractable by strong bases, and are further divided into humin, humic acid and fulvic acid according to their water solubility and resistance to precipitation by acids (Stevenson, 1982). Recently, the definition of humic substances has been clarified to mean those organic substances in water or extracted from soils that can be adsorbed by hydrophobic XAD resins (Thurman and Malcolm, 1981). Although the theories of humus formation in soils differ considerably and the origin of humus is not well understood, it is generally agreed that humic substances are complicated heterogeneous organic compounds somewhat modified by microbial activity, with diverse functional groups and various degrees of polymerization and branching. Significant heterogeneity in composition and structure exists within and among the various fractions of humic substances (Stevenson, 1982). An important characteristic of humic substances is the simultaneous presence of aromatic ring structures and the abundance of carboxylic, hydroxylic and other hydrophilic functional groups. One very important consequence of these structural features is the presence of both hydrophobic and hydrophilic sites or subunits on the same polyelectrolyte.

More recently, the importance of the interactions between hydrophobic organic

solutes and dissolved humic substances has been recognized, especially considering the fate of xenobiotics and pesticides in the environment. It is clear that complexation of organic solutes with soluble naturally occurring humic substances is an important process in both soils and aquatic systems. However, due to the large number of pesticides and industrial chemicals, and the complexity and diversity of dissolved humic substances, our current understanding of solute-humic complexation and subsequent implications for the fate of these solutes in the environment is limited. For example, the primary binding (association) of hydrophobic organic solutes with dissolved humic substances in aqueous solutions is thought to be caused by hydrophobic partitioning (Carter and Suffet, 1982, 1983; Chiou et al., 1983, 1986, 1987). But to what extent does hydrophobic partitioning of pesticides occur in soluble organic C? Which techniques are suitable for studying the interaction of solutes with soluble organic C? How do solution conditions affect the interactions of pesticides with soluble organic C? Are there other mechanisms for binding? In addition to water solubility, what are other important physical-chemical parameters for organic solutes in nature? Does binding of an organic solute by dissolved humic substances influence such parameters? What is the effect of herbicide-humic interaction on the bioavailability and phytotoxicity of the herbicide? In order to address these questions, a study was conducted to examine the interactions between several pesticides and several dissolved humic and fulvic acids, using fluorescence spectroscopy, static headspace gas chromatography and phytotoxicity bioassays.

CHAPTER 2

COMPLEXATION OF 1-NAPHTHOL BY HUMIC AND FULVIC ACIDS

Introduction

The interaction between organic solutes and humic substances may alter the fate of pollutants in soil or aquatic systems (Ballard, 1971; Caron et al., 1985; Gschwend and Wu, 1985; Poirrier et al. 1972). Significant amounts of anthropogenic organic solutes present in aquatic systems may be in the form of soluble organic complexes, as illustrated by the study on the pesticide mirex (1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta(cd)pentalene) in surface waters (Yin and Hassett, 1986). Complex formation of organic solutes with water soluble organic carbon (C) may result in water solubility enhancement of hydrophobic compounds (Chiou et al., 1986, 1987), reduction of biotoxicities, and a change in rate of bioaccumulation (Dell'Agnola et al., 1981; Landrum et al., 1987).

Complexation of nonpolar organic solutes with humic substances has resulted in a decrease in their adsorption to soil surfaces (Gschwend and Wu, 1985; Caron et al., 1985) and an increase in transport rates in porous media (Enfield and Bengtsson, 1986). A number of techniques including fluorescence quenching, solubility enhancement, gel permeation (size exclusion chromatography), equilibrium dialysis, reverse-phase separation, ultrafiltration and head space gas chromatography have been used to estimate the extent of interaction of organic solutes with dissolved organic matter (DOM), and to elucidate the mechanisms of complexation (Carter and Suffet, 1982, 1983; Chin and

Weber, 1989; Gauthier et al., 1986; Hassett and Milicic, 1985; Landrum et al., 1984; Lee and Farmer, 1989; Madhun et al., 1986; McCarthy and Jimenez, 1985; Traina et al., 1989).

Despite considerable effort from various researchers, the mechanism and extent of complexation of organic solutes with humic substances are poorly understood. McCarthy and Jimenez (1985) have shown that the association of some polycyclic aromatic hydrocarbons with soluble humic substances is fully reversible, but more recent work by Lee and Farmer (1989) demonstrated that the interaction between napropamide (N,N-diethyl-2-(1-naphthalenyloxy)-propanamide) and dissolved peat humic acid was not fully reversible. To explain the effect of dissolved humic materials on the transport of hexachlorobenzene and anthracene in soil columns, West (1984) postulated that irreversible formation of humic-solute complexes occurred during transport. Clearly, further study is needed in order to understand the interaction between organic solutes and dissolved organic C in natural systems.

Hassett et al. (1981) found that the mobility of naphthol increased as the hydrophobicity of the mobile phase (solvent) increased. They concluded that factors increasing the affinity of the solute for solvent resulted in decreased sorption and increased mobility of the solute. Consequently, one would expect a significant amount of complexation of naphthol with humic and fulvic acids. 1-Naphthol is the major component of many pesticides such as the herbicide, napropamide and the insecticide, carbaryl (methylcarbamate-1-naphthalenol). It is also the main degradation metabolite of naphthalene which is on the EPA list of priority pollutants. 1-Naphthol is relatively

nonpolar, with a pKa of 9.34 (Weast, 1985) and exists as a nonionic compound at neutral pH. Because of its fused aromatic rings, 1-naphthol fluoresces strongly and shows a fair degree of hydrophobicity (octanol-water partition coefficient, K_{ow} = 700±62, Hassett et al. 1981). Considering these properties, 1-naphthol is an ideal model compound to study the interaction between organic solutes and dissolved humic materials.

The objectives of this study were to (i) determine whether quenching of 1-naphthol fluorescence by humic acid (HA) and fulvic acid (FA) is dynamic or static, (ii) obtain the complex formation equilibrium constants of a variety of HA and FA, (iii) test the effects of solution ionic strength, pH, and cation composition on the association of 1-naphthol with HA and FA, and (iv) suggest possible modes of 1-naphthol-HA association.

Materials and Methods

Preparation Of Solutions

1. 1-Naphthol stock solution

A $1x10^{-2}$ *M* solution of 1-naphthol in methanol was prepared by dissolving high purity 1-naphthol (Aldrich Chemical Co., Milwaukee, WI. Gas chromatography-mass spectrometry analysis showed the purity was essentially 100%) into spectrum grade methanol (EM Science, Cherry Hill, NJ). The solution was transferred to a glass bottle wrapped with aluminum foil and stored at 5° C. Since this naphthol stock solution contained methanol and contributed a final methanol concentration of 0.1% (v/v) to treatments that received 1-naphthol, methanol was added to treatments that received no naphthol so that the final concentration of methanol was 0.1%. Preliminary studies

showed that the presence of 0.1% (v/v) methanol did not alter the fluorescence of 1-naphthol.

2. Dissolved humic substances solutions

Five different HA or FA were used in the fluorescence experiments with 1-naphthol (Table 1). Stock solutions of these acids were prepared by dissolving the solid phase in KOH (pH ≈ 8 - 9), filtering through 0.45 μm filters, then adjusting the pH to 7.0. The total dissolved organic carbon (DOC) of the stock solutions was measured with a Dohrmann DC-80 carbon analyzer.

Fluorescence Experiments

A typical fluorescence experiment consisted of 3 treatments in triplicate: naphthol, naphthol plus HA or FA, and HA or FA alone (Fig. 1). Twenty-five-ml volumetric flasks were used as reaction vessels and at least 12 h was allowed for equilibration before fluorescence measurements. With the exception of experiments designed to vary the ionic strength, pH, and metal cations, all other experiments were performed in 0.01 *M* KCl at pH 7.0. All fluorescence measurements were made with a Spex fluorolog-2 spectrofluorometer (Spex Industries, Inc., Edison, NJ) equipped with a 150 W Xe lamp. All fluorescence intensity measurements were made with the excitation wavelength fixed at 294 nm and emission wavelength at 468 nm, with 10 scans for each measurement. The inner filter effect was corrected for each measurement by manually adjusting the cuvette position via an x-y translation stage. The position of the micrometer was recorded for the naphthol plus HA or FA treatment and used for the intensity measurement of the HA or FA blank treatment. The effective bandpass was 2.25 nm and 4.5 nm for the excitation

Table 1. Chemical properties of humic substances used in this study.[†]

Humic Substance	C	H	O	N	S	P	Ash	COOH	OH	Percentage of
	g kg^{-1}							(mmol charge/g)	(mmol charge/g)	Aromatic[§] C
IHSS[¶] Reference HA (1R106H)	541.3	49.1	353.9	50.3	6.0	4.0	15.2	ND[‡]	ND	24
Commercial HA	399.0	39.4	301.9	5.2	ND	ND	155.6	ND	ND	ND
Montana Soil HA	432.7	40.2	354.9	30.9	ND	ND	152.4	ND	ND	ND
IHSS Standard FA (1S101F)	537.5	42.9	404.8	06.8	0.50	0.01	0.82	6.0	1.2	18
Wheat Straw FA	555.0	52.0	357.0	08.0	ND	ND	2.40	5.5	0.7	30

[†] All items on a moisture-free basis

[‡] ND: not determined

[§] Percentage Aromaticity = $\frac{\text{Peak area of } ^{13}\text{C-NMR spectrum 110-160 ppm}}{\text{Total peak area of } ^{13}\text{C-NMR spectrum 0-230 ppm}}$

[¶] IHSS = International Humic Substances Society

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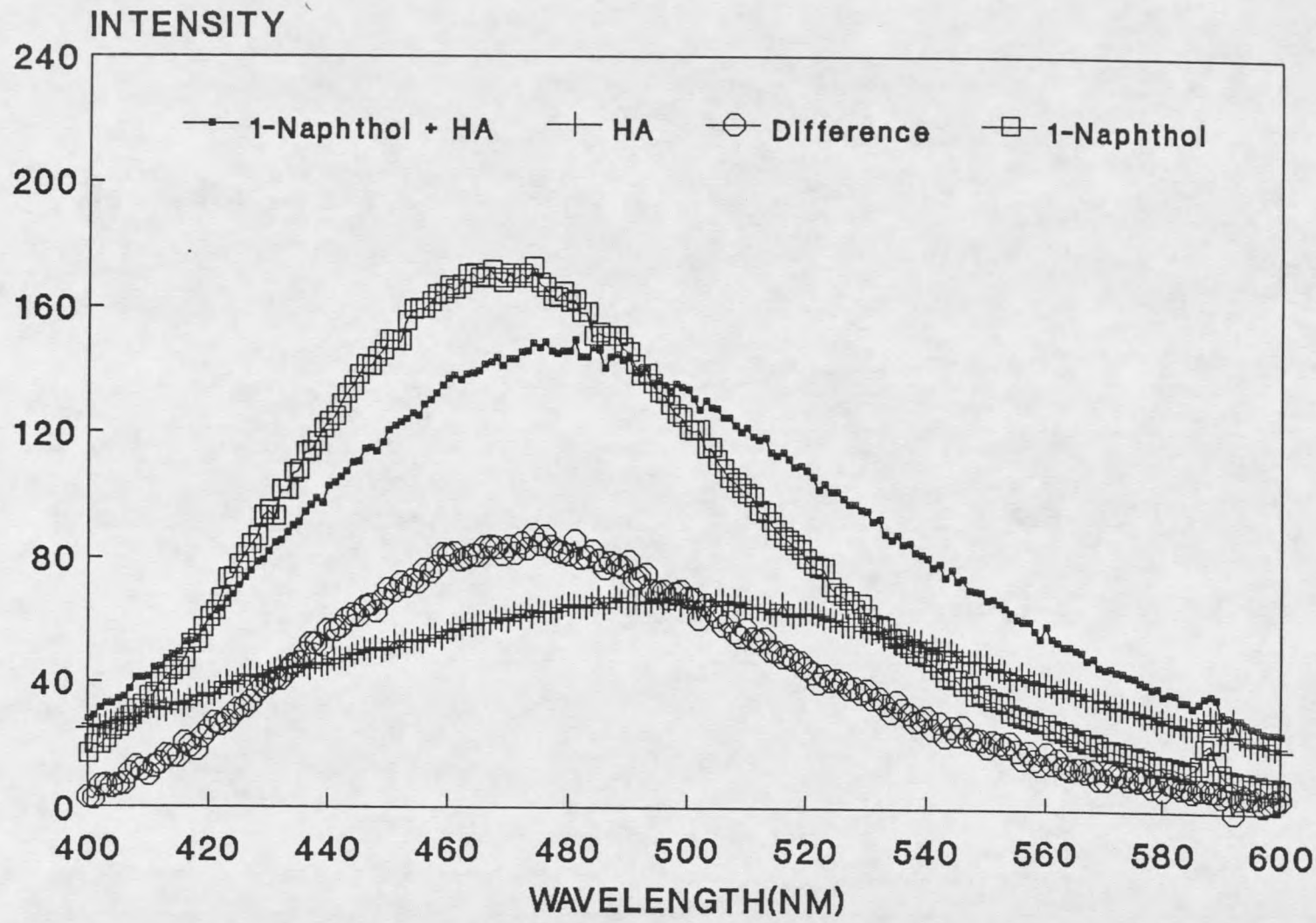

Fig. 1. Emission spectra of 1-naphthol, 1-naphthol plus HA, HA, and the difference spectrum between 1-naphthol plus HA and HA. [1-naphthol] = 1×10^{-5} *M*, commercial [HA] = 2.55×10^{-3} *M* DOC.

monochromator and the emission monochromator, respectively.

Static vs. Dynamic Quenching

Fluorescence quenching can be described by the Stern-Volmer equation (Lakowicz, 1983):

$$F_o/F = 1 + K_{sv}[Q] \quad [1]$$

where F_o and F are the fluorescence intensities in the absence and presence of the quencher Q, [Q] is the concentration of quencher, and K_{sv} is the Stern-Volmer constant. If the quenching is static (due to the formation of complexes of the fluorophore and the quencher), K_{sv} is equal to the complexation stability constant K:

$$K_{sv} = K = [F\text{-}Q]/([F][Q]) \quad [2]$$

where [F] and [F-Q] are the concentrations of free fluorophore and fluorophore-quencher complex, respectively. If the quenching is collisional (due to the collision of the excited fluorophore with the quencher molecules), the Stern-Volmer constant is the product of the bimolecular quenching constant, k_q, and the fluorescence life time, τ, of the fluorophore in the absence of the quencher:

$$K_{sv} = k_q\tau \quad [3]$$

For collisional quenching, the constant can be expressed as:

$$K_{sv} = \gamma k_0\tau \quad [4]$$

where k_0 is the bimolecular rate constant and γ is the quenching efficiency (Lakowicz, 1983).

In order to predict the influence of temperature on collisional quenching, it is assumed that $\gamma = 1$ in Eq. [4] (i.e. every collision between the excited fluorophore and the

quencher results in quenching). The Smoluchowski equation can be used to calculate k_0:

$$k_0 = 4\pi RDN/1000 = (4\pi N/1000)(R_f + R_q)(D_f + D_q) \quad [5]$$

where R is the collision radius, D is the sum of the diffusion coefficients of the fluorophore (D_f) and the quencher (D_q), and N is Avogadro's number. The collision radius is generally assumed as the sum of the molecular radii of the fluorophore (R_f) and quencher (R_q). Assuming that the collision radius does not change with temperature, and allowing $y = (4\pi N/1000)(R_f + R_q)$, we have

$$k_0 = y(D_f + D_q) \quad [6]$$

The diffusion coefficients for species i may be obtained from the Stokes-Einstein equation:

$$D_i = kT/6\pi\eta R_i \quad [7]$$

where k is the Boltzmann constant, T is the temperature, η is the solvent viscosity and R is the radius of species i. Letting $x_i = k/6\pi R_i$, Eq. [7] becomes

$$D_i = x_i T/\eta \quad [8]$$

The Stern-Volmer constants at T_1 and T_2 can be calculated from Eq.'s [4], [6] and [8]:

$$K_{sv,1} = k_{0,1}\tau_1 = y(x_f + x_q)(T_1/\eta_1)\tau_1 \quad [9]$$

$$K_{sv,2} = k_{0,2}\tau_2 = y(x_f + x_q)(T_2/\eta_2)\tau_2 \quad [10]$$

where η_1 and η_2 are the solvent viscosities at T_1 and T_2, respectively. By definition, the quantum yield $Q = \tau/\tau_o$, where τ is the lifetime of fluorescence and τ_o is the intrinsic lifetime (the lifetime of the fluorophore in the absence of nonradiative process (Lakowicz, 1983)) which is independent of T. If the conditions for the fluorescence intensity measurements are identical at temperature T_1 and T_2 (i.e. 313.15 and 283.15

K), then:

$$Q_1/Q_2 = (\tau_1/\tau_o)/(\tau_2/\tau_o) = I_1/I_2 \quad [11]$$

where Q_1 and Q_2 are the quantum yields, and I_1 and I_2 are intensities at T_1 and T_2. In the current study at $T_1 = 313.15$ and $T_2 = 283.15$ K, $I_1/I_2 \approx 0.903$ so $\tau_1/\tau_2 \approx 0.903$. For water at $T_1 = 313.15$ K, $\eta_1 = 0.6529$ cp and at $T_2 = 283.15$ K, $\eta_2 = 1.307$ cp (Weast, 1985). The ratio of Stern-Volmer constants at T_1 and T_2 can be estimated from Eq.'s [9] and [10] as

$$K_{sv,1}/K_{sv,2} = (k_{0,1}\tau_1)/(k_{0,2}\tau_2) = (T_1\tau_1\eta_2)/(T_2\tau_2\eta_1) \approx 1.999 \quad [12]$$

If the quencher concentration is constant at T_1 and T_2, then

$$\{(F_o/F)_1 - 1\}/K_{sv,1} = \{(F_o/F)_2 - 1\}/K_{sv,2} \quad [13]$$

Combination of Eq.'s [12] and [13] yields

$$(F_o/F)_{313.2\,K} \approx 1.999(F_o/F)_{283.2\,K} - 0.999 \quad [14]$$

In the presence of a quencher, $(F_o/F)_{283.2\ K} > 1$; consequently, for collisional quenching we would expect that the quenching ratio increases with temperature.

Two experiments were conducted to test whether fluorescence quenching of 1-naphthol by HA was collisional (dynamic) or static (complex-forming). In the first experiment, a brass water-jacketed cuvette holder was used to make fluorescence measurements at temperatures ranging from 283 K to 313 K($\pm 1^0$). In the second experiment, glycerol was added to the reaction vessels at 0%, 10% and 50% (v/v) to change the viscosity of the solution. Glycerol was added to all 3 treatments: 1-naphthol, 1-naphthol plus HA, and HA alone.

Effects of Solution Composition

To describe the quenching of 1-naphthol fluorescence by HA and FA, different amounts of HA or FA were added to $1x10^{-5}$ *M* 1-naphthol in 0.01 *M* KCl at pH 7. The fluorescence intensity of $1x10^{-5}$ *M* 1-naphthol in 0.01 *M* KCl at pH 7 was taken as F_o. The presence of HA or FA contributed a small amount of fluorescence to the naphthol plus HA or FA treatment, so F was determined from the difference spectra of HA or FA plus naphthol and the HA or FA blanks (Fig. 1). Linear regression was used to obtain a relationship between the quenching ratio F_o/F and the concentration of HA or FA expressed as molarity of DOC.

Two salts, KCl and $CaCl_2$, were employed to test the effects of ionic strength on fluorescence quenching. The concentration of 1-naphthol was fixed at $1x10^{-5}$ *M*, HA was fixed at $2x10^{-3}$ *M* DOC and ionic strength varied from 0.001 to 0.5 *M*. After equilibrating the reaction vessels for 12 h, we observed that concentrations equal to or greater than 0.033 *M* $CaCl_2$ caused the HA to flocculate. For these treatments, fluorescence intensity was determined only on the supernatants.

In the pH experiment, the concentration of HA was fixed at $2x10^{-3}$ *M* DOC for all the naphthol plus HA, and HA blank treatments. The desired pH values (3.0, 7.0 and 10.0) were achieved by drop-wise addition of HCl or KOH solutions.

To assess the role of cations on the formation of 1-naphthol-HA complexes, Cu and Zn were added to reaction vessels containing 1-naphthol and HA. The experiment was performed at two pH values, 5.0 and 9.5, with and without metal ions. Additions of Cu^{2+} and Zn^{2+} to the reaction vessels were made from 1 m*M* stock solutions of each ion,

which were prepared from $CuCl_2 \cdot 2H_2O$ (>99% purity, Aldrich) and $ZnSO_4 \cdot 7H_2O$(99% purity, Aldrich) respectively so that the final concentrations of Cu and Zn were $1x10^{-4}$ *M*. Several preliminary experiments were conducted to determine acceptable levels of Cu and Zn that could be used at pH 9.5 without precipitation of the metal hydroxides. At the concentrations of Cu and Zn used in these experiments, no visible hydroxide precipitates were observed.

Results and Discussion

Static vs. Dynamic Quenching

1. Temperature

Results of the temperature experiment at pH 7 show that the quenching ratio did not change significantly from 283.2 K to 313.2 K (Fig. 2). The slope of the plot F_o/F vs. temperature was essentially zero (t-test indicated that it was not significantly different than 0 at $\alpha = 0.01$). If the quenching was solely a result of collisional processes, we estimated that $(F_o/F)_{313\ K}$ would be approximately 3.74 (Eq. [14]). However, the measured quenching ratio at 313 K was 2.37, much smaller than expected. Therefore, quenching of 1-naphthol fluorescence by HA did not follow the temperature dependence expected for a collisional mechanism.

One form of the van't Hoff equation,

$$\ln(K_2/K_1) = (\Delta H^0/R)(1/T_1 - 1/T_2) \qquad [15]$$

describes the influence of temperature on the equilibrium constants, K_1 and K_2 at temperatures, T_1 and T_2 respectively, where ΔH^0 is the standard enthalpy change and R

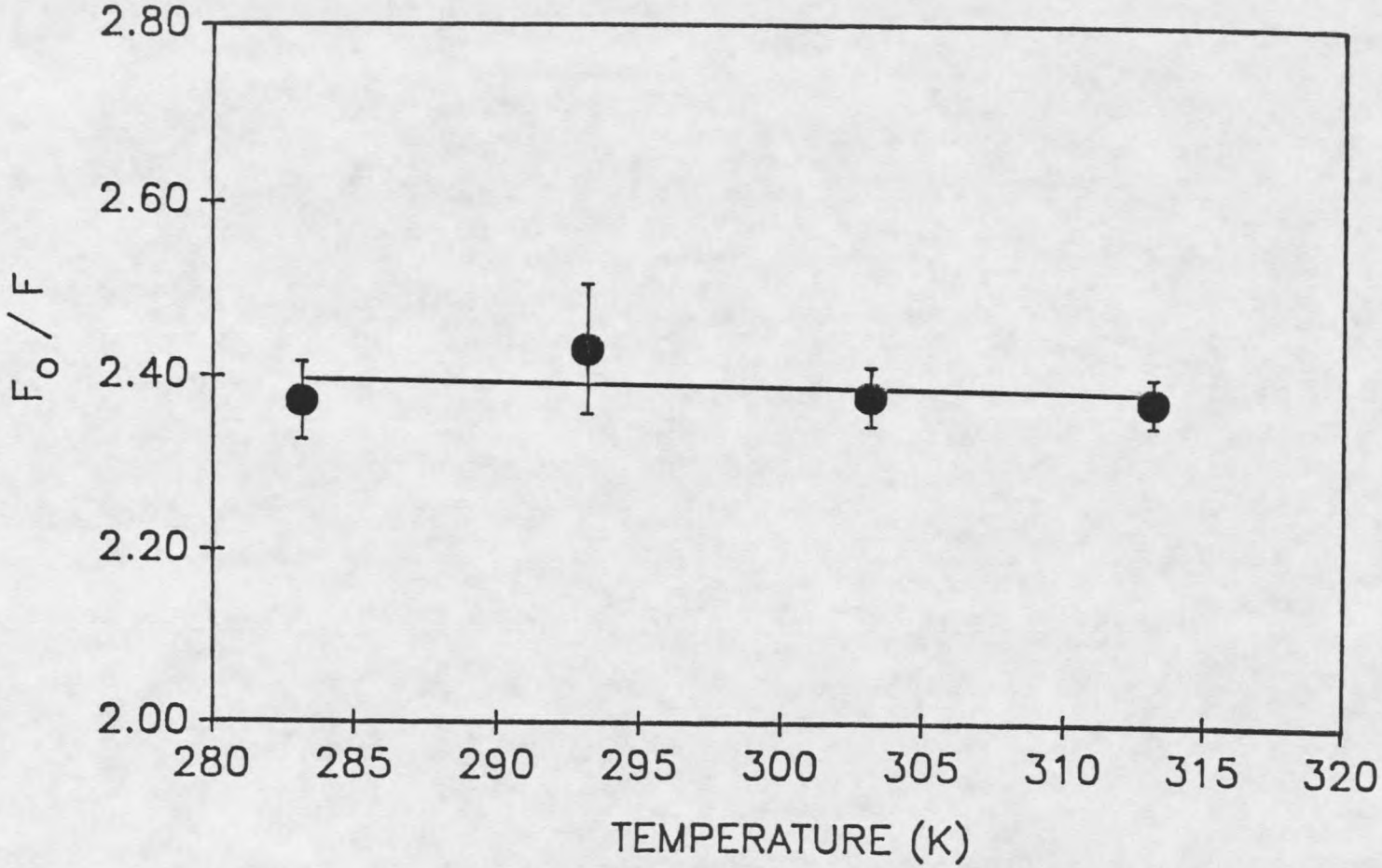

Fig. 2. Effects of temperature on the quenching ratio. [1-naphthol] = $1x10^{-6}$ *M*, IHSS [HA] = $2.00x10^{-3}$ *M* DOC.