

Effects of Iron on the Molecular Weight Distribution, Light Absorption, and Fluorescence Properties of Natural Organic Matter

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ABSTRACT

This study investigated the effects of iron on natural organic matter (NOM) absorbance, fluorescence, and molecular weight. Addition of iron(III) to 5 and 10 mg C/L XAD-8 isolated NOM samples at pH 5.5 caused absorbance of visible light to increase, fluorescence intensity of peaks at excitation/emission wavelengths 230/435 nm and 320/450 nm to decrease, and a small shift from intermediate to higher molecular weight components. Iron(II) had little or no effect on XAD-8 isolate properties. Addition of iron(III) to XAD-4 isolated NOM solutions also caused absorbance of visible light to increase. Iron(III) addition to XAD-4 isolate solutions caused a greater decrease in fluorescence intensity than was observed for the XAD-8 isolate, but little or no change in the molecular weight distribution. Overall, our results suggest that the effects of iron on NOM molecular weight, absorbance, and fluorescence properties depend upon the redox state of iron and the composition of the NOM. These results also suggest that the potential effects of iron should be considered when using fluorescence to infer NOM provenance or structure.

Key words: natural organic matter; dissolved organic matter; fulvic acid; humic acid; iron; SUVA; fluorescence

INTRODUCTION

NATURAL ORGANIC MATTER (NOM) is ubiquitous in aquatic and terrestrial environments, and plays an important role in many biogeochemical processes, affecting the fate and transport of metals, radionuclides,

and hydrophobic organic compounds (Cabaniss *et al.*, 2000, and references therein); helping to control the penetration of potentially harmful UV radiation in surface waters (Scully *et al.*, 1995), and serving as a primary carbon source to micro-organisms (Findlay *et al.*, 1986; Pullin *et al.*, 2004a; Young *et al.*, 2005). The effects of

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NOM on water treatment processes and on the removal of NOM from drinking water have been the topics of much ongoing investigation in environmental engineering (e.g., Allpike *et al.*, 2005). NOM can present problems in drinking water production by clogging filtration systems, serving as a carbon source to bacteria within distribution systems, and leading to disinfection byproducts when bromide and chloride react with humic substances. Because NOM is a complex mixture, investigators often have relied on relatively easily measured properties of mixtures, such as molecular weight (MW) distribution, light absorbance, and fluorescence, to trace NOM evolution through "natural" and engineered systems and to quantify NOM responses to photodegradation, adsorption, biodegradation, coagulation, and other processes (e.g., Cabaniss *et al.*, 2000; McKnight *et al.*, 2001; Allpike *et al.*, 2005).

Iron(III) has long been known to be a coagulant of NOM, removing NOM from solution and hence changing the NOM MW distribution remaining in solution (e.g., Maurice *et al.*, 2002a; Nierop *et al.*, 2002). Additionally, iron binding by NOM could potentially bridge NOM molecules, altering its molecular weight. Jones *et al.* (1993) observed that the addition of Fe(III) to several different water samples from humic lakes caused a relative increase in NOM MW, as measured by gel filtration. NOM is responsible for membrane fouling during drinking water treatment. Such fouling appears to be controlled at least in part by molecular size of the NOM, with most of the clogging occurring due to lower molecular weight components (e.g., Clark *et al.*, 2005) that are also less subject to coagulation by ferric salts (e.g., Volk *et al.*, 2000). Therefore, understanding how iron affects NOM molecular weight distribution is important to provide insight on coagulation and the potential for membrane fouling.

Iron also has the potential to alter light absorption properties of NOM, including the specific UV absorption (SUVA), generally measured at 254 nm. Weishaar *et al.* (2003) found that the addition of iron(III) increased UV absorbance at 254 nm and 280 nm of Suwannee River fulvic acid and a lake sample, but only by a very small amount. However, the absorbance increase appeared to be directly related to the light absorbance properties of iron(III), not due to changes in the inherent NOM absorbance. They concluded, based on an analysis of a wide range of samples, that the effects of iron(III) on SUVA₂₅₄ and SUVA₂₈₀ would be unimportant for most waters, although not necessarily for ground waters or some surface waters with high iron concentrations.

The humic/fulvic components of NOM are well known to fluoresce, and fluorescence spectroscopy has been used to infer properties and/or provenance of NOM (e.g.,

McKnight *et al.*, 2001; Chen *et al.*, 2002, 2003; Albert and Takacs, 2004). Yet, fluorescence yields can be sensitive to binding of iron and other metals such as aluminum and magnesium (Cabaniss, 1992; Pullin and Cabaniss, 1997). Because of the known sensitivity of fluorescence yields to metal binding, McKnight *et al.* (2001) acidified samples as one means of decreasing the overall amount of metal binding prior to fluorescence analysis. Using synchronous fluorescence spectroscopy, Cabaniss (1992) found that iron(III) quenched fulvic acid fluorescence very weakly at pH 7.5 due to formation of colloidal iron oxyhydroxides that sequestered most of the iron away from the fulvic acid, but quenched more strongly at pH 5.0 where iron(III) is more soluble. He also found that aluminum enhanced NOM fluorescence at pH 5.0, but quenched NOM fluorescence at pH 7.5. Our study used fluorescence excitation emission matrices (EEMs) rather than synchronous fluorescence spectroscopy because the effects of iron on EEMs need to be evaluated if EEMs are to be used to infer NOM composition and provenance.

Considering the close association that often occurs between iron and NOM, and previous observations that iron can affect NOM properties (e.g., Cabaniss, 1992; Jones *et al.*, 1993; Weishaar *et al.*, 2003), a systematic and quantitative evaluation of the effects of iron on NOM is necessary. To that end, we compared the effects of iron both as iron(II) and as iron(III) on selected properties of XAD-8 and XAD-4 resin NOM isolates from an organic-rich stream in the Upper Peninsula of Michigan (USA). The research described herein focused specifically on the effects of dissolved iron both as iron(II) and iron(III) on the molecular weight distribution, light absorption, and fluorescence properties of NOM.

MATERIALS AND METHODS

Materials and general methodology

The NOM used in these experiments was collected in June 2002 from Nelson's Creek (NLC) in the Upper Peninsula of Michigan (USA). The surface water sample had a pH of 5.5 and a dissolved organic carbon (DOC) concentration of 30 mg C/L. The sample was filtered on site through a series of cartridge filters (20 micron, Omnifilter; 1.0 micron, Parker Filtration; 0.4 micron, Nuclepore) and transported by overnight courier on ice to the Boulder, CO, USGS laboratory of Dr. George Aiken, where it was sequentially extracted and fractionated using XAD-8 and XAD-4 resins according to Aiken *et al.* (1992), and freeze dried. NOM stock solutions were prepared by dissolving either the XAD-8 base extract or the XAD-4 base extract in deionized water several hours be-

fore each experiment and diluted to a concentration of 5 mg C/L for use in experiments, unless otherwise stated. These solutions will simply be referred to as "XAD-8" or "XAD-4" throughout.

All experiments were conducted in acid-washed polypropylene bottles. All reagents were ACS grade, unless noted otherwise. Ultraviolet (UV)-treated deionized water was used for all solutions and reagents, hereafter referred to as deionized water. Experiments were carried out at pH = 5.5, at room temperature, and in the dark to prevent photo-induced effects on the NOM characteristics and iron speciation. This pH was chosen because it is near the natural pH of NLC water and in the range where iron can be expected to stay in solution when bound to NOM. Although kinetics experiments revealed equilibration in terms of NOM properties within 8 h (C. Anthony, unpublished M.S. thesis, University of Notre Dame, 2005), experiments were allowed to equilibrate for 24 h and were carried out in triplicate. All samples were filtered with 0.10- μm Millipore filters after reaction (prior to the analytical measurements).

Descriptions of specific experiments

Iron(III) addition to XAD-8. Iron(III) stock solution was prepared by dissolving ferric chloride (Acros Organics, Morris Plains, NJ) in deionized water to a concentration of 2.50×10^{-4} M iron. To prevent precipitation in the iron stock solution, the stock solution was adjusted to pH = 2 using trace metal grade HCl. Varying amounts of the iron stock solution were added to XAD-8 solutions in 100-mL polypropylene volumetric flasks to achieve NOM solutions with iron concentrations from 0.87 to 9.75 μM iron and [DOC] of 5 mg C/L (later repeated at 10 mg C/L). The pH of the NOM solution, which dropped to pH 3–3.5 during the iron(III) addition (due to the acidity of the iron stock solution), was then immediately adjusted to 5.5 using dilute NaOH as needed.

Iron(II) addition to XAD-8, with and without hydroxylamine HCl. Iron(II) stock solutions were prepared fresh daily from the dissolution of ferrous ammonium sulfate hexahydrate (Aldrich, Milwaukee, WI) in deionized water. The pH of the iron(II) stock solution was adjusted to 2.0 using HCl to prevent oxidation. Varying amounts of iron(II) stock solution were added to XAD-8 solutions in 100-mL polypropylene volumetric flasks to achieve NOM solutions with total iron concentrations that were later measured to be from 0.43 μM iron to 9.50 μM iron. The pH was then immediately adjusted to 5.5 as above.

In a separate experiment, the iron(II) additions were made as above, but the reductant hydroxylamine HCl

(Aldrich) was also added to the NOM solutions at a concentration of 10.0 mM to keep the iron in the reduced iron(II) form. We specifically used hydroxylamine (instead of ascorbic acid or some other reductant) because it is a simple, small, UV-VIS transparent, nonfluorescent, carbon-free molecule, with simple oxidation products.

Iron removal from XAD-8 by desferrioxamine B addition. A stock solution of desferrioxamine B (DFO-B; Sigma-Aldrich, St. Louis, MO, mesylate salt) was made in deionized water at a concentration of 95 mM. In order to complex some of the iron (0.34 μM) inherently found in the Nelson's Creek XAD-8 isolate, varying amounts of DFO-B were added to XAD-8 stock solution in 100-mL polypropylene volumetric flasks. Samples were made in triplicate with DFO-B concentrations of: no-added DFO-B, 50, 100, 500, and 1,000 μM .

Iron(III) addition to XAD-4. The iron(III) addition was repeated using XAD-4 as the NOM instead of XAD-8. Due to the XAD-4 having less iron initially than the XAD-8, the iron concentrations tested in this experiment were from 0.02 μM (no added iron) to 10.0 μM .

Description of analyses

Absorbance was measured using a Varian Cary 300 UV-VIS spectrophotometer from 200 to 800 nm in 1.0-cm quartz cells using dual-beam mode with deionized water as the reference.

Fluorescence EEMs were collected with a Photon Technology International (PTI) (Birmingham, NJ) Quantmaster fluorometer with a 75-W Xe arc lamp and a photon-counting PMT. EEMs were collected with excitation wavelengths ranging from 230–500 nm in steps of 10 nm and emission wavelengths ranging from 230–700 nm in steps of 5 nm in 1-cm quartz cells. The integration time was 0.05 s. The EEM data were water subtracted to remove Raman scattering peaks and zeroed where $\lambda_{\text{em}} \leq \lambda_{\text{ex}}$. The EEMs also were corrected for wavelength-dependent instrumental response using an excitation source monitoring device and monochromator correction factors provide by PTI. The EEMs were corrected for both excitation and emission inner filter effects by using the absorbance data and the equations of Kubista *et al.* (1994). The fluorescence data presented here have been normalized to 1 ppb quinine sulfate (intensity units are in quinine sulfate units, QSU) to allow for interlaboratory comparison.

High-pressure size-exclusion chromatography (HPSEC) was conducted using a Waters 2695 high-performance liquid chromatograph (HPLC) with a Waters 2996 photodiode array detector, a Waters 2475 fluorescence detector, and an in-line DOC detector (a modified Sievers

Turbo Portable TOC, Boulder, CO). The DOC detector was not operational during the XAD-8 experiments, but it was operational during the XAD-4 experiments. When only UV and fluorescence detection were used, the method followed that of Zhou *et al.* (2000). When in-line DOC detection was conducted, a TSK gel G3000PW_{XL} (300 mm long, 7.8 mm diameter) column was used with a mobile phase consisting of Na₂SO₄ and calibrated with polyethylene glycol standards (Her *et al.*, 2002, 2003). Because the two types of HPSEC work used different columns, different standards, and the DOC detector is subject to a small amount of peak broadening, the data from the different detectors could not be compared directly in terms of molecular weight. We therefore display the DOC detection data in terms of retention time rather than molecular weight. HPSEC was always conducted on filtered samples.

A Perkin-Elmer (Norwalk, CT) inductively coupled plasma optical emission spectrometer (ICP-OES) was used to measure total iron at 238.204 nm. Iron(II) was determined by the ferrozine method, as detailed in Pullin *et al.* (2004b). Iron(III) was determined as the difference between total iron and iron(II).

Dissolved organic carbon concentration ([DOC]) was measured using a Shimadzu TOC5000A (Woods Dole, IL) using potassium hydrogen phthalate as a carbon standard.

RESULTS AND DISCUSSION

Characteristics of the NOM samples

Table 1 presents results of elemental analysis, and other characteristics of the Nelson's Creek (NLC) NOM

isolates. Values from the literature for the International Humic Substances Society (IHSS) Suwannee River fulvic acid (SRFA) sample and an XAD-8 and an XAD-4 isolate from McDonalds Branch (McDBr) basin in the New Jersey Pinelands (USA) (previously characterized by Maurice *et al.*, 2002b) are included for comparison.

The ratio of O:C of the two NLC isolates is similar, but the value for NLC XAD-4 is slightly higher, consistent with XAD-4 being more hydrophilic. The concentrations of aluminum and iron are higher in the NLC XAD-8 than in the NLC XAD-4. The weight average molecular weight (M_w) of the NLC XAD-8 isolate is considerably higher than the M_w of the IHSS Suwannee River fulvic acid (SRFA) sample as is the molar absorptivity at 280 (ϵ_{280}), suggesting that the NLC XAD-8 isolate is larger and more aromatic (Traina *et al.*, 1990; Chin *et al.*, 1994). The NLC XAD-8 isolate would contain both humic and fulvic acids, but it is probably mostly fulvic acid, as most surface waters are more fulvic-rich (Aiken, 1985).

The M_w and ϵ_{280} are greater for the NLC XAD-8 isolate than for the NLC XAD-4 isolate, which agrees with the previous comparison of XAD-8 and XAD-4 isolates by Maurice *et al.* (2002b) and shown in Table 1. Absorbance and fluorescence characteristics of XAD-8 and XAD-4 (without iron addition) that were observed in this study are reported in Table 2. The corrected fluorescence of each isolate is higher at the "A" peak (excitation 230 nm, emission 435 nm) than at the "H" peak (excitation 320 nm, emission 450 nm).

Results of iron addition and removal experiments

Effects of iron(III) addition to XAD-8. When iron(III) was added to XAD-8, several changes were observed in

Table 1. Results of analysis of Nelson's Creek NOM samples and comparison with several other samples from the literature.

	XAD-8 isolate	XAD-4 isolate	IHSS SRFA*	McDBr XAD-8*	McDBr XAD-4*
% Carbon	47.75	46.83	53.49	47.71	NM
% Hydrogen	4.34	4.31	4.29	4.29	NM
% Oxygen	46.27	47.9	41.02	46.94	NM
% Nitrogen	1.16	1.37	0.70	0.45	NM
% Sulfur	0.48	0.00	0.56	0.50	NM
% Ash	1.51	1.55	0.85	3.15	NM
Aluminum (ppm)	223	97	NM	NM	NM
Iron (ppm)	1,910	1,120	NM	NM	NM
Silicon (ppm)	1,120	2,840	NM	NM	NM
OC	0.73	0.76	0.77	0.98	NM
Mw (Da)	3,112	1,242	2,170	2,217	1,693
ϵ_{280} (M ⁻¹ cm ⁻¹)	513	416	415	454	344

Data from Maurice *et al.* (2002b)

NM = not measured.

%C, % H, %O, %N, and %S were calculated on an ash-free basis.

Table 2. Characteristics of NOM without iron addition.

	Absorbance (intensity)			Fluorescence peak (quinine sulfate units)		Ratio of H/A peaks
	254 nm	280 nm	600 nm	"A" peak Ex 230 nm Em 435 nm	"H" Ex 320 nm Em 450 nm	
	XAD-8 4.73 mg C/L	0.232	0.181	0.000	109.15	
XAD-4 5.89 mg C/L	0.208	0.155	0.001	179.95	3.49	0.019

the NOM's characteristics. Nothing was added to the NOM-iron mixture to stop NOM-facilitated redox reactions from occurring, so that at the end of the experiment (i.e., after 24 h), both iron(II) and iron(III) were present, as shown in Fig. 1. NOM is known to cause the "dark" or thermal reduction of iron(III) to iron(II) (Pullin and Cabaniss, 2003b). However, it is important to note that the total [Fe] in solution was almost unaffected by filtration (slope = 0.88 \approx 1), which suggests that if iron colloids formed, they were small enough to pass through the 0.10- μ m filter. The [DOC] did not change significantly upon iron addition (data not shown), indicating that NOM precipitation did not occur. Nierop *et al.* (2002) observed little coagulation at iron(III)/C mole ratios of up to 0.02. The iron(III)/C mole ratios in our experiment were relatively low, ranging from 0.0009 to 0.026.

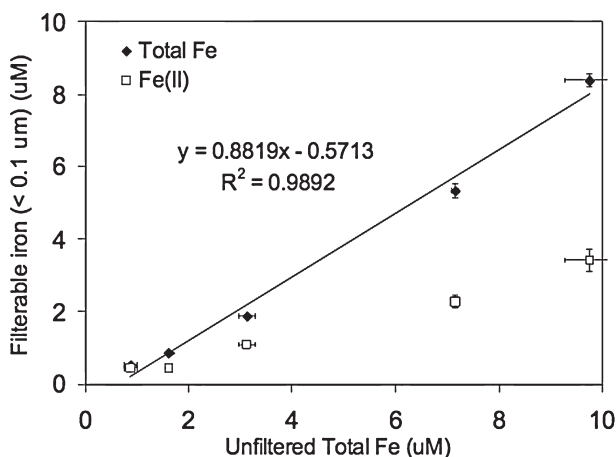


Figure 1. Graph showing the measured total [Fe] (Fe(II) + Fe(III); closed diamonds) and [Fe(II)] (open squares) that pass through a 0.1- μ m filter vs. the total iron (Fe(II) + Fe(III)) measured by ICP-OES on unfiltered samples for the iron(III) addition experiment to 5-mg C/L XAD-8. Iron(II) was measured by the ferrozine method. Error bars are \pm 1 standard deviation.

Iron(III) addition to XAD-8 affected the light absorbance properties of the NOM at some wavelengths. Absorbance of XAD-8 at 254 nm and at 280 nm did not exhibit any statistically significant changes (at the 99% confidence level, *t*-test, *n* = 3) with increasing iron(III). However, absorbance in the visible range showed a large relative increase with added iron(III) (Fig. 2a). It is important to note that the absolute changes in absorbance observed are small (Fig. 2b), even if the relative changes are large. Nevertheless, the changes in absorbance in the 500–600 nm range at the two highest added iron concentrations are statistically significant at the 95% confidence level (*t*-test, *n* = 3), though not at the 99% level.

When added to circumneutral pH water, iron(III) can precipitate and form light absorbing and/or scattering colloids (Pullin and Cabaniss, 2003a). Sherman and Waite (1985) have published the absorbance spectra of a variety of iron oxyhydroxide minerals, which show absorbance bands located in the UV and visible regions. However, the published spectra all have the general trend of increasing absorbance with decreasing wavelength, with absorbance tapering off as the wavelength exceeds 500 nm. Additionally, changes in measured absorbance caused by scattering from colloid-sized particles are expected to follow the trend of increasing scatter (to the fourth power) with decreasing wavelength (Bohren and Huffman, 1983). Finally, Pullin and Cabaniss (2003a) found that the absorbance maxima of freshly precipitated iron(III) colloids was in the range of 300–400 nm. In the experiments described here, the changes in absorbance after iron(III) addition were mainly observed in the visible region (>400 nm) with little or no reproducible change in the UV region. Therefore, we interpret the observed changes in absorbance upon iron(III) additions as likely to result from the formation of iron(III)-NOM complexes that absorb light in the visible light region. However, we can not eliminate the possibility that the observed changes in absorbance after iron(III) additions are the result of iron(III) colloid scattering or absorbance.

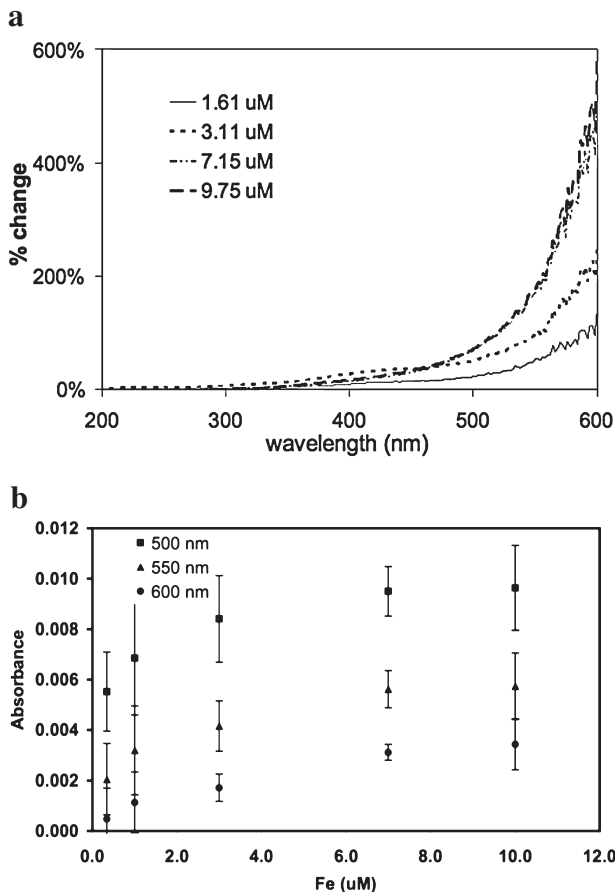


Figure 2. (a) Percent change in absorbance upon iron(III) addition to 5-mg C/L XAD-8. Results are the averages of three separate experiments at each iron concentration. (b) Increase in NOM absorbance with added iron(III). Absorbance data were collected using a 1-cm cell. Error bars are ± 1 standard deviation. The changes in absorbance from the lowest to the two highest iron concentrations are statistically significant at the 95% confidence level.

These results have important implications for understanding the role of iron–NOM interactions in the optical properties in natural waters. Absorbance by NOM in visible light range is generally low, in the range 0.010–0.001 cm^{-1} . Admittedly, the increases in absorbance observed here represent small changes in the absolute magnitude of absorbance. However, they do represent large relative changes in the total absorbance of these solutions in the visible light range. Increased NOM visible wavelength absorbance could increase the involvement of the NOM in photochemical reactions and affect the penetration of visible wavelength light through the water column. For example, at 500 nm, absorbance was observed to increase from 0.005 (± 0.002) cm^{-1} to 0.010 (± 0.002) cm^{-1} at 10 μM added iron(III). This

change in absorbance represents a 50% decrease in light penetration depth at that wavelength.

The “spectral slope” of the linear relationship between log absorbance and wavelength from 220–380 nm was determined by least-squares linear regression. This wavelength range was chosen because it was the region over which the log absorbance vs. wavelength data were linear. This parameter is currently being used as a single-number value to describe NOM absorbance over a range of wavelengths, especially by oceanographers (Twardowski *et al.*, 2004, and references therein).

A variety of spectral slope determination methods are currently in use, and therefore values determined by others (Stedmon *et al.*, 2000; Twardowski *et al.*, 2004) may not be directly comparable to ours. The absolute value of the spectral slope, as determined by our method, decreased with increasing iron(III) addition, from $-5.47 \pm 0.02 \times 10^{-5} \text{ cm}^{-1}$ at 0.87 μM total iron to $-5.18 \pm 0.06 \times 10^{-5} \text{ cm}^{-1}$ at 9.75 μM total iron. This change reflects the increase in absorbance observed in the visible region with added iron(III). This result, and the observation of increased absorbance in the visible wavelength region, indicates that changes in iron concentrations (as can occur in estuaries) may affect the value of this spectral slope parameter.

The addition of iron(III) to XAD-8 caused fluorescence quenching of the NOM. Figure 3 also shows that iron caused changes in fluorescence intensity of both the XAD-8 and XAD-4 materials.

No changes in the MW distribution were observed at added iron(III) concentrations between 0.87 μM and 7.15 μM total dissolved iron, but at 9.75 μM total dissolved iron, a clear loss of low to intermediate MW components and an increase in higher MW components was observed

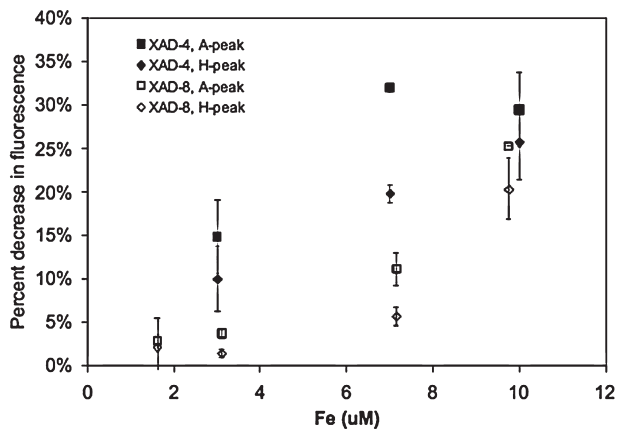


Figure 3. Percent decrease in fluorescence at both peaks for the 5 mg C/L XAD-8 and XAD-4 solutions with added iron(III). Error bars are ± 1 standard deviation.

(Fig. 4a). Since absorbance at 254 nm is not affected by the iron(III) addition (see discussion above), we interpret these changes to result from a shift in the MW distribution of the UV light-absorbing components of the NOM sample. The changes in the MW distribution demonstrate that iron(III) may affect the MW distribution of NOM, most likely by bridging NOM molecules. Figure 4b shows that essentially no change was apparent with increasing iron(III) addition in the data collected from the HPSEC fluorescence detector at 350/450 nm, even though quenching was apparent in the fluorescence EEMs at 320/450 nm.

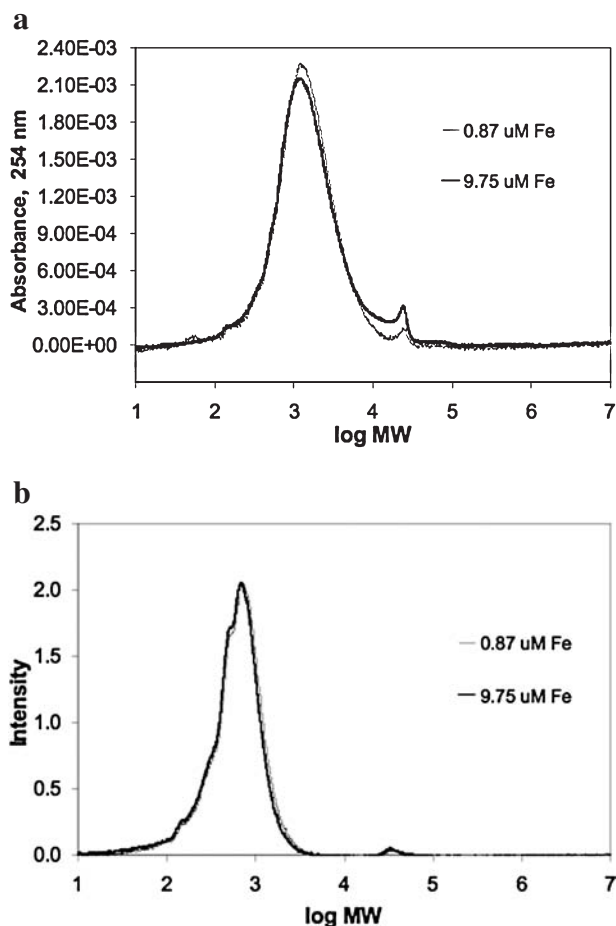


Figure 4. (a) Size-exclusion chromatogram for the iron(III) addition to the 5-mg C/L XAD-8 experiment, using the UV absorbance detector at 254 nm, showing a small loss of intermediate molecular weight components and an increase in high molecular weight components with a total of 9.75 μM dissolved iron. (b) Size-exclusion chromatogram for the iron(III) addition to the 5-mg C/L XAD-8 experiment, using the fluorescence detector set to excitation = 350 nm and emission = 450 nm, showing no change between 0.87 μM total iron and 9.75 μM total iron.

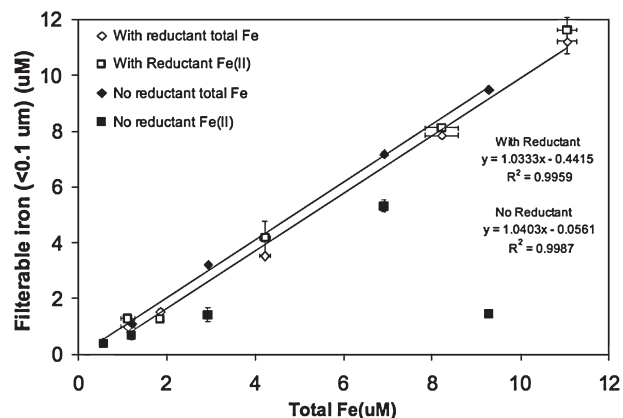


Figure 5. During the iron(II) addition without reductant experiment, oxidation of iron(II) to iron(III) occurred. Closed diamonds are total iron (II+III) and closed squares are iron(II). When iron(II) was added with the reductant hydroxylamine HCl, the added iron remained as iron(II) throughout the experiment. Open diamonds are total iron (II+III) and open squares are iron(II). Iron(II) measured by the ferrozine method. Error bars are ± 1 standard deviation.

Accurate calculation of changes in average molecular weight upon Fe(III) addition could not be accomplished because the molecular weight distribution contained not only a broad peak, as commonly observed for NOM samples (e.g., Cabaniss *et al.*, 2000), but also a second small peak at $\sim 25,000$ Da, which increased in magnitude upon Fe(III) addition (Fig. 4a). Methods for calculating average molecular weights rely on a single log-normal distribution (Zhou *et al.*, 2000) and become invalid when multiple peaks occur.

Iron(III) addition to a 10 mgC/L XAD-8 solution showed similar or slightly less pronounced changes in the NOM characteristics compared to the more dilute NOM solution exhibited (data not shown).

Iron(II) addition to XAD-8. When iron(II) was added to XAD-8 without a reductant, substantial oxidation of iron(II) to iron(III) occurred over the 24-h equilibration time (Fig. 5). The data for changes in absorbance, fluorescence, and MWs showed similar results as the iron(III) addition experiments, presumably because both additions resulted in exposure of the samples to iron(III).

To eliminate the effect of iron(III) during iron(II) additions, hydroxylamine (a chemical reductant) was added to rereduce any iron(III) formed during the experiment. The inclusion of the hydroxylamine in the solutions prevented the net oxidation of the added iron(II) (Fig. 5). Upon addition of iron(II) plus reductant, very little change was observed in the NOM bulk properties. Fluorescence at either EEM peak was unchanged to ~ 8 μM

total [Fe], and it increased by only a few percent at ~ 10 μM ; absorbance changed only very little or not at all. There was a much smaller change in the MW distribution with added iron(II) with reductant than for iron (III), only a slight increase in the higher MW components with 11.05 μM total dissolved iron (data not shown here).

As can be seen from Figs. 1 and 5, it is impossible to add iron(II) or iron(III) to NOM solutions without the NOM inducing changes in the iron redox state. These observations are consistent with prior work (Pullin and Cabaniss, 2003b; Langford *et al.*, 1981). In an effort to examine the effect of iron(II) on NOM properties in the absence of iron(III), hydroxylamine was used to keep the added iron in the iron(II) redox state by rereducing any iron(III) that was formed after the addition. This reductant was chosen because it is a small inorganic molecule and does not interfere in the spectroscopic, carbon, or size-exclusion chromatography analyses. A mechanism for the reduction of aqueous inorganic Fe^{3+} and FeOH^{2+} by hydroxylamine has been proposed (Bengtsson *et al.*, 2002). The mechanism of hydroxylamine with iron(III)–NOM complexes is not known. However, the existence of a preequilibrium step involving the formation of a $\text{Fe}(\text{NH}_2\text{OH})^{3+}$ complex in the proposed mechanism for inorganic iron(III) reduction suggests that the hydroxylamine may be able to compete with NOM for iron(III) binding, as evidenced by the lack of increase in [Fe(III)] observed in our experiments (Fig. 5).

The lack of effect of added iron(II) in the presence of hydroxylamine is interpreted to mean that any iron(II)–NOM binding has little effect on the optical and bulk chemical properties of the NOM. Due to their differences in charge and electronic structure, iron(II) and iron(III) are known to have vastly different interactions with organic ligands. According to the Irving-Williams series of stability, iron(III) is expected to form high stability complexes with carboxylate and hydroxyl ligands (which are found in abundance in NOM), whereas iron(II) is expected to bind strongly to N-containing ligands. However, indirect evidence of weak iron(II) binding to NOM does exist (Pullin and Cabaniss, 2003b, and references therein). It is possible that hydroxylamine–iron(II) complex formation could have prevented iron(II)–NOM complex formation, thereby causing our observation of a lack of effect. However, no published stability constants exist for hydroxylamine and iron(II), and the existence of this complex was not needed in the above-mentioned mechanism.

DFO-B has a high affinity for iron (III) ($10^{30.6}$; Martell and Smith, 2001), and was used to complex some or all of the iron that remained bound to the XAD-8 after the isolation/fractionation method. The XAD-8 fluorescence increased with added DFO-B at the H peak. The fluo-

rescence increased by $\sim 5\%$ at ~ 50 – 100 μM added DFO-B, and by 12% at 1,000 μM added DFO-B. The effect on the H peak was opposite to that observed when Fe(III) was added to the XAD-8 without the DFO-B, suggesting that the iron present in the XAD-8 isolate (before any iron additions) was bound to the NOM and quenched fluorescence of the material.

In contrast to the effect of DFO-B on the H peak intensity, the fluorescence of the A peak decreased with increasing DFO-B addition, by 8% at 50 μM DFO-B added to $\sim 60\%$ when 1,000 μM DFO-B was added. The fluorescence of DFO-B throughout the EEM is negligible (data not shown). One possible explanation for these results is that iron removed from the site responsible for fluorescence at 320/450 nm (H peak) is somehow becoming bound to the NOM at the site responsible for the peak at 230/435 nm (A peak), quenching its intensity. Perhaps more likely, the decrease could be due to DFO-B binding of aluminum that is inherent in the XAD-8 sample. The binding constant of DFO-B for aluminum is also high, $10^{24.14}$ (Martell and Smith, 2001), and aluminum can both quench and enhance NOM fluorescence (Cabaniss, 1992).

Unfortunately, size-exclusion chromatography with detection at 254 nm could not be used for quantifying changes in molecular weight distribution upon addition of DFO-B to NOM because the DFO-B peak overlapped with the NOM peak (Anthony, 2005, unpub. M.S. thesis, Univ. of Notre Dame).

Effects of iron(III) addition to XAD-4. In agreement with the experiments using XAD-8 isolate, we observed no evidence for XAD-4 coagulation or for formation of 0.10- μm filterable iron colloids (no statistically signifi-

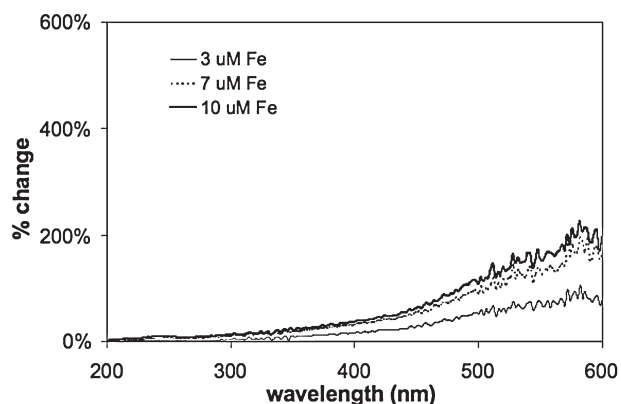


Figure 6. Percent change (+ = increase) in absorbance of XAD-4 with increasing iron concentrations (iron added as iron(III)). Results are the average of three separate experiments at each iron concentration.

cant loss of measured iron upon filtration) under the conditions of our experiments. Iron(III) addition to XAD-4 caused a very slight increase in absorbance at 254 and 280 nm. The visible absorbance of XAD-4 (Fig. 6) increased less than that of XAD-8 with the same amount of added iron(III). The ϵ_{280} of the XAD-4 sample increased slightly with iron addition, from $416 \text{ M}^{-1}\text{cm}^{-1}$ at no added iron to $482 \text{ M}^{-1}\text{cm}^{-1}$ at $10 \mu\text{M}$ total iron concentration, suggesting that iron may affect estimates of aromaticity for XAD-4 isolates (Traina *et al.*, 1990; Chin *et al.*, 1994). It is important to note that ϵ_{280} correlations with aromaticity were originally developed for XAD-8 isolates rather than for XAD-4 isolates.

The XAD-4 isolate's fluorescence decreased more with added iron(III) than the XAD-8 isolate's fluorescence (Fig. 3). For both isolates, the A peak has a higher percent decrease than does the H peak. The change in the

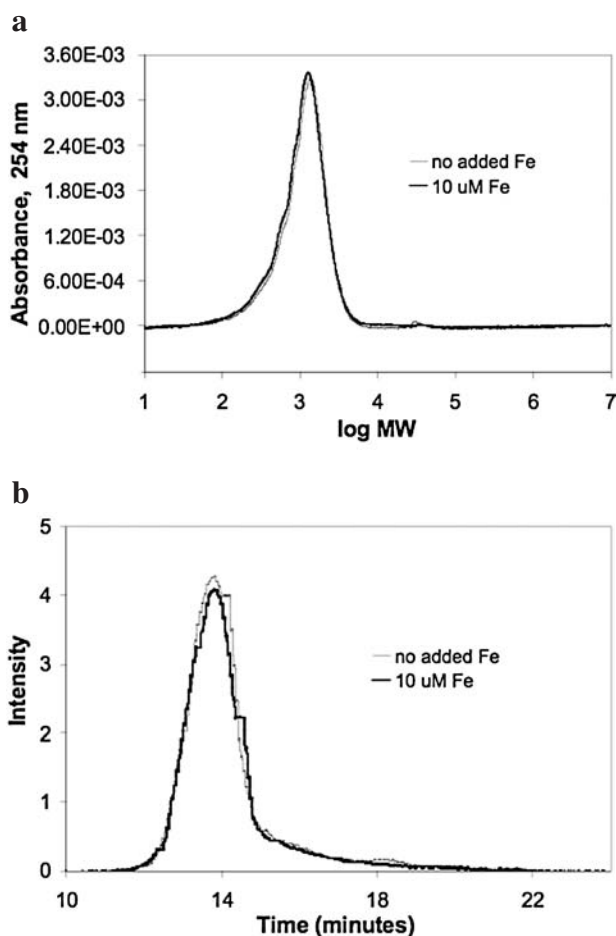


Figure 7. (a) SEC chromatograms from the UV absorbance detector, set to 254 nm, for the iron(III) addition to the 5-mg C/L XAD-4 experiment. (b) SEC chromatograms from the TOC detector for the iron(III) addition to the 5-mg C/L XAD-4 experiment.

Table 3. Iron speciation of XAD-8 and XAD-4 isolates with and without iron(III) addition.

	XAD-8 Fe(III) addition no added Fe	9.74 μM added Fe	XAD-4 Fe(III) addition no added Fe	10.73 μM added Fe
Fe(II)	83.32%	39.71%	15.58%	0.74%
Fe(III)	16.68%	60.29%	84.42%	99.26%

ratio of the percent decrease of the H peak to that of the A peak does not follow a linear trend, making it complicated to interpret. However, the difference in the ratios of the two isolates further indicates that the fluorescence characteristics of the isolates are affected differently with added iron(III), so that fluorescence would not be a good method for differentiating XAD-8 from XAD-4 components of NOM, at least in iron-containing samples.

Figure 7a and b show size-exclusion chromatograms for XAD-4 with increasing iron addition, with data obtained from the UV absorbance detector. There is no noticeable effect on the MW distribution of the XAD-4 sample. This is in contrast to the loss of intermediate MW components and the increase in high MW components observed upon addition of iron to the XAD-8 isolate, suggesting that there are properties other than MW alone that are responsible for how the NOM MW is affected by iron(III) addition. The molecular weight of different NOM samples may be affected differently by the presence of iron(III), presumably due to differences in chemical structure.

Another difference between the XAD-8 and XAD-4 isolate behaviors with respect to iron could be observed when the concentrations of iron(II) and iron(III) were measured in the two samples, with and without iron(III) addition (Table 3). In either case, the percent iron(II) present in the XAD-8 sample was much greater than in the XAD-4 sample. This difference in iron speciation in the presence of XAD-8 vs. XAD-4 may have implications for our understanding of the mechanisms of iron-NOM complexation and the role of NOM in redox cycling of iron as studied by Pullin and Cabaniss (2003b), because it suggests that XAD-4 components may play a less important role in iron reduction than do XAD-8 components.

CONCLUSIONS

Our results showed that addition of iron(III) to XAD-8 caused, within the first 8 h of reaction, absorbance of

visible light to increase, fluorescence intensity to decrease, and a small shift from intermediate to higher MW components. Addition of iron(II) to XAD-8 with reductant to prevent oxidation caused little, if any, change in NOM characteristics, indicating that iron(II) has a much smaller effect on XAD-8 than does iron(III). Addition of iron(III) to XAD-4 caused similar effects in absorbance as observed in XAD-8 with iron(III) addition. Iron(III) addition to XAD-4 caused a greater decrease in fluorescence intensity than that observed for the XAD-8, but no change in its MW distribution.

These results suggest that the potential for iron to affect NOM MW should be taken into consideration when comparing XAD-8 isolate MWs from different sites and in other similar applications. Given that soils often contain substantial iron, the high MWs of soil NOM could be related in at least some small part to the presence of iron. We agree with the conclusion by Weishaar *et al.* (2003) that the effects of iron on SUVA 254 and 280 of XAD-8 are likely to be negligible for many DOM-rich waters, but our results further suggest that waters containing abundant XAD-4 components may show more sensitivity in SUVA measurements to iron content. Because iron binding changes the visible light absorbance of NOM, water color, and light penetration may be altered. Our results suggest that caution is needed when using fluorescence EEMs to infer provenance or structure of NOM. Because iron can affect different fluorescence peaks differently, we recommend that iron content of samples be measured in conjunction with fluorescence studies, and that iron addition and/or removal experiments be incorporated when possible in conducting detailed fluorescence studies.

The results of our study suggest that lower molecular weight components of NOM are less susceptible to iron(III) induced aggregation and/or coagulation than are higher molecular weight components, so that the former are less likely to be removed when ferric salts are added in water treatment. In water treatment approaches, the pH and the dose of iron coagulant are optimized for the particular organic carbon present at a site (see, e.g., Volk *et al.*, 2000). Application of size-exclusion chromatography combined with spectroscopy, such as described herein, should be a useful approach for understanding semi-quantitatively why certain components of an NOM mixture are more or less amenable to coagulation than others at any particular site.

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